Metabolomics based metabolite analysis and exploring therapeutic potential of traditional medicinal plant of NER against metabolic and life style related disorders

Thesis submitted for the degree of Doctor of Philosophy

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Dedicated to my family, friends, and to all PhD scholars whose unfinished dreams continue to inspire

Certificates from Supervisors

This is to certify that the thesis entitled "Metabolomics based metabolite analysis and exploring therapeutic potential of traditional medicinal plant of NER against metabolic and life style related disorders" submitted by Mr. Barun Das Gupta, who got registered (registration no D-7/ISLM/65/21 of 21-22, dated 06.01.2022) his name under the Faculty of Interdisciplinary Studies, Law & Management for the award PhD (Pharmacy) degree of Jadavpur University is absolutely based upon his own work under the supervision of Prof. Pulok Kumar Mukherjee and Prof. Pallab Kanti Haldar and that neither his thesis nor any part of the thesis has been submitted for any degree / diploma or any other academic award anywhere before.

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Statement of Originality

I, Barun Das Gupta (D-7/ISLM/65/21 of 21-22) registered on 06.01.2022 do hereby declare that this thesis entitled "Metabolomics based metabolite analysis and exploring therapeutic potential of traditional medicinal plant of NER against metabolic and life style related disorders" contains literature survey and original research work done by the undersigned candidates as part of Doctoral studies.

All information in this thesis has been obtained and presented in accordance with existing academic rules and ethical conduct. I declare that, as required by thesis rules and conduct, I have fully cited and referred all materials and results that are not original to this work.

I also declare that I have checked this thesis as per the "Policy on Anti Plagiarism, Jadavpur University, 2019", and the level of similarity as checked by iThenticate software is 3%.

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5a) Publications related to Doctoral work:

i. **Das Gupta, B.**, Kar, A., Singha, S., Gayen, S., Jana, S., Sharma, N., Haldar, P.K., Mukherjee, P.K., 2025b. Metabolite Profiling and Integrated Network Pharmacology Based Mechanism of *Benincasa hispida* (Thunb.) Cogn. Fruit Against Non-insulin-Dependent Diabetes Mellitus. Phytochemical Analysis 36, 884–895. https://doi.org/10.1002/pca.3476.

ii. Mukherjee, P.K., Banerjee, S., **Das Gupta, B.**, Kar, A., 2022. Chapter 1 - Evidence-based validation of herbal medicine: Translational approach, in: Mukherjee, Pulok K. (Ed.), Evidence-Based Validation of Herbal Medicine (Second Edition). Elsevier, pp. 1–41. https://doi.org/10.1016/B978-0-323-85542- 6.00025-1

iii. **Das Gupta, B.**, Gayen, S., Chowdhury, S., Chatterjee, T., Kar, A., Duangyod, T., Charoensup, R., Haldar, P. K., & Mukherjee, P. K. Integrative metabolomics and network pharmacology - Exploring the

antidiabetic and antiobesity potential of *Allium hookeri* Thwaites in C57BL/6J mice. Phytomedicine. Elsevier. [Communicated]

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5b) Other Publications during the period of Doctoral Research (if any):

i. **Das Gupta, B.**, Kar, A., Gayen, S., Jana, S., Sarkar, S., Mukherjee, M., Sarkar, G., Sinha, S., Sharma, N., & Haldar, P. K. (2025). UPLC-QTOF-MS/MS driven network pharmacology approach for elucidating antiproliferative pharmacological mechanisms of *Plumbago zeylanica* L. Industrial Crops and Products, 225, 120441. https://doi.org/10.1016/j.indcrop.2024.120441.

ii. **Gupta, B. D.,** Kar, A., Narayan, S., Thakur, C. P., Mukherjee, P. K., & Haldar, P. K. (2023). Ultra-performance liquid chromatography-Quadrupole time-of-flight tandem mass spectrometry-based metabolite profiling, quality evaluation, and marker analysis of *Trachyspermum ammi* (L.) Sprague by high-performance thin-layer chromatography. Journal of separation science, 46(10), e2200872. https://doi.org/10.1002/jssc.202200872.

iii. Banerjee, R., **Das Gupta, B.**, Kar, A., Bhardwaj, P. K., Sharma, N., Haldar, P. K., Bandyopadhyay, R., & Mukherjee, P. K. (2023). Quality evaluation of different black rice varieties of northeastern region of India. Phytochemical analysis : PCA, 34(5), 507–517. https://doi.org/10.1002/pca.3230.

iv. Chowdhury, S., **Das Gupta, B.**, Ghosh, S., Gayen, S., Kar, A., Mukherjee, P. K., & Haldar, P. K. (2025). Metabolite profiling and neuroprotective potential of *Clitoria ternatea* L. through *in-vitro* and *in-vivo* experimental models. Fitoterapia, 185, Article 106772. https://doi.org/10.1016/j.fitote.2025.106772.

v. Kar, A., Biswas, S., **Das Gupta, B.**, Banerjee, S., Minz, A. P., Jeyaram, K., Senapati, S., Somkuwar, B. G., Sharma, N., & Mukherjee, P. K. (2025). Immunomodulatory activity of bamboo biosilica from *Dendrocalamus longispathus* Kurz. – Metabolomics integrated network pharmacology approach. Phytomedicine. https://doi.org/10.1016/j.phymed.2025.156797.

vi. Singha, S., **Das Gupta, B.**, Sarkar, A., Jana, S., Bharadwaj, P. K., Sharma, N., Haldar, P. K., Mukherjee, P. K., & Kar, A. (2024). Chemo-profiling and exploring therapeutic potential of *Momordica dioica* Roxb. ex Willd. for managing metabolic related disorders: *In-vitro* studies, and docking based approach. Journal of Ethnopharmacology, 331, 118351. https://doi.org/10.1016/j.jep.2024.118351.

vii. Gayen, S., Jana, S., **Das Gupta, B.**, Ghosh, A., Kar, A., Bala, A., Mukherjee, P. K., & Haldar, P. K. (2024). Exploration of anti-diabetic activity and metabolite profiling of *Bruguiera cylindrica* (L.) Bl.— *In vivo* anti-diabetic activity, exploration of molecular mechanism, and network pharmacological analysis. Journal of Pharmacy and Pharmacology, 76(7), 798–812. https://doi.org/10.1093/jpp/rgae030.

viii. Jana, S., Gayen, S., **Dasgupta, B.**, Singha, S., Mondal, J., Kar, A., Nepal, A., Ghosh, S., Rajabalaya, R., David, S. R., Balaraman, A. K., Bala, A., Mukherjee, P. K., & Haldar, P. K. (2023). Investigation on anti-diabetic efficacy of a Cucurbitaceae food plant from the North-East region of India: Exploring the molecular mechanism through modulation of oxidative stress and glycosylated hemoglobin (HbA1c). Endocrine, metabolic & immune disorders drug targets, 10.2174/1871530323666230907115818. Advance online publication. https://doi.org/10.2174/1871530323666230907115818.

ix. Sarkar, S., Kar, A., Shaw, P., **DasGupta, B.**, Keithellakpam, O. S., Mukherjee, P. K., Bhardwaj, P. K., Sharma, N., Haldar, P. K., & Sinha, S. (2023). Hydroalcoholic root extracts of *Houttuynia cordata* (Thunb.) standardized by UPLC-Q-TOF-MS/MS promotes apoptosis in human hepatocarcinoma cell HepG2 via GSK-3β/β-catenin/PDL-1 axis. Fitoterapia, 171, 105684. https://doi.org/10.1016/j.fitote.2023.105684.

x. Kar, A., Chowdhury, S., **Das Gupta, B.**, Gayen, S., & Mukherjee, P. K. (2025). Adaptogenic health benefits of Ashwagandha (*Withania somnifera* L. Dunal): Phytochemical and pharmacological perspectives. In Ashwagandha (1st ed., pp. 1–28). CRC Press. https://doi.org/10.1201/9781032675961-9.

xi. Gayen, S., Ghosh, S., **Das Gupta, B.**, & Haldar, P. K. (2024). Apoptosis: Natural product-derived small molecules as therapeutics. In K. Jana (Ed.), Apoptosis and human health: Understanding mechanistic and therapeutic potential (pp. 375–414). Springer. https://doi.org/10.1007/978-981-97-7905-5_18.

xii. Jana, S., **Das Gupta, B.**, Kar, A., Ghosh, A., Pervin, M., Bala, A., Matsabisa, M. G., Das, R., Ghosh, S., Mukherjee, P. K., & Haldar, P. K. (2024). Production of biopharmaceuticals on genetically modified organisms. In Concepts in pharmaceutical biotechnology and drug development (pp. 91-101). Springer Nature Singapore. https://doi.org/10.1007/978-981-97-1148-2_6

xiii. Kar Amit, Das Mahapatra A, **Das Gupta B**, Chattopadhyay D, 2022. Chapter 10 - Validation of antiviral potential of herbal ethnomedicine, Editor(s): Pulok K. Mukherjee, In: Evidence-Based Validation of Herbal Medicine (Second Edition), Elsevier, Pages 251-281, https://doi.org/10.1016/B978-0-323-85542-6.00008-1.

xiv. Haldar, P. K., Ghosh, S., Debnath, M., **Das Gupta, B.**, & Gayen, S. (2025). Traditional medicinal plants of India: Unveiling ethnopharmacological wonders (Vol. 2, pp. 1-327). Current Books International. ISBN: 978-93-85274-67-1.

xv. Haldar, P. K., Ghosh, S., Debnath, M., **Das Gupta, B.**, & Jana, S. (2024). Traditional medicinal plants of India: Unveiling ethnopharmacological wonders (Vol. 1, pp. 1-450). Current Books International. ISBN: 978-93-85274-57-2.

6. **List of Patents (if any):** NIL

7. **Presentations in National/International Conferences:**

i. **Das Gupta, B.**, Gayen, S., Chowdhury, S., Kar, A., Haldar, P. K., & Mukherjee, P. K. Evaluation of therapeutic potential of polyherbal product derived from medicinal food plants of Eastern Himalayan Region against diabetes. In 12th International Congress of Society for Ethnopharmacology & International Conference on Innovations in Drug Technology & Phytopharmaceuticals at KLE College of Pharmacy, KLE Academy of Higher Education and Research, Belagavi, Karnataka, India during March 6–8, 2025.

ii. **Das Gupta, B.**, Kar, A., Haldar, P. K., Sharma, N., & Mukherjee, P. K. UHPLC-QTOF-MS/MS analysis coupled with validated network pharmacology approach to reveal synergy-based mechanism of action of medicinal food plants from NER in the treatment of diabetes and related disorders. In P. K. Haldar (Ed.), Proceedings of the International Conference on Indian Medicinal Plants in Drug Discovery: Tradition, Science & Innovation (ISBN: 978-93-48215-95-6). School of Natural Product Studies, Jadavpur University, Kolkata, India during January 21–22, 2025.

iii. **Das Gupta, B.**, Gayen, S., Haldar, P. K., Sharma, N., Mukherjee, P. K., & Kar, A. Metabolomics integrated network pharmacology analysis for combination synergy-based approach for exploring traditionally used medicinal plants of NER for the management of diabetes and obesity. In 11th Convention of the Society for Ethnopharmacology & International Conference (SECON 2024) at BRIC-Institute of Bioresources and Sustainable Development, Gangtok, Sikkim, India during November 15-16, 2024.

iv. **Das Gupta, B**., Kar, A., Singha, S., Jana, S., Gayen, S., Chowdhury, S., Haldar, P. K., & Mukherjee, P. K. LC-QTOFMS-based metabolite profiling and evaluation of α-glucosidase and α-amylase inhibitory potential of combined plant extract-based nutraceutical formulation from NER. In International Bioresource Conclave & Ethnopharmacology Congress: 22nd International Congress of International Society for Ethnopharmacology & 10th International Congress of Society for Ethnopharmacology: at Institute of Bioresources and Sustainable Development, Imphal, Manipur, India, during February 24–26, 2023.

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ii. Participated in the "Training & Capacity Building Program on Extraction and Downstream Processing" at Jadavpur University, Kolkata during September 24-26, 2024.

iii. Participated in the "Hands-on Training on Basic Techniques in Animal Handling and Research" at Jadavpur University, Kolkata and Centre for Translational Animal Research (Centralized Animal Facility), Bose Institute, Madhyamgram, Kolkata during August 29-30, 2024.

iv. Participated in the National workshop on "Instrumental analysis for quality evaluation and validation of natural products" and hands-on training of HPLC, HPTLC, rotary vacuum evaporator at Jadavpur University, India during August 08-09, 2024.

v. Participated and presented paper in Oral session in the National seminar on "Role of medicinal plants to ameliorate diabetes and related disorders" held at Jadavpur University, India during March 08-09, 2024.

vi. Participated and presented paper in Oral session in the International conference on Traditional medicine and phytopharmaceuticals at CSIR-IIIM, Jammu and Kashmir during February 16-18, 2024.

vii. Participated in the 9th International congress of Society for Ethnopharmacology on "Redefining Ethnopharmacology for the global health and wellbeing", India at JSS College of Pharmacy, Mysore during April 22-24, 2022.

Abstract

The Northeastern region of India, with its unique ecological conditions, is home to a significant portion of India's biodiversity and serves as a gateway to its indigenous flora and fauna. The local tribes utilise traditional herbs of this region to lower blood glucose levels, regulate blood pressure, and manage metabolic disorders such as diabetes and obesity. This study employed metabolite profiling, integrative network pharmacology analysis, and *in vitro* and *in vivo* exploration to assess the therapeutic potential and mechanisms of action of three Northeast Indian food plants, selected for their traditional significance: *Allium hookeri* Thwaites, *Benincasa hispida* (Thunb.). Cogn., and *Houttuynia cordata* Thunb. To find druggable compounds in this extract, pseudotargeted metabolomic dereplication and enzyme inhibition with kinetic validation were performed. Integrative metabolomics and network pharmacology were employed to elucidate the mechanisms of phytomolecules. *In vivo* testing in diet-induced hyperglycaemia and hyperlipidaemia models proved the preventive efficacy of the extracts. Metabolite profiling and integrated network pharmacology analysis of hydroalcoholic extracts from *A. hookeri*, *B. hispida*, and *H. cordata* identified 27, 16, and 17 compounds, respectively, demonstrating a multi-molecule, multi-target approach to modulating molecular pathways. The dose-dependent inhibition of α-glucosidase, α-amylase, and pancreatic lipase was observed *in vitro*. *A. hookeri* had the lowest IC_{50} value in an *in vitro* enzyme inhibition potential test and was tested *in vivo* for its ability to prevent hyperglycemia and hyperlipidaemia from a high-fat, high-sugar diet. *A. hookeri* extract at 200 and 400 mg/kg body weight, taken orally daily, significantly ($p < 0.05$) maintained normal blood glucose levels compared to the disease control group. Moreover, the extract maintained anthropometric parameters and HbA1c levels. This study utilises established evidence of *A. hookeri*, a well-known medicinal food plant native to northeastern India, to develop an improved extract that prevents diabetes and obesity associated with modern diets. The findings will help develop safe and effective *A. hookeri* nutraceuticals to prevent metabolic disorders, such as diabetes and obesity.

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List of abbreviations

Abbreviations	Full form
%	percentage
°C	degree Celsius
ALP	Alkaline phosphatase
ALT	Alanine transaminase
ANOVA	Analysis of Variance
Apo A-I	Apolipoprotein A-I
Apo B	Apolipoprotein B
AST	Aspartate aminotransferase
b.w.	Body weight
BA	Bioactive/phytocompounds
BMI	Body mass index
BUN	Blood urea nitrogen
CAT	Catalase
cm	Centimeter
cm^2	square centimeter
COMB	A combination of the test drug and the reference control drug
DC	Disease control
DIS	Disease
DM	Diabetes mellitus
DPPH–FRSA	2,2-diphenyl-1-picrylhydrazyl free radical scavenging assay
FBG	Fasting blood glucose
g	gram
g/mol	grams per mole
GC-MS	Gas chromatography–mass spectrometry
GO	Gene Ontology
GSH	Glutathione
HbA1c	Glycated haemoglobin
HD	Test drug – High dose
HDL	High-Density Lipoprotein
HEAH	Hydroalcoholic extracts of *A. hookeri*
HEBH	Hydroalcoholic extracts of *B. hispida*

HEHC	Hydroalcoholic extracts of *H. cordata*
HFSD	High-fat and high-sugar diet
HPTLC-MS	High-Performance Thin-Layer Chromatography-Tandem Mass Spectrometry
i.p.	Intraperitoneal
IC_{50}	Half-maximal inhibitory concentration
KEGG	Kyoto Encyclopedia of Genes and Genomes
K_i	Inhibitor constant
K_m	Michaelis constant
LC-MS	Liquid chromatography-tandem mass spectrometry
LD	Test drug – Low dose
LD_{50}	Median Lethal Dose or Lethal Dose 50%
LDL	Low-Density Lipoprotein
LI	Lees index
LPO	Lipid peroxidation
m/z	Mass-to-charge ratio
mg/dL	milligrams per deciliter
mg/kg	milligrams per kilogram
min	Minutes
mL	milliliter
mM	millimolar
NC	Normal control
NER	Northeastern region of India
NIDDM	Non-Insulin-Dependent Diabetes Mellitus
NMR	Nuclear magnetic resonance
NO	Nitric oxide antioxidant potential
OGTT	Oral Glucose Tolerance Test
OH	Hydroxyl radical scavenging activity
p.o.	Per oral
PBS	Phosphate Buffered Saline
PCA	Principal Component Analysis
pH	Potential of Hydrogen
p-NPG	4-nitrophenyl-α-D-glucopyranoside
PPI	Protein–protein interaction

ppm	Parts per million
RC	Reference control
rpm	Revolutions per minute
RT	Retention time
SD	Standard deviation
SGOT	Serum glutamic-oxaloacetic transaminase
SGPT	Serum glutamic pyruvic transaminase
SOD	Superoxide dismutase
TAR	Target/gene
TC	Total cholesterol
TFC	Total flavonoid content
TG	Triglycerides
TPC	Total phenolic content
UHPLC-QTOF-MS	Ultra-high performance liquid chromatography-quadrupole time-of-flight mass spectrometry
V_{max}	Maximum reaction velocity
w/w	weigh/weight
WAT	White adipose tissue
WHO	World Health Organization
α	Alpha
α-amylase	Alpha-amylase
α-glucosidase	Alpha-glucosidase
µm	Micrometer

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Chapter 1

Metabolomics and network pharmacology in validation of traditional medicine

1.1. Translation of traditional knowledge through metabolomics

1.2. Network pharmacology in the validation of traditional medicine

1.3. Metabolic and lifestyle-related disorders: An overview

1.4. Traditional medicinal plants of northeast India in the management of metabolic disorders

1.5. Conclusion

1.6. Publications and conference presentations

1.1. Translation of traditional knowledge system through metabolomics

The World Health Organization (WHO) reports that over 80% of the global population utilises traditional herbal medicine as the primary approach to healthcare and the promotion of health and well-being (World Health Organization, 2023). The application of medicinal plants and polyherbal formulations in Indian healthcare dates back to approximately 5000 BC, as evidenced in ancient texts such as the "Charak Samhita" and the "Sushruta Samhita" (Mukherjee et al., 2022a). This traditional knowledge system initiates a translational approach in developing value-added phytopharmaceuticals for human health and well-being. The quality control and regulation of traditional medicinal plants and formulations present considerable challenges that can be addressed through thorough metabolite profiling (Mukherjee, 2019a).

The metabolomics of medicinal plants provides a detailed analysis of secondary metabolites, serving as an essential resource in drug discovery and development (Mukherjee et al., 2016). Metabolomics can be categorised as either targeted, focusing on specific metabolites, or untargeted, which aims to identify all metabolites within a defined mass range (Mukherjee, 2019a). Untargeted metabolomics, along with hybrid methods that integrate both untargeted and targeted metabolomics (pseudotargeted metabolomics), serve as an effective strategy for identifying metabolites in plant extracts, which encompass a wide variety of molecules. This method offers flexibility for extensive metabolite profiling in untargeted approaches, while enabling precision-based quantification in targeted metabolomics, thereby supporting simultaneous high-throughput detection (Du et al., 2025). Metabolomics involves analytical methods such as liquid chromatography-tandem mass spectrometry (LC-MS), nuclear magnetic resonance (NMR) spectroscopy, and high-performance thin-layer chromatography-tandem mass spectrometry (HPTLC-MS), integrated with modern multivariate statistics for metabolite data analysis (Mukherjee et al., 2016).

LC-MS-based metabolomics utilises chromatographic separation before mass spectrometric analysis, which decreases sample complexity and alleviates matrix

effects during ionisation (Zhou et al., 2012). Reverse-phase liquid chromatography typically employs C_{18} columns for the separation of semipolar compounds, including phenolic acids, flavonoids, glycosylated steroids, alkaloids, and other glycosylated species. Mass spectrometric analysis employs an ion source within a mass spectrometer to convert samples or extracts into ions. The mass analyser subsequently separates these ions, utilising either a time-of-flight (ToF) tube or an electromagnetic field, followed by a detector for the identification of metabolites (Mukherjee, 2019a). Gas chromatography–mass spectrometry (GC-MS) is utilised in metabolomics to analyse metabolites that exhibit low polarity, low boiling points, or volatility after a derivatisation step. The main limitation of GC–MS is its applicability exclusively to volatile compounds or those that can be made volatile through derivatisation, often requiring extensive sample preparation (Putri et al., 2022). NMR spectroscopy represents a significant analytical platform utilised in plant metabolomics studies. NMR can trace metabolic pathways and fluxes through isotope labelling and quantify abundant compounds without requiring complex sample preparation or fractionation (Markley et al., 2017).

These analytical techniques are essential for the scientific validation and standardisation of traditional medicine, facilitating quality evaluation through the use of key biological and phytochemical reference standards (Mukherjee et al., 2016). Plant metabolomics facilitates the rapid dereplication and effective identification of biomarkers, contributing to the discovery of novel bioactive compounds. Metabolomics can also be employed to dereplicate the biosynthesis of natural products across different developmental stages in their biological sources, while simultaneously screening for bioactivity. The use of various analytical techniques streamlines the bioassay-guided isolation process, facilitating the rapid reproduction of established activity (Tawfike et al., 2013). Thus, plant metabolomics differs from conventional phytochemical analysis in fundamental ways, notably as a data-driven approach with predictive capabilities that aims to investigate all detectable metabolites without preconceived notions or pre-selection (Bijttebier et al., 2016). The geographic origin of a plant has a significant influence on its metabolite fingerprints. Metabolite fingerprints can be

employed in quality control using a metabolomics approach. Metabolite accumulation variations directly affect sensory properties, such as flavour and aroma, as well as bioactivity. Regional chemical profiling is crucial for ensuring quality control and validating authenticity. The metabolomic profiles of medicinal plants show considerable variation depending on their geographical origin. A study revealed that the compounds in a plant extract exhibited regional variation, allowing for targeted breeding and quality improvement through metabolomics (Jing et al., 2024).

The metabolomics-based translational strategy employs chemometrics and statistical modelling to interpret data. Chemometrics combines mathematics, statistics, and computer science to identify significant patterns in complex multivariate data, facilitating the analysis of chemical fingerprints in medicinal plant extracts for quality assessment and standardisation. Principal component analysis (PCA) is an unsupervised method for identifying patterns in multivariate data, applied without prior knowledge of the samples involved. PCA minimises the dimensionality of a dataset characterised by numerous interrelated variables, while preserving the maximum variance. PCA evaluates the discriminative capacity of common components by utilising the relative peak areas of common peaks as input data, instead of the complete fingerprint (Nahar and Sarker, 2022).

1.1.1. *Metabolomics in quality control of herbal drugs*

The increasing demand for herbal medicines in both developing and developed countries requires the preservation of quality and purity in herbal raw materials and finished products. Herbal drugs are formulated using extracts or fractions derived from whole plants or specific plant parts. The intricate chemical composition of medicinal plants necessitates the standardisation of herbal drugs to ensure the preparation of safe and effective products. The quality of the precursor material is crucial for ensuring the therapeutic reproducibility of herbal remedies (Mukherjee, 2019b). The assessment of quality control techniques in herbal medicine has been conducted through the evaluation of one or two types of indicators. Medicinal plants contain various compounds, suggesting that

relying on one or two markers would be insufficient for effective quality control. Consequently, quality control, which incorporates the assessment of all compounds through metabolomics, has become increasingly important in preventing the production of substandard and adulterated pharmaceuticals derived from natural products (Lee et al., 2017).

Metabolomics plays a critical role in ensuring the quality control and authenticity of plant-derived products, thereby safeguarding consumer safety and meeting regulatory standards. Metabolomics aids in the classification of medicinal plants and the creation of metabolic fingerprints for the authentication and quality control of natural products, as well as in understanding the metabolic pathways that produce bioactive compounds (Adeeyo et al., 2024). Thus, untargeted and pseudotargeted metabolomics of herbal medicine, utilising techniques like LC-MS or NMR alongside multivariate statistical methods, facilitates the identification of biomarkers between authentic and counterfeit samples (Jiang et al., 2024). The applications of metabolomics in quality control are given in Figure 1.1.

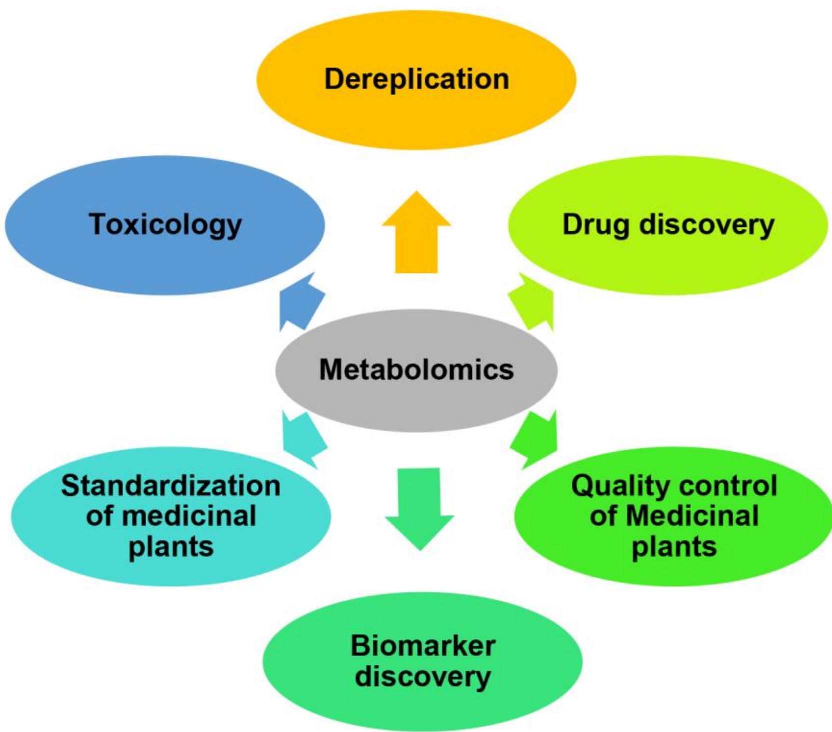


Figure 1.1. Metabolomics in medicinal plant research

1.1.2. *Metabolomics in drug discovery against metabolic disorders*

Metabolic disorders, including diabetes and obesity, continue to pose a global challenge, and contemporary drug discovery research involving medicinal plants emphasises the mechanistic pathways through which phytoconstituents exert their effects. Metabolomics-driven drug discovery involves the identification of bioactive phytochemicals and their associated biological markers, along with the application of in silico methods to elucidate the underlying mechanisms (Nafie et al., 2025). Metabolomics, a leading omics technology, is the preferred method for correlating drug or gene expression patterns with the modulation of signalling pathways (Pan et al., 2024). Metabolomics provides insight into the key pathways through which bioactive phytoconstituents act, employing a multi-molecule, multi-target approach (Mukherjee et al., 2022b).

Recent studies have utilised metabolomics as an efficient tool in the mechanistic elucidation and scientific validation of traditional medicine. A metabolomics-based network pharmacology study revealed that the fruits of *Benincasa hispida* (Thunb.) Cogn., which contain the compounds kaempferol, catechin, and naringenin, inhibit Non-insulin-Dependent Diabetes Mellitus (NIDDM) via critical bioactive pathways, including insulin resistance, the AMPK signalling pathway, the PPAR signalling pathway, and the PI3K-Akt signalling pathway (Das Gupta et al., 2025b). *Lagenaria siceraria* Stand. metabolomics showed that p-Coumaric acid, Ferulic acid, monolignol, coniferyl alcohol, hesperidin, and apigenin-7-glucoside enhanced lipid profiles in hyperlipidaemic rats via IRS–Akt–Foxo1 regulation (Banerjee et al., 2023). A metabolomics analysis of *Bruguiera cylindrica* (L.) Bl. displayed anti-hyperglycaemic potential through the modulation of both PPAR and PI3-AKT signalling pathways by Urolithin A, Urolithin B, and Esculetin (Gayen et al., 2024).

1.1.3. *Synergy of herbal medicine*

The processes underlying the synergistic effects of herbal components can be investigated to develop novel multitarget nutraceutical combinations, as well as to identify effective combinations that are individually subtherapeutic yet potent when used together. Synergistic actions entail interactions with several locations,

targets, and pathways that are intricately affected by genetic, environmental, behavioural, and temporal characteristics. It is asserted that herbs and herbal formulations exhibit synergistic effects. There is substantial *in vitro and/or in vivo* evidence supporting the existence of synergism among ingredients in specific herbal extracts. Synergy is also understood as the reduction of adverse effects, with another fundamental principle of herbs and herbal formulations positing that the toxicity of plant extracts is inferior to that of a singular isolated component. Thus, synergy plays a significant role in the therapeutic efficacy of medicinal plants and plant-derived formulations. The mechanisms of action of numerous phytomedicines remain unidentified, and there are multiple cases where a complete herbal extract demonstrates superior efficacy compared to an equivalent dosage of an isolated compound. Synergistic effects are typically regarded as advantageous, with the utilisation of low doses viewed as beneficial; yet, it is evident that negative interactions may also exist (Mukherjee et al., 2022a).

1.1.4. *System biology and metabolomics*

Systems biology seeks to comprehend the biological complexity of various measurements without resorting to hypotheses. Systems biology focuses on the dynamics of genetic, regulatory, and metabolic processes within cells, aiming to understand the complexity of cellular networks. Adopting a systems biology approach would help to explore the scientific implications of herbal medicine and modernise traditional medicine (Kitano, 2002). Metabolomics aims to identify a diverse range of compounds both qualitatively and quantitatively. Chromatographic techniques, combined with mass spectrometry and nuclear magnetic resonance spectrometry, are used in these analyses. Combining the results of these analyses with other parameters can reveal unexpected correlations, such as the relationship between the presence of specific compounds in extracts and their biological activity (Hood and Perlmutter, 2004). Metabolomics-validated herbal products can be rapidly investigated using innovative approaches, such as reverse pharmacology and systems biology, both of which are grounded in traditional medicine knowledge (Mukherjee et al.,

2022a). Metabolomics thus becomes an effective tool for studying the function and safety of herbal drugs (Figure 1.2).

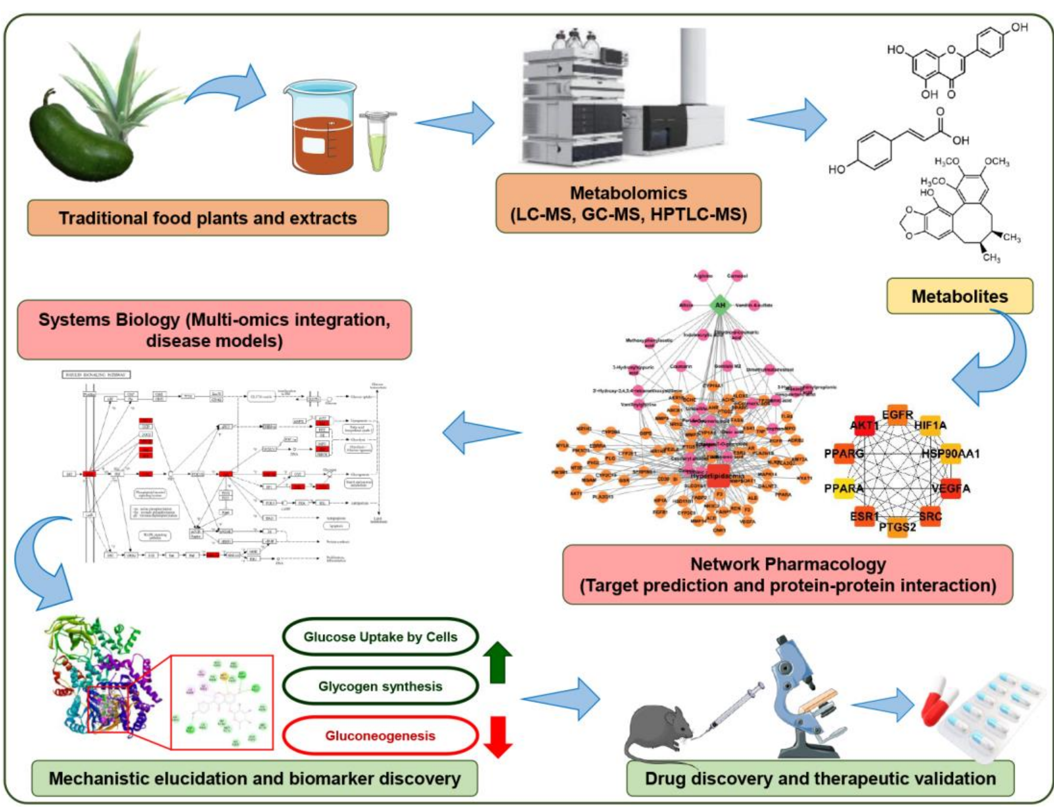


Figure 1.2. Metabolomics and system biology in drug discovery

1.2. Network pharmacology in the validation of traditional medicine

To address the limitations of the reductionist approach in drug discovery, systems biology has emerged as an integrative method to comprehend biological complexity. It represents biological systems as networks of interacting elements – genes, proteins, and metabolites – within cells or among tissues. In this context, network pharmacology has emerged as an innovative instrument that correlates drugs with various targets and pathways, synthesising information from genomics, transcriptomics, proteomics, and cheminformatics (Hopkins, 2007). Introduced by Andrew L. Hopkins, network pharmacology transcends the "one-drug-one-target" paradigm of the reductionist approach to adopt "multi-drug,

multi-target" approaches, which are ideally suited for examining traditional and herbal medicinal systems.

Network pharmacology provides (Hopkins, 2008):

- Prediction of targets for various compounds in a conventional formulation.
- Identification of critical hub genes or proteins implicated in disease modulation.
- Visualisation of compound-target-pathway networks.
- Integration with disease ontology and pathway databases (e.g., KEGG, Reactome).

The integration of ethnopharmacology, computational biology, and bioinformatics now provides a systematic and evidence-based framework for the scientific validation and global recognition of traditional medical systems, such as AYUSH, Thai traditional medicine, and traditional Chinese medicine. Metabolite profiling, combined with network pharmacology analysis, systems biology, multidirectional pharmacology, and bioinformatics, is widely used to determine the synergy (samyoga) between phytomolecules, predict and validate medicinal plants and their products, and discover novel therapeutic prospects (Das Gupta et al., 2025a).

1.2.1. *Network Pharmacology in Systems Biology*

Traditional herbs and formulations comprise multiple phytoconstituents that act in synergy to exhibit an overall pharmacological potential by targeting multiple sites (Chandran, 2015). Network pharmacology within systems biology provides integrated multi-omics data and computational methodologies to elucidate the mechanism of action and molecular interactions related to a disease (Imran et al., 2025). The reductionist method in pharmacological research emphasises the examination of individual components in isolation to understand their function in disease and therapeutic efficacy. Network pharmacology adopts a holistic perspective, examining the intricate interactions within a biological system to comprehend the overall impact of drugs on that system (Azmi, 2012). This methodology for understanding multi-targeted therapeutics and synergy

assessment, as provided by traditional medicinal plants, comprises numerous small clusters of phytomolecules, genes, proteins, and pathways integrated to create a biological network (Talevi, 2015).

This methodology for synergy assessment offers significant benefits in herbal drug discovery and the validation of traditional herbs and formulations, which include –

- Addressing multiple targets and pathways, demonstrating a cluster of therapeutic effects that reduce drug resistance (Li et al., 2023).
- Identification of synergistic interactions that minimise therapeutic concentrations to reduce side effects (Mukherjee et al., 2022b).
- Forecast secure pharmacological combinations and provide an overview of herb-herb and herb-drug interactions (Zhang et al., 2013).
- Identification of novel targets and repurposing of existing drugs/traditional formulations through the analysis of interaction potential to expedite drug discovery (Ye et al., 2016).
- Elucidating intricate disease mechanisms through gene mapping, protein-protein interactions, and the identification of disease-associated pathways (Mukherjee et al., 2022b).
- Advancement of precision medicine derived from traditional herbs and formulations through the identification of critical bioactive targets and pathways, resulting in optimised traditional formulations tailored for precision (Mukherjee et al., 2022b).

Consequently, network pharmacology elucidates the intricacies of biological systems to formulate innovative, precision-based therapeutics and drug repositioning strategies, aiming to create safe and effective alternative treatments.

1.2.2. *Network Pharmacology tools in the validation of Traditional Medicine*

The network pharmacology approach combines network biology, systems pharmacology, and bioinformatics to elucidate the complex relationships between

various herbal components in traditional medicine. It challenges the conventional "one drug-one target" paradigm by recognising that most diseases are complex and involve multiple genes, proteins, and signalling pathways (Mukherjee et al., 2022b). Traditional medicinal formulations comprise various compounds that target distinct genes and proteins, thereby affecting multiple pathways with a collectively beneficial impact on disease conditions. Utilising research and accessible molecular datasets in the formulation, one can develop and assess networks to delineate distinct features. Follow these procedures to authenticate the assertions of traditional medicines through a network pharmacology methodology (Noor et al., 2022).

Identification of active compounds: The preliminary step in network pharmacology analysis involves identifying phytoconstituents present in herbs and formulations through an exhaustive literature survey or by conducting LC–MS/MS analysis.

Protein–target interactions and pathway analysis: This phase involves predicting the targets of the identified phytoconstituents using databases such as Binding DB (www.bindingdb.org), Swiss Target Prediction (www.swisstargetprediction.ch), and DrugBank (go.drugbank.com). The protein targets are examined for their corresponding gene encoding in Uniprot (www.uniprot.org). The genes are subsequently utilised to investigate disease interactions in the Therapeutic Target Database (TTD; db.idrblab.net), DrugBank (go.drugbank.com), the Online Mendelian Inheritance in Man database (OMIM; www.omim.org), the DisGeNET database (www.disgenet.org), and STRING (string-db.org), among others. The disease-associated genes are used to create pathway analysis through which proteins interact, as determined by STRING (string-db.org), Database for Annotation, Visualisation, and Integrated Discovery (DAVID; davidbioinformatics.nih.gov), and ShinyGO (bioinformatics.sdstate.edu) (Mukherjee et al., 2022b).

Network construction and analysis: The network illustrating botanical–bioactive, bioactive–target, and target–disease interactions is generated using Cytoscape (cytoscape.org) (Das Gupta et al., 2025a). Cytoscape is an open-source software

platform that enables the visualisation and analysis of complex networks. Network analysis techniques allow the identification of central nodes (highly interconnected proteins) and clusters (groups of strongly interacting proteins) (Shannon et al., 2003). The amalgamation of individual interacting networks produces a composite network that demonstrates multi-molecule, multi-target interactions to establish synergy. The methodology of integrative metabolomics-based network pharmacology is illustrated in Figure 1.3.

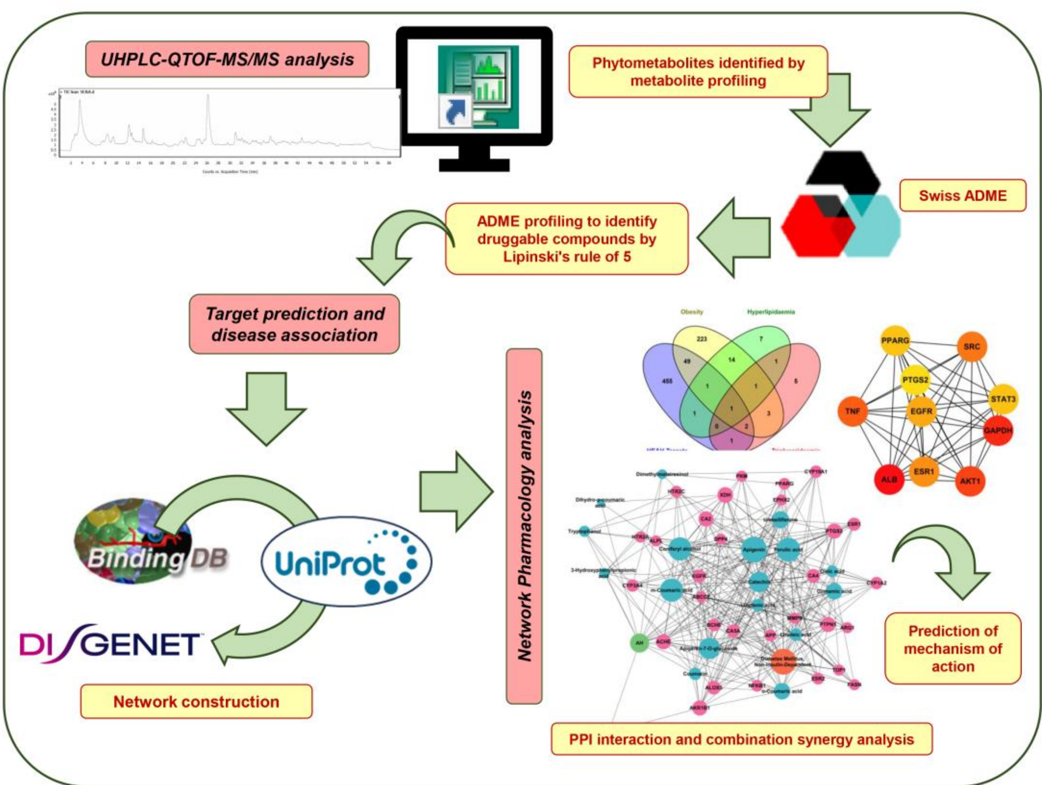


Figure 1.3. Integrative metabolomics-based network pharmacology approach in natural product drug discovery

Validation and hypothesis generation: Validation involves comparing network findings with established assertions to ensure consistency, thereby corroborating both sources of knowledge. It is crucial to juxtapose network and pathway analysis with the established claims of traditional medicine/formulations within the framework of network pharmacology. It validates the alignment between network findings and conventional wisdom. The outcome confirms the ability of network

pharmacology to identify pathways consistent with traditional uses. It may unveil new insights and correct inconsistencies (Noor et al., 2022). A combination of traditional knowledge with contemporary methodologies facilitates a more thorough comprehension of the effectiveness of traditional medicinal flora and formulations. This validation may be succeeded by experimental cross-validation to enhance reliability. The development of hypotheses arises from previously unrecognised pathways identified during research, facilitating new insights into mechanisms of action and highlighting the capacity of network pharmacology to validate traditional medicine while revealing novel mechanisms (Srikanth, 2021).

1.2.3. *Network pharmacology in personalised healthcare systems*

Recent research on healthcare treatment emphasises the importance of minimising unnecessary side effects and addressing the underlying causes of diseases, while also promoting personalised preventive measures (Pandey and Gupta, 2024). Network pharmacology employs molecular interactions between bioactive compounds and their respective gene targets to customise the specific application of traditional herbs and their derived formulations (Li et al., 2023). Precision medicine, inspired by traditional medicine, is made possible by the combination of network pharmacology with omics technologies such as transcriptomics, proteomics, metabolomics, and epigenomics, which focus on synergistic network targets. The discovery of bioactive metabolites in herbs and herbal formulations provides valuable evidence that can be used in large clinical trials to evaluate their efficacy (Poornima et al., 2016). A study reveals that two traditional Chinese medications, WeiFuChun (WFC) and MoLuoDan (MLD), which are used to address gastritis, have pharmacological effects through distinct mechanisms. MLD demonstrated greater specificity of action compared to WFC, underscoring the need for network pharmacology-based elucidation of the mechanism of action in the development of precision medicine (Zhang et al., 2024).

1.2.4. *Role in drug discovery and repurposing*

The network pharmacology method for expedited drug discovery initiates with target identification through metabolite profiling and the determination of corresponding human gene targets, subsequently leading to disease association and the establishment of pertinent mechanistic pathways. In drug repurposing, established metabolites and gene targets are integrated with disease-gene networks from an alternative disease to explore potential pathway modifications (Joshi et al., 2025). Gene mapping and disease association in network pharmacology has markedly decreased the time and expense associated with drug development and repurposing (Hopkins, 2007).

The recent outbreak of COVID-19 has underscored the necessity for high-throughput screening of traditional herbs and herbal formulations to identify leads through network pharmacology-based computational approaches. These leads can be validated through experimental validation and the exploration of alternative therapeutics in the management of various diseases. *Plumbago zeylanica* L., commonly referred to as Chitrak, is an esteemed Ayurvedic herb noted for its therapeutic efficacy in addressing digestive insufficiency, intestinal disorders, haemorrhoids, abdominal pain, and perianal swelling (Government of India, 2001a). The repurposing of *P. zeylanica* for exhibiting antiproliferative potential was studied using metabolomics-based network pharmacology analysis. The study provided mechanistic insights into the potential apoptotic and antitumour activity of *P. zeylanica* extract, mediated by Plumbagin and other phytometabolites, against prostate cancer, hepatocellular carcinoma, breast cancer, and tumour growth (Das Gupta et al., 2025a).

The repurposing of *Curcuma longa* L., traditionally utilised for wound healing, skin ailments, and metabolic disorders, has been reported as a potential lead for drug development against Alzheimer’s disease via network pharmacology (Kadiri and Tiwari, 2025). Another example of drug repurposing includes the fresh fruits of *Lagenaria siceraria* (Mol.) Standl., which are used in fever, cough, dyspnoea/respiratory distress and Inflammation (Government of India, 2001b). Recent research has indicated the involvement of *L. siceraria* in hyperlipidaemia

management through mechanistic assessment utilising metabolite profiling and network pharmacology methodologies (Banerjee et al., 2023). In addition to natural products, drug repurposing through network pharmacology has identified selegiline, a monoamine oxidase inhibitor used in the treatment of depression, as a potential anticancer agent (Kumkar et al., 2024). Thus, network pharmacology is a practical approach in the drug discovery and scientific validation of traditional medicine.

1.3. Metabolic and lifestyle-related disorders: An overview

Lifestyle-related disorders like Diabetes mellitus (DM) and Obesity are chronic metabolic disorders recognised as a global public health concern. WHO identifies Diabetes and Obesity as one of the ten most significant global health issues (Ghosh, 2022; Basile et al., 2022). ICMR has identified India as the most important contributor to metabolic diseases (Anjana et al., 2023). Diabetes, especially non-insulin-dependent diabetes mellitus and Obesity, are heterogeneous and multifaceted disorders leading to conditions like insulin resistance, hyperglycaemia, hyperlipidaemia, hypertension, hypertriglyceridemia and hypercholesterolaemia (Kar et al., 2024; Pi-Sunyer, 2003). NIDDM is a persistent metabolic condition characterised by abnormalities in both the production and function of insulin. Obesity is caused by low rates of physical activity and chronic overeating despite its genetic and epigenetic influences (Safaei et al., 2021).

1.3.1. *Current treatment strategies and limitations*

Contemporary approaches to managing NIDDM and obesity encompass non-pharmacological interventions, specifically dietary adjustments and the incorporation of physical activity (Dent et al., 2012). The pharmacological treatment options comprise appetite suppressants, digestion and absorption inhibitors (α-glucosidase, α-amylase, and pancreatic lipase inhibitors), metabolic enhancers, glucose reabsorption blockers (SGLT2 inhibitors), glucose uptake enhancers (activators of GLUT2 and GLUT4), and incretin mimetics (GLP-1 receptor agonists) (Mukherjee et al., 2020). Nevertheless, numerous therapeutic

alternatives lack sustained efficacy and holistic management. Lifestyle modifications yield varied individual responses and are most effective as preventive strategies; nonetheless, the aforementioned drugs often result in flatulence, diarrhoea, indigestion, vomiting, soft and oily stools, and gastrointestinal disturbances (Das Gupta et al., 2025b). In addition to the aforementioned adverse effects, these medications are contraindicated for those with a history of seizures, hypertension, congestive heart failure, myocardial infarction, or arrhythmias. Moreover, discontinuation of these medications often leads to a reversal of effects, including considerable weight gain (Bonnet and Scheen, 2017). These limitations in modern treatment strategies direct the therapeutic focus towards the traditional system of medicine for the exploration of novel therapeutic targets, cost-effective treatment alternatives, and the holistic management of NIDDM, obesity, and related comorbidities.

1.3.2. *Alternative therapeutics with herbal medicine*

Plant-based therapy in the management of NIDDM and obesity has been used since ancient times and is documented in traditional texts of Ayurveda and Charak Samhita. Natural alternatives include whole extracts and plant-derived phytochemicals. Phytomolecules like quercetin **(1)** and daidzein **(2)** act by AMPK activation and enhancing glucose uptake by activating GLUT1 and GLUT4 transporters (George Thompson et al., 2015). Phenolic compounds like kaempferol **(3)**, resveratrol **(4)**, luteolin **(5)** have shown promising α-glucosidase inhibitory potential, while epicatechin **(6)**, myricetin **(7)**, rosmarinic acid **(8)** and caffeic acid **(9)** exhibited α-amylase inhibitory potential (Kar et al., 2024). Flavones such as apigenin **(10)** have been shown to stimulate lipolysis in rat adipocytes, whereas luteolin has been demonstrated to decrease triglyceride formation in murine 3T3-L1 preadipocytes in a dose-dependent manner (Williams et al., 2013). Flavonoids, such as naringenin **(11)**, have been found to inhibit hepatic steatosis and dyslipidaemia by increasing fatty acid oxidation, reducing VLDL overproduction, improving insulin sensitivity, and decreasing cholesterol synthesis (Yoshida et al., 2013). Genistein **(12)** has been shown to exhibit

antiobesity effects by enhancing the methylation of six cytosine-guanine sites, thereby modifying obesity susceptibility (Dolinoy et al., 2006).

(1) (2) (3)

(4) (5) (6)

(7) (8) (9)

(10) (11) (12)

Many compounds have been identified as beneficial in pancreatic β-cell regeneration. These compounds have been reported to regulate oxidative stress, inflammation, and apoptotic pathways, thereby improving β-cell function and regeneration (Kimani et al., 2023). Swertisin **(13)**, a flavonoid, was found to promote islet neogenesis via the Activin-A-driven MEK-TKK pathway in mice subjected to partial pancreatectomy (Dadheech et al., 2015). In another study, Salidroside **(14)**, a phenylpropanoid glycoside, was shown to enhance β-cell mass and replication by diminishing oxidative stress, activating the AMPK-AKT pathway, and restoring mitochondrial membrane potential through the reduction

of NOX2 expression (Ju et al., 2017). Capsaicin **(15)**, a capsaicinoid, has been shown to demonstrate remarkable efficacy in managing metabolic disorders, including obesity and NIDDM. Capsaicin has been shown to mitigate obesity-induced insulin resistance, enhance SIRT-1 expression to induce the browning of white adipose tissue, augment AMPK activity, regulate genes associated with gluconeogenesis and glycogen synthesis, and inhibit ghrelin release in animal models (Ghosh, 2022).

(13) **(14)** **(15)**

In addition to isolated phytomolecules, various plant extracts, encompassing both medicinal and culinary species, have demonstrated significant antidiabetic and antiobesity properties. *Aegle marmelos* Correa. leaves were reported to contain coumarins **(16)**, umbelliferone **(17)**, and esculetin **(18)**, which enhanced lipolysis. *Allium cepa* L. peels, which contain quercetin, were found to suppress preadipocyte differentiation and inhibit adipogenesis. The bulbs comprising cycloalliin **(19)**, S-methyl-L-cysteine **(20)**, S-propyl-L-cysteine sulfoxide **(21)**, and dimethyl trisulfide **(22)** were observed to reduce serum triglyceride and free fatty acid levels in diabetic rats (Mukherjee et al., 2015). *Benincasa hispida* (Thunb.) Cogn. whole fruits containing kaempferol, catechin **(23)**, linolenic acid **(24)** and linoleic acid **(25)** were found to inhibit α-glucosidase and α-amylase enzymes comparable to reference standard acarbose (Das Gupta et al., 2025b). Fruits of *Momordica dioica* Roxb. ex Willd. containing β-sitosterol **(26)**, gypsogenin **(27)**, stigmasterol **(28)**, kaempferol 7-O-neohesperidoside **(29)**, and balsaminoside-A **(30)** have been documented to inhibit α-glucosidase, α-amylase, and pancreatic lipase in a dose-dependent manner, demonstrating antihyperglycaemic and

antihyperlipidaemic potential comparable to standard inhibitors (Singha et al., 2024).

Fruits of *Lagenaria siceraria* (Molina) Standl. containing coniferyl alcohol **(31)**, ferulic acid **(32)**, and p-coumaric acid **(33)**, these compounds inhibited carbonic anhydrase activity in a dose-dependent manner, potentially aiding in the management of oedema, hypertension, obesity, and associated metabolic disorders (Chanda et al., 2021). In a report by Chanda et al., the fruits of *Coccinia grandis* (L.) Voigt, which contain caffeic acid, sinapic acid **(34)**, ferulic acid, caffeoylquinic acid **(35)**, p-coumaroylquinic acid **(36)**, feruloyl quinic acid **(37)**, Cucurbitacin E **(38)**, and Sophorol **(39)**, exhibit inhibitory effects on the α-glucosidase enzyme and may function as a functional food for managing diabetes-related disorders (Chanda et al., 2020). The extract of *Dalbergia sissoo* Roxb. bark, which includes β-amyrone **(40)**, Ergosta-4,6,8(14),22-tetraen-3-one **(41)**, Lup-20(29)-en-3-one **(42)**, Lupeol **(43)**, and Soyasapogenol B **(44)**, exhibits various anti-diabetic effects against the enzymes α-amylase, α-glucosidase, and DPP-4, as demonstrated by molecular docking analysis (Vijh and Gupta, 2024). The extensive heritage of medicinal and food plants in India has consistently captivated researchers' interest in the development of innovative and alternative therapies for managing metabolic disorders. Medicinal plants are cultivated throughout India, with particular emphasis on the northeastern region due to its distinctive diversity and traditional heritage. The medicinal plants of the northeastern region of India are explored in detail in the subsequent section.

(16) **(17)** **(18)**

(19) **(20)** **(21)**

(22) **(23)** **(24)**

(25) **(26)** **(27)**

(28) **(29)** **(30)**

(31) **(32)** **(33)**

(34) (35) (36)

(37) (38) (39)

(40) (41) (42)

(43) (44)

1.4. Traditional medicinal plants of northeast India in the management of metabolic disorders

The northeastern region of India (NER) is a notable biodiversity hotspot owing to its ecological conditions. NER comprises roughly fifty per cent of India's biodiversity and is acknowledged as the geographical conduit for the majority of indigenous flora and fauna. The traditional communities of the NER possess profound ethnobotanical knowledge, largely due to their deep connection with

local natural resources. This indigenous traditional knowledge is crucial for the development of novel therapeutics with improved efficacy and safety (Das et al., 2024).

1.4.1. *Medicinal plants of NER: Traditional uses in diabetes, obesity and associated complications*

Natural products play a vital role in daily nutrition, significantly enhancing our health and well-being. Traditional medicinal systems have acknowledged food plants as powerful healing agents, emphasising the principle of "food as medicine". This concept emphasises that a comprehensive diet abundant in natural bioactive compounds can significantly contribute to the prevention and management of diverse health concerns (Mukherjee et al., 2022). This diet, inspired by traditional healthcare practices, is deeply rooted in the northeastern region of India. The unique climatic and geographical characteristics of the region significantly contribute to the diverse array of plant species containing potent bioactive compounds (Sheikh et al., 2015).

Most of the medicinal food plants available in the NER are an integral part of the daily diet. *Allium hookeri* Thwaites, belonging to the family Liliaceae, also known as hooker chive and locally referred to as Napakpi in Manipur, is consumed whole as a vegetable (Deb et al., 2023). *Parkia roxburghii* G.Don (Fabaceae), popularly known as the tree bean and locally known as Yongchak in Manipur, has pods and seeds that are consumed as a vegetable in various forms, including raw, salad, boiled, or pickled (Chhikara et al., 2018). *Houttuynia cordata* Thunb. (Saururaceae) commonly known as Toningkok is mostly consumed raw as a salad (Kumar et al., 2014). Apart from being a part of the daily diet, these food plants are also consumed together in dishes known as 'Singju' and 'Eromba' in Manipur (Dagawal et al., 2023; Devi and Basumatary, 2017; Singha et al., 2021). *Polygonum posumbu* Buch. Ham. Ex D. Don (Polygonaceae), commonly referred to as Phak-pai, is traditionally utilised for the treatment of fever and dyspepsia. The delicate shoots and leaf portions were utilised as cooked vegetables or chutney by the natives in the northeastern states of India (Das et al., 2025a). *Hogdsonia heteroclita* (Roxb.), belonging to the family Cucurbitaceae and

commonly known as Hagrani jwgwnar, is widely used on an empty stomach for the treatment of diabetes and various other health ailments (Basumatary et al., 2024).

Lagenaria siceraria (Molina) Standl., commonly known as the bottle gourd, is traditionally used as a cardiac tonic, aphrodisiac, general tonic, hepatoprotective agent, analgesic, anti-inflammatory, expectorant, and diuretic (Das et al., 2024). *Diplazium esculentum* (Retz.) Sw., a member of the Athyriaceae family, is predominantly utilised as a vegetable by the hill tribes of NER. The decoction of the plant is utilised to address haemoptysis and cough, and is traditionally reported for the treatment of dysentery, glandular swellings, indigestion, diarrhoea, and various skin infections (Junejo et al., 2018). *Phlogacanthus puninervius* T. Anderson, commonly referred to as Dein kajut, is a member of the Acanthaceae family. The leaves and flowers are utilised to manage diabetes, while an infusion of the leaves and a curry made from the aerial parts are consumed with rice (Kripasana and Xavier, 2020). *Glinus oppositifolius* (L.) Aug. DC, a member of the Molluginaceae family, is utilised by certain traditional healers for diabetes treatment through leaf decoction (Ragasa et al., 2015).

Andrographis paniculata (Burm.f.) Wall. ex Nees (Acanthaceae) is referred to as Kalmegh and Hnakhapui. Traditionally, the leaves and aerial components of the plant are utilised to address diabetes, hepatic disorders, and splenomegaly (Das et al., 2025b). *Momordica charantia* L. (Cucurbitaceae), commonly referred to as Changkha, Kerela Tita-kerala, and Karon Akhabi in NER, possesses a bitter flavour and is documented to lower elevated cholesterol levels and promote fat reduction (Ahmad et al., 2016). The familial distribution of the traditional utilisation of some ethnopharmacologically potential medicinal plants in NER is presented in Table 1.1 (Deb et al., 2023).

Table 1.1. Ethnopharmacological uses of some well-known medicinal plants of NER

Family	Scientific Name	Local Name	Traditional Use
Acanthaceae	*Andrographis paniculata* (Burm.f.) Wall.	Chirata (K), Kalmegh, Bhubati	Decoction of the entire plant, Juice and powder derived from the dried leaves of the whole plant, as well as juice from the flowers and stems.
Aizoaceae	*Glinus oppositifolia* L.	Bakhate	Boiled plant extract
Asteraceae	*Stevia rebaudiana* (Bertoni) Bertoni	Stevia	Extract of the whole plant
Athyraceae	*Diplazium esculentum* (Retz.) Sw.	Hiikahamang	Young fruit extract
Bignoniaceae	*Oroxylum indicum* L.	Tokharung, Shamba, Sonaka, Totola, Phagorip, Kakidziihe (Mao tribe)	Decoction of bark and seed, 15–20 ml of freshly peeled or dried stem bark decoction administered twice daily.
Caryophyllaceae	*Drymaria cordata* (Linn.) Willd. and Schult.	Tandan mana, Abhijalo	Whole aerial plants in cooked form with *Channa orientalis*
Cucurbitaceae	*Momordica charantia* L.	Karela, Kairu, Changkhate, Korola, Karot akhabi	25 ml of fruit extract administered twice daily for a duration of 12 to 14 weeks, or raw fruit juice

			(50 ml) administered orally once daily for a duration of 5 to 6 weeks. Extract of branches can also be used. Leaf and fruit are utilised as a vegetable
	Solena heterophylla Lour.	Bankundri	Roots decoction
Cyperaceae	*Cyperus rotundus* L.	Mutha, Lam-thang, Sembang Kaothum	Fresh juice of rhizome or decoction of tuber or boiled extract of whole plant and rhizome
Lauraceae	*Cinnamomum tamala* (Buch.-Ham.) T.Nees & Eberm.	Tespata, Sinkauli, Napsor, Mensing, Tejpat, Tezpata	Oral administration of 5 g/per day of powdered dried leaves for a duration of 5 to 6 weeks or a decoction of the stem bark thrice daily for a duration of 3 to 4 weeks. Infusion of leaves
Liliaceae	*Allium hookerii* Thwaites	Zawngtuipui, Napakpi	Decoction of the whole plant or the fresh whole plant as a vegetable
Mimosaceae	*Parkia timoriana* (A. DC.) Merr	Yongchak	Decoction of bark with *Centella asiatica* and *Ficus glomerata* fruits
Passifloraceae	*Passiflora edulis* Sims	Shitaphal, Bel (Angami tribe)	Fresh fruits and decoction of leaves

1.4.2. *Molecular mechanism of medicinal plants of NER against NIDDM and obesity*

The *P. posumbu* hydroalcoholic extract was reported to exhibit antidiabetic potential by inhibiting the in *vitro* α-amylase enzyme. When administered orally to STZ-induced diabetic mice, the extract showed improved glucose tolerance, lower fasting glucose, and HbA1c levels compared to the disease control group (Das et al., 2025a). The ethanolic extract of *H. heteroclita* fruit demonstrated a dose-dependent inhibition of the α-amylase enzyme, effectively reducing elevated glucose, lipid, and triglyceride levels in diabetic mice (Basumatary et al., 2024). The rhizome extract of *Paris polyphylla* Sm. containing diosgenin **(45)**, the compound inhibited α-amylase and α-glucosidase, reduced fasting blood glucose and HbA1c levels, and enhanced oral glucose tolerance in streptozotocin (STZ)-induced diabetic rats. It also normalised liver enzyme markers while reducing serum urea and creatinine, indicating hepatoprotective and renoprotective effects (Kshetrimayum et al., 2023). A separate study demonstrated that the hydroalcoholic extract of dried rhizomes of *P. polyphylla*, which contains Paris saponin VII **(46)**, Dioscin **(47)**, and Polyphyllin V **(48)**, significantly accelerated diabetic wound healing in STZ-induced diabetic rats compared to both control and standard povidone iodine ointment groups (Kshetrimayum et al., 2024).

The hydroalcoholic extract of dried aerial parts of *Ageratina adenophora* (Spreng.) R. King & H. Robinson, containing chlorogenic acid **(49)** and caffeic acid, showed significant inhibitory effects on α-amylase and α-glucosidase activities. It was also found to lower fasting glucose and HbA1c levels, improve serum biochemical markers, boost tissue antioxidant levels, and partially regenerate pancreatic islets in STZ and nicotinamide-induced diabetic rats (Chanu et al., 2023). Methanolic leaf extract of *Drymaria cordata* (Linn.) Willd. and Schult. demonstrated antidiabetic and antihyperlipidemic properties through α-amylase and α-glucosidase inhibition, enhanced glycaemic regulation, and reduced serum lipid and triglyceride concentrations in STZ and nicotinamide-induced type 2 diabetic rats (Patra et al., 2020). The ethyl acetate fraction of *Solena heterophylla* Lour. fruits, containing Cucurbitacin B **(50)**, demonstrated antidiabetic and hepatoprotective effects by lowering fasting blood glucose and

HbA1c levels, as well as normalising liver antioxidant enzyme levels (Jana et al., 2024). The hydroalcoholic extract of *Cyperus rotundus* L. rhizome demonstrated a reduction in weight gain and enhanced glucose and lipid metabolism in olanzapine induced obese rats. The bioactive compounds identified using network pharmacology were shown to regulate PTP1B and HSD11B1, thereby downregulating adipogenesis (Kanagali et al., 2022). The ethanolic extract of *Oroxylum indicum* (L.) Kurz bark, rich in oroxylin A **(51)**, chrysin **(52)** and baicalein **(53),** was reported to inhibit pancreatic lipase in a dose-dependent manner and downregulate adipogenesis in 3T3-L1 preadipocytes (Mangal et al., 2017). The mechanism of action of medicinal plants of NER has been listed in Table 1.2.

(45) **(46)** **(47)**

(48) **(49)** **(50)**

(51) **(52)** **(53)**

Table 1.2. The pharmacological action of medicinal plants of NER against T2DM and obesity.

Sl. No.	Plant name	Mechanism of action			Reference
		In vitro	*In silico*	*In vivo*	
1.	*Polygonum posumbu* Buch. Ham. Ex D. Don	Inhibition of the α-amylase enzyme ↓ postprandial hyperglycaemia	Modulation of PI3K-AKT, MAPK and TGF-β pathways	↓ fasting glucose (FBG) & glycated haemoglobin (HbA1c) ↓ inflammation normalised AST/ALP/creatinine ↑ increased glucose uptake	(Das et al., 2025a)
2.	*Hogdsonia heteroclita* (Roxb.)	Inhibition of the α-amylase enzyme ↓ postprandial hyperglycaemia	–	Improve glycemic control, lipid and triglyceride levels Maintain body weight	(Basumatary et al., 2024)
3.	*Paris polyphylla* Sm	–	–	Formulated ointments accelerated diabetic wound healing ↑ collagen deposition and fibroblast regeneration	(Kshetrimayum et al., 2024)

				↑ hair follicle regeneration and skin architecture restoration ↑ re-epithelialization and keratin layer formation ↑ angiogenesis	
4.		Inhibition of α-amylase and α-glucosidase enzymes ↓ postprandial hyperglycaemia	–	↓ FBG ↓ HbA1c Restored body weight and serum protein levels. Corrected dyslipidaemia ↓ TC, TG, LDL ↑ HDL hepatoprotective and renoprotective ↓ serum urea and creatinine ↑ β-cell regeneration	(Kshetrimayum et al., 2023)
5.	*Ageratina adenophora* (Spreng.) R. King & H.	Inhibition of α-amylase and α-glucosidase enzymes ↓ postprandial hyperglycaemi	–	↓ FBG and HbA1c levels ↑ glycaemic control Restored body weight	(Chanu et al., 2023)

	Robinson	a		Hepatoprotective and renoprotective Normalisation of SGPT, SGOT, ALP, total protein, urea, and creatinine	
6.	*Drymaria cordata* (Linn.) Willd. and Schult.	Inhibition of α-amylase and α-glucosidase enzymes ↓ postprandial hyperglycaemia	–	↓ FBG ↓ HbA1c ↓ TC, TG, LDL ↑ HDL ↓ serum urea and creatinine ↑ β-cell regeneration ↑SOD and GSH, ↓MDA	(Patra et al., 2020)
7.	*Solena heterophylla* Lour.	Inhibition of α-amylase and α-glucosidase enzymes ↓ postprandial hyperglycaemia	–	↓ FBG ↓ HbA1c ↓ TC, TG, LDL ↑ HDL ↑ β-cell regeneration ↑ SOD, CAT to improve redox balance	(Jana et al., 2024)
8.	*Osbeckia nepalensis* Hook. f.	↑ glucose uptake in CC1 Rodent Primary Hepatocytes	Taxifolin-3-O-glucoside and Quercetin-	Reduction of hyperglycaemia by ↓ FBG ↑ serum insulin	(Gurumayum et al., 2023)

		Inhibition of hepatic gluconeogenesis ↓ gluconeogenic enzymes, G6Pase & PEPCK.	3-O-rhamnoside in n-butanol fraction gave stable hydrogen bonds, and showed hydrophobic & Pi interactions Taxifolin-3-O-glucoside – G6Pase complex was found to be stable	↓ triglycerides ↓ LDL ↑ HDL ↓ SGOT, SGPT ↑ AMPK phosphorylation ↓ G6Pase ↓ PEPCK ↓ FOXO1 ↓ HNF4α ↓ PGC1α	
9.	*Cyperus rotundus* L.	–	PTP1B and HSD11B1 identified as hub targets in obesity regulation ↑ insulin signalling	↓ Body weight, BMI ↓ abdominal circumference ↓ food intake ↑ locomotor activity ↓ FBG ↓ TC, TG, LDL, VLDL	(Kanagali et al., 2022)

			(PTP1B modulation) ↓ adipogenesis (HSD11B1 modulation) ↓ appetite and improved energy balance	↑ HDL ↓ adipocyte size	
10.	*Oroxylum indicum* (L.) Kurz	Inhibition of pancreatic lipase enzyme Anti-adipogenesis in 3T3-L1 preadipocytes	–	–	(Mangal et al., 2017)

1.5. Conclusion

The metabolomics-integrated network pharmacology approach elucidates the interactions of phytoconstituents with diverse physiologically active targets, highlighting the multi-molecule, multi-target characteristics of plant extracts in delivering therapeutic effects. This distinctive approach finds bioactive metabolites and therapeutic pathways to elucidate the mechanism of action. The technique for screening bioactive phytometabolites expedites the identification of leads. This systems biology technique is essential for advancing India's traditional medicine system in the global market by facilitating the identification of novel phytochemical leads for complex diseases and ensuring the safety and

efficacy of treatments through diverse methodologies. The northeastern region of India is abundant in a diverse array of medicinal herbs that have been traditionally utilised for generations to treat and manage metabolic diseases. Despite their traditional use, the absence of scientific validation and safety research diminishes the popularity of these medicinally active plants as alternatives in contemporary healthcare. Consequently, the incorporation of metabolomics in the evidence-based validation of traditional medicine and formulations from India, especially in the northeastern region, will yield novel leads and accelerate the drug discovery process.

1.6. Publications and conference presentations

1.6.1. *Paper communicated*

- Mukherjee, P.K., Banerjee, S., Das Gupta, B., Kar, A., 2022. Chapter 1 - Evidence-based validation of herbal medicine: Translational approach, in: Mukherjee, Pulok K. (Ed.), Evidence-Based Validation of Herbal Medicine (Second Edition). Elsevier, pp. 1–41. https://doi.org/10.1016/B978-0-323-85542-6.00025-1
- Gayen, S., Das Gupta, B., Mukherjee, P.K., Haldar, P.K., Network Pharmacology in the Scientific Validation of Traditional Medicine for Management of Metabolic Disorders, in: Nissapatron, V., Bodade, R., Bala, A., Pandey, R., Gaurav, A. (Ed.), Network Pharmacology : Exploring New Horizons in Drug Discovery. Elsevier. ISBN: 9780443440892. [Communicated]

Chapter 2

Scope, objectives and plan of work

2.1 Scope and rationale of the work
2.2 Objectives of the study
2.3 Work Plan

2.1. Scope and rationale of the work

Medicinal plants have been employed for centuries for their therapeutic properties and nutritional purposes. Medicinal plants have been utilised in disease mitigation since antiquity and have continually evolved as a form of adjunctive medicine, attributable to their accessibility and cost-efficiency as alternative healthcare solutions (Chaachouay and Zidane, 2024). Indian healthcare has utilised medicinal plants and polyherbal formulations since approximately 5000 BC, as documented in the "Charak Samhita" and "Sushruta Samhita". There is an increasing interest in utilising crude extracts and dry powder samples from medicinal and aromatic plants to develop alternative therapeutics and food additives. Herbal medicines garner the attention of both patients and researchers in all facets of drug development, encompassing natural products and the validation of traditional medicine (Mukherjee et al., 2022).

The pursuit of alternative medicine has prompted investigations into biodiversity hotspots in India. NER contains a substantial share of the nation's biodiversity, owing to its distinct ecological conditions, and functions as the geographical entry point for the majority of India's native flora and fauna. NER contains approximately 50% of India's biodiversity and possesses a unique geography and climate that support the growth of medicinally important flora and fauna (Devi et al., 2022). The residents of the Indo-Burma region in Northeast India have practised traditional healing methods, passing down knowledge through generations by more than 200 tribal groups, each possessing distinct cultural traditions (Das et al., 2025). These conventional practices underpin innovative pharmacological discoveries and bioprospecting.

The extensive ethnopharmacological potential of medicinal and food plants in NER serves as a significant resource for the development of drugs influenced by Ayurveda, local healing practices, and indigenous knowledge systems (Das et al., 2024). This extensive repository of medicinal plants remains unexamined in modern phytochemistry and pharmacology, which will enhance the search for novel bioactive compounds with therapeutic relevance. Preserving ethnomedicinal knowledge protects cultural heritage and biodiversity. The

sustainable utilisation of resources can provide economic opportunities for local communities (Devi et al., 2022).

Among the numerous extensive methods in drug discovery, metabolomics offers a distinct advantage in the discovery and scientific validation of traditional medicine. The metabolomics of medicinal plants provides an in-depth analysis of secondary metabolites, which are essential for drug discovery and development (Mukherjee et al., 2016). Untargeted and pseudotargeted metabolomics serve as an efficient approach for identifying metabolites in plant extracts, encompassing a diverse array of molecules. Analytical techniques, including LC-MS, are utilised in conjunction with synergy estimation methods and contemporary multivariate statistical approaches for metabolite data analysis (Mukherjee et al., 2022). Metabolomics integrated network pharmacology research emphasises advanced systems biology methodologies to elucidate the prediction and validation of mechanisms of action. Network Pharmacology is an innovative approach to elucidate the systems pharmacology of drug combinations and their synergistic effects (Hopkins, 2008).

The importance and preventative function of traditional medicinal plants in the management of lifestyle-related metabolic diseases cannot be overstated. The medicinal flora of NER has been traditionally employed in culinary practices and used to regulate metabolic disorders, particularly in the context of diabetes, obesity, and hypertension. However, the absence of evidence regarding the mechanisms of action and phytochemical constituents of traditional medicinal food plants from NER underscores the necessity for scientific validation of traditional claims and the pursuit of novel leads in drug discovery initiatives.

2.2. Objectives of the study

The concept of "food as medicine" gives a substantial advantage in the development of herbal drugs with negligible adverse effects (Mukherjee et al., 2015). This type of food, enriched with medicinal value, is a mainstay in the northeast Indian diet, contributing to the management of various metabolic disorders. The development of evidence-based and value-added alternative

therapeutics from food plants in the northeastern region of India may expand the regional bioeconomy (Das et al., 2024).

The present study was done to evaluate three (03) traditional food plants of NER of India, selected based on their traditional use, viz. *Allium hookeri* Thwaites, *Benincasa hispida* (Thunb.) Cogn. and *Houttuynia cordata* Thunb. against NIDDM and obesity with their metabolite profiling and network pharmacology analysis to elucidate the mechanism of action.

The following objectives were taken into consideration:

- Selection of medicinal plants of NER on the basis of traditional uses.
- Development of hydroalcoholic extracts utilising a green extraction technique.
- Chemoprofiling using UHPLC-QTOF-MS for the identification and characterisation of bioactive metabolites that contribute to therapeutic potential.
- Investigation of molecular mechanisms and therapeutic targets through network pharmacology analysis.
- Enzyme inhibition potential of enriched extracts *in vitro*, focusing on α-amylase, α-glucosidase, and pancreatic lipase for the management of metabolic disorders.
- Enzyme inhibition kinetics assay to determine the mode of inhibition.
- Evaluation of anti-hyperglycaemic and anti-hyperlipidaemic potential of the most promising bioactive extract using *in vivo* animal models.

2.3. Work Plan

The current study involved the preparation of enriched extract of *A. hookeri*, *B. hispida* and *H. cordata* through microwave-assisted extraction (MAE), followed by extensive pseudotargeted metabolomic dereplication, enzyme inhibition with kinetic validation. The work plan has been illustrated as a schematic diagram (Figure 2.1).

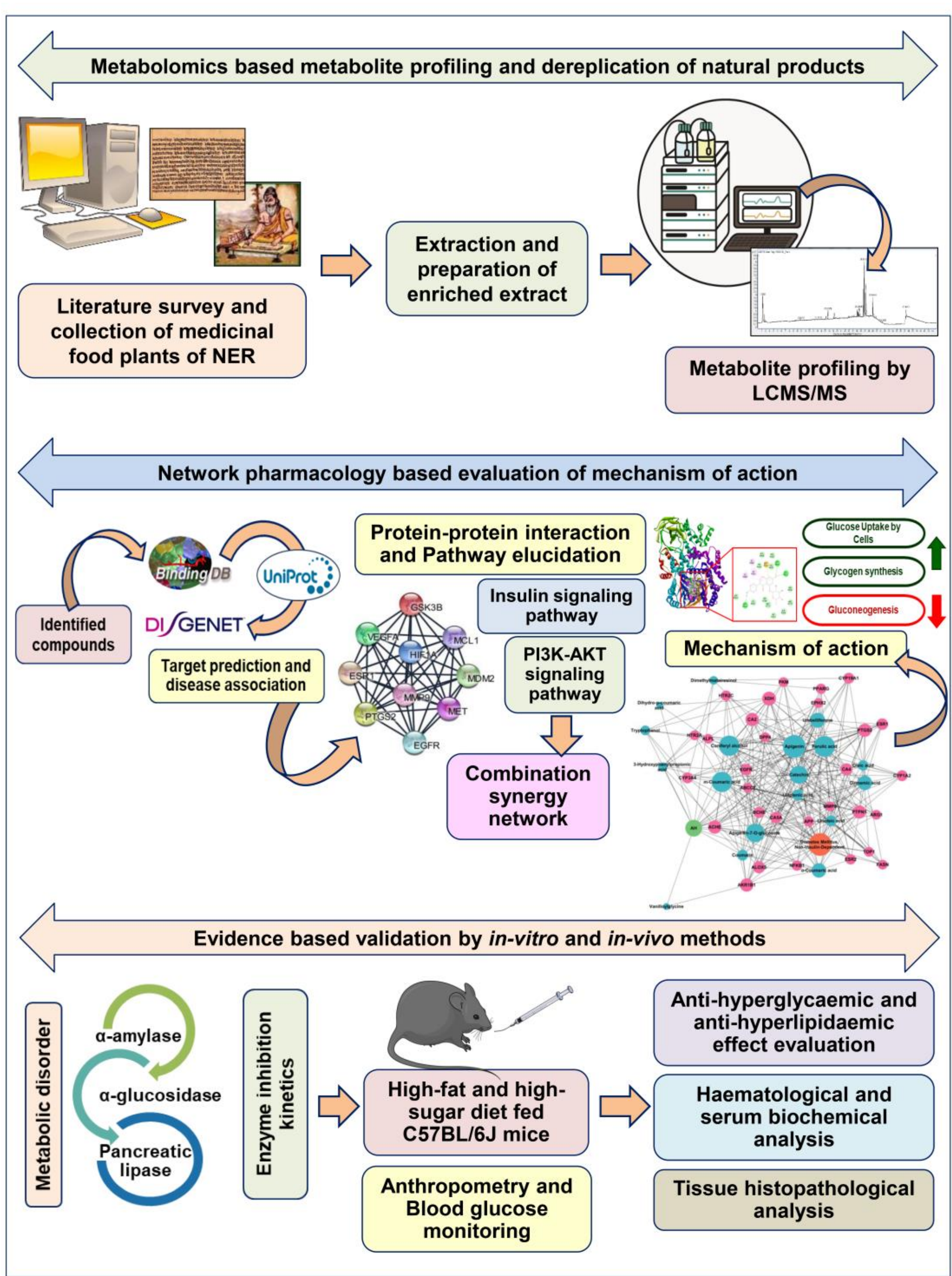


Figure 2.1. Schematic representation of the work plan

A thorough literature search was conducted using the search engines like Scopus, ScienceDirect, Google Scholar and PubMed to search literatures from 2001-2025. The literature search was conducted using keywords such as northeastern medicinal plants, ethnopharmacology, traditional medicine, metabolic disorders, diabetes, obesity, phytochemistry, pharmacology etc. The exclusion criteria consisted of articles available solely as abstracts, bibliographies, and images, as well as the texts in languages other than English. The inclusion criteria consisted of full-text research articles, reviews, mini-reviews, short communications, book chapters, government datasheets, and books written in the English language were included. Finally, 167 articles including reviews and original research papers were included in the thesis. Integrative metabolomics and network pharmacology analyses were employed to elucidate the probable mechanism of action for the phytomolecules. The network pharmacology analysis was followed by in *vivo* testing in models of diet-induced hyperglycaemia and hyperlipidaemia to demonstrate preventive efficacy. The current study aims to validate the traditional claim, bridge the gap by linking the phytochemical composition, and establish mechanistic insights to promote the advancement of safe and effective alternative therapeutics derived from *A. hookeri*, *B. hispida*, and *H. cordata* for the management of metabolic dysregulations induced by non-insulin-dependent diabetes mellitus (NIDDM) and obesity.

Chapter 3

Allium hookeri Thwaites – Metabolomics integrated network pharmacology and *in vitro* evaluation

3.1. *Allium hookeri* Thwaites – A profile
3.2. Materials and methods
3.3. Results
3.4. Discussion
3.5. Publications and conference presentations

3.1. *Allium hookeri* Thwaites – A profile

Scientific classification

Kingdom: Plantae

Division: Pteridobiotina

Class: Angiosperms

Order: Asparagales

Family: Liliaceae/Amaryllidaceae

Genus: *Allium*

Species: *Allium hookeri* var. hookeri

Vernacular names

English: East-Himalayan Chives

Manipuri: Maroi napakpi

Mizo: Runphek

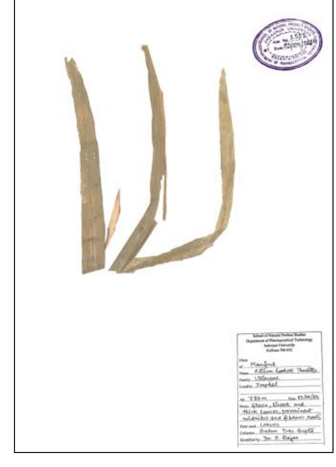
Nagamese: Zhiva (Sangtam)

Tangkhul: Namrei

3.1.1. *Plant description*

Allium hookeri Thwaites, a member of the Liliaceae family, is distributed over India, southwestern China, Korea, Bhutan, Myanmar, and Sri Lanka. *A. hookeri* is found in the northeastern parts of India and is utilised in culinary applications. The plant blooms from August to September, producing white flowers. The rhizomes are diminutive and possess fibrous roots; the leaves are green, linear, and thick, featuring prominent midribs (Deka et al., 2022). *A. hookeri* is often found in the wild but is now cultivated domestically and in kitchen gardens because of its therapeutic significance (Ayam, 2011). The aerial parts of *A. hookeri* are shown in Figure 3.1.

A



B

Figure 3.1. A: Aerial parts of *Allium hookeri* Thwaites; B: Herbarium

3.1.2. *Traditional uses of A. hookeri*

A. hookeri has been utilised traditionally for various ailments. The roots have been utilised to remedy coughs and treat burn injuries. The leaf juice and decoction have been utilised for stomach ulcers, fever, and to lower blood pressure (Deka et al., 2022). Traditional applications for antidiabetic effects include the consumption of a decoction made from the entire plant or the use of the fresh whole plant as a vegetable (Deb et al., 2023). The local tribes of the northeast have used the leaf powder *A. hookeri* to manage diabetes (Singh et al., 2024; Deka et al., 2021).

3.1.3. *Phytochemical profile of A. hookeri*

A. hookeri was identified as abundant in bioactive phytomolecules, predominantly organosulfur compounds. Rhyu and Park documented that Dimethyl thiosulfinate **(54)**, Allyl methyl thiosulfinate **(55)**, 1-Propenyl methyl thiosulfinate **(56)**, Methyl propyl thiosulfinate **(57)**, Allyl propyl thiosulfinate **(58)**, 1-Propenyl allyl thiosulfinate **(59)**, and 1-Propenyl propyl thiosulfinate **(60)** are the alkyl thiosulfinates identified in the hydroalcoholic extract of *A. hookeri* root. The phytomarker Allicin **(61)** was found to be more abundant in the roots than in the stem (Rhyu and Park, 2013). Non-volatile organosulfur compounds were identified in both the stem and roots of *A. hookeri*. The HPLC-PDA analysis of stem and root identified 11 non-volatile compounds, including γ-L-glutamyl-S-allyl-L-cysteine **(62)**, γ-L-glutamyl-S-(trans-1-propenyl)-L-cysteine **(63)**, γ-L-glutamyl-S-methyl-L-cysteine **(64)**, γ-glutamyl-phenylalanine **(65)**, (+)-S-(trans-1-propenyl)-L-cysteine **(66)**, (+)-S-methyl-L-cysteine **(67)**, Alliin **(68)**, Isoalliin **(69)**, Methiin **(70)**, Cycloalliin **(19)** and Allicin. The study documented that Alliin and Allicin were detected only in the roots (Kim et al., 2016, 2015). Furthermore, numerous reports have been issued identifying volatile compounds in the leaves and roots of *A. hookeri*. The volatile compounds were identified as belonging to the class of aldehydes, ketones, sulphides, and vinyldithiins (Deka et al., 2022).

(54) (55) (56)

(57) (58) (59)

(60) (61) (62)

(63) (64) (65)

(66) (67) (68)

(69) (70)

3.1.4. *Pharmacological activities of A. hookeri*

The methanolic extract of *A. hookeri* root was examined for its anti-inflammatory properties in *in vitro* LPS-induced RAW264.7 macrophage cells. The extract effectively inhibited the production of nitric oxide and reactive oxygen species, and partially suppressed the activation of nuclear factor-kappa B (Jang et al., 2017). *In vitro* studies of 3T3-L1 adipocytes demonstrated that the *A. hookeri* root extract significantly diminished fat accumulation and inhibited adipogenesis. The root extract effectively decreased liver weight, hepatic steatosis, and triglyceride concentrations (Kim et al., 2019). The impact of *A. hookeri* on cognitive health was examined in scopolamine-induced mice with cognitive impairment. The ethanolic extract from the root of *A. hookeri* elevated acetylcholine levels and enhanced choline acetyltransferase expression in the hippocampus, concurrently reducing acetylcholinesterase activity. Behavioural assessments indicated that the extract was efficacious for strengthening spatial learning and memory (Choi et al., 2021).

The antiulcer efficacy of *A. hookeri* leaf methanolic extract was examined *in vivo* in rats with indomethacin-induced gastric ulcers. The extract was reported to exhibit a dose-dependent gastroprotective effect (Singh et al., 2018). The fermented extract of *A. hookeri* roots has demonstrated *in vitro* inhibition of angiotensin-converting enzyme, resulting in reduced systolic and diastolic blood pressure, a phenomenon also observed *in vivo* in hypertensive rats. The extract effectively modulated oxidative stress *in vivo* and demonstrated an antihypertensive effect (Tong et al., 2021).

3.2. Materials and methods

3.2.1. *Chemicals and reagents*

The α-glucosidase enzyme (*Saccharomyces cerevisiae*) type I (≥10 units/mg protein), 4-nitrophenyl-α-D-glucopyranoside (p-NPG, purity ≥99%), α-amylase (from *Aspergillus oryzae*), powder-30U/mg, starch from potato (Soluble), 3,5-dinitrosalicylic acid (98%), lipase (from porcine pancreas), type II (≥125 units/mg protein), Orlistat (≥98%, solid), 4-methylumbelliferyl oleate (≥95%, HPCE), potassium sodium tartrate tetrahydrate ACS reagent (99%), sodium dihydrogen phosphate, disodium hydrogen phosphate were procured from Sigma-Aldrich, USA. Acarbose extrapure, 95%, was procured from Sisco Research Laboratories Pvt. Ltd, India. Acetonitrile, water, and formic acid (LC–MS grade) were obtained from Sisco Research Laboratories (Maharashtra, India). Other chemicals and reagents were obtained from e-Merck (Mumbai, India).

3.2.2. *Instrumentation*

The plant samples were weighed using a weighing balance purchased from Sarto vision scales & systems (West Bengal, India) bearing Model No. CST-1K. Extraction and drying of the dried sample were performed using a microwave extraction unit (Catalyst microwave synthesizer-CATA R), a rotary vacuum evaporator (Hahnshin, Republic of Korea; HS–2005 V–N), and a lyophiliser (Indian Instrumentation, Kolkata, West Bengal), respectively. Kinetics assays of the enzyme were performed using a microplate reader (SpectraMax ID3, Molecular Devices LLC, USA), and metabolite profiling was conducted using UHPLC-QTOF-MS (Agilent, Santa Clara, CA, United States).

3.2.3. *Plant collection and extraction*

The leaves of *A. hookeri*, fruits of *B. hispida* and leaves of *H. cordata* were purchased from the local market in Imphal, Manipur, in the month of April 2024 and 2022, and the voucher specimen (SNPS-JU/2024/1521; SNPS-JU/2022/1493 and SNPS-JU/2024/1522) is maintained at the School of Natural Product Studies, Jadavpur University. The leaves and fruits were cleaned and dried at 50°C in a leaf dryer for 48 hours, then converted into a coarse powder.

The dried leaves and fruits (500g) were extracted using a microwave-assisted extraction technique (Das Gupta et al., 2025b). The microwave-assisted extraction was performed at 60°C with a microwave power of 595 watts. The extraction time was set to 15 minutes, and a hydroalcoholic solvent consisting of 80% ethanol (v/v) was used. The material-to-solvent ratio was fixed at 1:10 g/mL. The menstruum was filtered and dried in a rotary vacuum evaporator at 60°C, followed by lyophilisation at -45°C, 0.120 m. barr pressure. The extracts were named the hydroalcoholic extracts of *A. hookeri* (HEAH), *B. hispida* (HEBH), and *H. cordata* (HEHC) and stored until further use.

3.2.4. *Metabolite profiling by UHPLC-QTOF-MS analysis*

The ultra-high performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UHPLC-QTOF-MS) analysis of HEAH and HEHC was conducted using the previously established method (Das Gupta et al., 2025a). The Agilent LC-QTOF 6500 system, equipped with an Agilent Zorbax Eclipse Plus C18 column (2.1 mm × 50 mm) and a particle size of 1.8 µm, was utilised for UHPLC-QTOF-MS analysis. A gradient elution method was employed with a flow rate of 0.3 mL/min. Water (A) and acetonitrile (C), each containing 0.1% formic acid, were utilised as solvent systems with the following gradient profile: 65% A at 2 minutes, 40% A at 10 minutes, 15% A at 35 minutes, 8% A at 45 minutes, and concluding with 65% A at 57 minutes. The injection volume was 5 µL, and the column temperature was sustained at 40°C. The elutions were analysed in positive ion mode, with a gas temperature of 320°C, a gas flow rate of 9 L/min, and a mass-to-charge ratio (m/z) range set between 50–3200 Da. The mass spectrometry data were analysed using Agilent Mass Hunter B.08.00 software (Qualitative navigator, Qualitative workflows) for target screening, utilising a custom library developed through a comprehensive literature review. The compounds were identified using the "Find by formula" algorithm, based on molecular formula, mass, score (≥ 75), and mass error (≤ 5 ppm).

3.2.5. *Network pharmacology analysis*

3.2.5.1. *Identification of Targets for Bioactive Phytoconstituents*

The targets for each phytocompound identified from HEAH, HEBH and HEHC, along with Acarbose, Orlistat, Metformin and Atorvastatin, were identified using Binding DB (https://www.bindingdb.org/) and DrugBank (https://go.drugbank.com/) with a similarity threshold of ≥0.7. The UniProt database was used to search the corresponding human gene targets (https://www.uniprot.org/). The target genes were filtered for status "Reviewed (Swiss-Prot)" and the popular organism "Human." The gene names in bold were taken for further analysis (Das Gupta et al., 2025b).

3.2.5.2. *Construction of Gene Diseases Association Network*

The obtained gene targets were screened against existing disease-based targets in DisGeNET and presented in a Venn diagram (Oliveros, 2007). The target corresponding genes were matched with the associated diseases to obtain a summary of gene–disease associations using DisGeNET (http://www.DisGeNET.org). The results were filtered to understand the potential link between the targets and the following conditions: "Non-insulin-Dependent Diabetes Mellitus", "Hyperglycaemia", "Obesity", "Hyperlipidaemia", and "Hypertriglyceridaemia". The gene−disease association score was set to 0.1. The gene-disease association results were downloaded in Excel format, containing the Gene, Disease, and disease ID (Banerjee et al., 2023).

3.2.5.3. *Protein–Protein Interaction, Target–Disease Network, and Pathway Analysis*

Protein–protein interaction (PPI) was analysed using STRING Version 12.0 (Szklarczyk et al., 2017). The gene list was uploaded in the "Multiple Proteins by Names/Identifiers" search, and the organism choice was set to *Homo sapiens*. The string network was created with a minimum required interaction score set to 0.4. The high-resolution string network was downloaded, and the top 10 interactions, determined by degree centrality and shortest path length, were visualised using Cytoscape. The gene targets were mapped to their respective

diseases, and a target–disease network was created. The pathway analysis was conducted using ShinyGO 0.82 (https://bioinformatics.sdstate.edu/go/). The gene list was uploaded, and GO Enrichment Analysis was performed to identify the pathways involved (Ge et al., 2020). The pathways were used to elucidate the mechanism of action of the protein targets. The pathways enriched with the genes were visualised using a Kyoto Encyclopedia of Genes and Genomes (KEGG) diagram (Kanehisa et al., 2021; Luo and Brouwer, 2013).

3.2.5.4. *Network analysis-based combination synergy*

The combined synergy of the phytocompounds of HEAH, HEBH and HEHC linked with Non-Insulin-Dependent Diabetes Mellitus (NIDDM), Hyperglycaemia, Obesity, Hyperlipidaemia, and Hypertriglyceridaemia was analysed based on neighborhood approach to obtain a bioactive–target–disease network in Cytoscape (http://Cytoscape.org/, Version 3.10.0) (Das Gupta et al., 2025b; Banerjee et al., 2023). Separate files of plant–phytomolecule (BOT-BA), phytomolecule–gene (BA-TAR), and gene–disease (TAR-DIS) were prepared in Excel format and uploaded separately in Cytoscape. The source node, target node, and target node attribute were set accordingly, and each network was created. The network was filtered for "self-loop" and "duplicate edge" and finally merged in union to create the bioactive–target–disease network.

3.2.6. *Total flavonoid and phenolic content determination*

The total flavonoid content (TFC) and total phenolic content (TPC) in test samples HEAH, HEBH, and HEHC were determined according to a previously reported high-throughput assay method with slight modifications (Das Gupta et al., 2025b).

In TFC, 90μL distilled water was added to a 96-well plate with $NaNO_2$ (10μL) and 25μL of standard (Rutin) and test sample solution in different rows. A solution of $AlCl_3$ (15 μL) was added after 15 minutes, followed by 50 μL of NaOH solution. Absorbance was measured at 510nm. In TPC, 70 μL of distilled water was added to a 96-well plate containing 30 μL of sample or standard (Gallic acid) and 20 μL of Folin–Ciocalteu (FC) reagent. After 6 minutes, 100 μL of Na_2CO_3 was added to

each well. The plate was left in the dark for 90 minutes, and absorbance was measured at 765nm after shaking the plate for 20 seconds orbitally in the plate reader. All analyses were performed in triplicate. The standard and test samples were prepared in concentrations ranging from 0.025 to 0.5mg/mL. The TPC and TFC were denoted as GAE mg/g and RE mg/g, respectively.

3.2.7. *Antioxidant potential of A. hookeri, B. hispida and H. cordata*

The DPPH radical scavenging capacity was assessed utilising the 2,2-diphenyl-1-picrylhydrazyl free radical scavenging assay (DPPH–FRSA) method, performed in triplicate according to the previously documented method (Das Gupta et al., 2025b). DPPH solution (0.1 mM) was introduced to each well containing the test sample or ascorbic acid standard (15.625-500 µg/mL). The plate was maintained at room temperature for one hour under dark conditions, and absorbance was subsequently measured using a spectrophotometer. The IC_{50} value was calculated through nonlinear regression utilising GraphPad Prism version 8.0.2 (Boston, Massachusetts, United States). A concentration-response curve was constructed by plotting the percentage of relative activity versus concentration.

The nitric oxide antioxidant potential (NO) of the hydroalcoholic extract was assessed by ascertaining the scavenging ability against nitric oxide radicals (Singha et al., 2024). Different concentrations (20–400 µg/mL) of test sample or ascorbic acid were combined with sodium nitroprusside in a 96-well plate and incubated for one hour. The Griess reagent was added to the mixture, and it was incubated for five minutes. Afterwards, the absorbance was measured at 532 nm.

The hydroxyl radical scavenging activity (OH) was determined using the 2-deoxy-D-ribose oxidation method with some modifications (Kazeem and Ashafa, 2015). The experiment (n = 3) involved the addition of $FeCl_3$, H_2O_2, 2-deoxy-D-ribose, and ascorbic acid (standard) or test sample (20–400 µg/mL) to a 96-well plate, followed by incubation at 37°C for 1 hour. The incubated mixtures were mixed with thiobarbituric acid (TBA) and tricarboxylic acid (TCA), followed by heating at 100°C for 30 minutes. Absorbance was measured at 532 nm using a microplate

reader after the sample reached room temperature. All the experiments were performed in triplicate (n = 3).

The percentage of antioxidant potential was calculated according to the following equation:

$$\% \text{ antioxidant potential} = [[(A_1\text{-}A_0)/A_1] \times 100$$

Where A_1 represents the absorbance of the control (without sample), while A_0 denotes the absorbance of the sample reaction mixture.

3.2.8. *In vitro assay for enzyme inhibition*

An *in vitro* α-amylase and α-glucosidase inhibition assay of test samples HEAH, HEBH and HEHC was performed in a 96-well plate according to the previous method (Das Gupta et al., 2025b). For α-amylase enzyme inhibition, the substrate (potato starch) and test sample or acarbose were prepared in a 20 mM phosphate buffer at pH 6.9. The reaction mixture consisted of 40 µL of α-amylase (1 U/mL) and 40 µL of sample solutions (0.8–0.1mg/mL). The 96-well plate was preincubated for 10min (37°C) before adding 40µL of 1% w/v potato starch solution and incubated for 30min (37°C). The reaction mixture was terminated by 80µL DNS colour reagent and heated for 10min (100°C). The absorbance was measured at 540nm, and percentage relative activity and IC_{50} were calculated.

In case of α-glucosidase enzyme inhibition assay, 20µL of 0.5U/mL α-glucosidase enzyme and 20µL of test sample were taken in dilutions 0.8–0.1mg/mL and incubated for 10 min (37°C), followed by the addition of 5.0 mM, 20 µL 4-nitrophenyl-β-D-glucopyranoside (p-NPG) substrate and incubated for 30min (37°C). The reaction was terminated by the addition of 50 µL of Na_2CO_3 (0.1 M), and the absorbance of the released p-nitrophenol was measured at 405 nm. Acarbose was used as the reference standard drug in both studies.

The *in vitro* pancreatic lipase inhibition assay was conducted according to a previously validated method (Singha et al., 2024). Porcine pancreatic lipase was dissolved in 50 mM phosphate buffer at pH 7.0. The substrate used was 4-methylumbelliferyl oleate (4-MUO), with Orlistat serving as the reference standard (positive control). Test samples and the standard were dissolved in a

Tris-HCl buffer at pH 8.0. The reaction mixtures were incubated further, and the reaction was terminated by the addition of sodium citrate (0.1 M). Absorbances were measured at 460 nm. Inhibitory activities were quantified as a percentage of inhibition. All the experiments were performed in triplicate (n = 3).

The % inhibitory activity was calculated as follows:

$$\text{Inhibitory activity (\%)} = (1 - A_s/A_c) \times 100$$

Where A_s represents the absorbance in the presence of the test substance, and A_c denotes the absorbance of the control.

3.2.9. *In vitro enzyme kinetic assay*

The inhibitory mechanism, whether reversible or irreversible, was assessed at varying sample concentrations by plotting reaction velocity (ΔOD/min) against enzyme concentrations [E], employing previously established methods with minor modifications (Das Gupta et al., 2025b; Singha et al., 2024). Substrate concentrations were taken in the range of 1.5–6.0mM, and the inhibitor (HEAH or HEBH or HEHC) concentration varied from 0.25–1mg/mL. The inhibitory effect was calculated by taking absorbance at every one-minute interval (0–30 min). The reaction rates were assessed to identify the type of inhibition: competitive, noncompetitive, uncompetitive, or mixed. The Michaelis constant (K_m) and maximum reaction velocity (V_{max}) were obtained from the Lineweaver–Burk double reciprocal plot using Sigma Plot 14.0 (CA, USA).

3.2.10. *Statistical analysis*

The results were visualised graphically and statistically analysed using GraphPad Prism 8.0.2 software (GraphPad Software, Inc., Boston, MA, USA). All results (n=3) were statistically analysed and presented as mean value ± standard deviation (SD). The Pearson correlation test was utilised to investigate the relationship between antioxidant levels, total phenolic content, total flavonoid content, and enzyme inhibitory activity.

3.3. Results

3.3.1. *Metabolite profile of A. hookeri*

The percentage yield of HEAH was found to be 19.34% w/w. The UHPLC–QTOF–MS revealed a variety of compounds, which are detailed in Table 3.1. The positive ion chromatogram is depicted in Figure 3.2., displaying the total ion chromatogram. The analysis identified 27 distinct compounds; the details of these compounds, including their chemical formula, theoretical mass (g/mol), observed mass-to-charge ratio (m/z), retention time (RT), mass error (Dif (Tgt, ppm)), and compound class, are presented in Table 3.1. The results confirmed the existence of several compounds known for their therapeutic properties in addressing metabolic disorders, such as diabetes and obesity.

Table 3.1. Compounds identified in the hydroalcoholic extract of *A. hookeri* leaves by UHPLC-QTOF-MS.

Sl. No.	RT	Name	Formula	Mass (g/mol)	m/z	Error (ppm)	Class of compounds
1.	2.127	Arginine	$C_6H_{14}N_4O_2$	174.1122	175.1195	3.22	Carboxylic acids and derivatives
2.	2.509	Indoleacrylic acid	$C_{11}H_9NO_2$	187.0639	188.0711	3.13	Indoles and derivatives
3.	3.150	Ferulic acid	$C_{10}H_{10}O_4$	194.18	195.0658	2.94	Cinnamic acids and derivatives
4.	4.158	Tryptophanol	$C_{11}H_{14}N_2O$	190.24	191.1155	-12.72	Indoles and derivatives
5.	4.188	Vanilloylglycine	$C_{10}H_{11}NO_5$	225.0636	226.0715	-0.41	Benzene and substituted derivatives
6.	4.989	Benzoyl meso-tartaric acid	$C_{11}H_{10}O_7$	254.19	255.0427	-28.41	Benzene and substituted derivatives

7.	5.700	Coumarin	$C_9H_6O_2$	146.0367	147.044	-0.39	Coumarins and derivatives
8.	6.417	Umbelliferone	$C_9H_6O_3$	162.0318	163.0393	0.67	Coumarins and derivatives
9.	7.903	3'-Hydroxy-3,4,5,4'-tetramethoxystilbene	$C_{17}H_{18}O_5$	302.1152	303.1208	-0.88	Stilbenes
10.	8.147	Catechin	$C_{15}H_{14}O_6$	290.27	291.0772	-30	Flavonoids
11.	10.835	Apigenin 7-O-glucoside	$C_{21}H_{24}O_9$	420.1406	421.1488	-3.35	Flavonoids
12.	12.497	Apigenin	$C_{15}H_{10}O_5$	270.0521	271.0587	-2.74	Flavonoids
13.	12.767	Vanillin 4-sulfate	$C_8H_8O_6S$	232.005	233.0129	3.82	Organic sulfuric acids and derivatives
14.	14.265	Allicin	$C_6H_{10}OS_2$	162.27	163.0246	-0.16	Thiosulfinic acid esters
15.	21.423	Dimethylmatairesinol	$C_{22}H_{26}O_6$	386.1729	387.1801	-0.03	Furanoid lignans
16.	21.423	Gomisin M2	$C_{22}H_{26}O_6$	386.1729	387.1801	-0.03	Tannins
17.	24.018	3-Hydroxyphenylpropionic acid	$C_9H_{10}O_3$	166.0636	167.071	3.52	Phenylpropanoic acids
18.	24.018	Dihydro-p-coumaric	$C_9H_{10}O_3$	166.0636	167.071	3.52	Phenylpropanoic acids

		acid					
19.	24.018	Methoxyphenylacetic acid	$C_9H_{10}O_3$	166.0636	167.071	3.52	Benzene and substituted derivatives
20.	29.732	m-Coumaric acid	$C_9H_8O_3$	164.0481	165.0554	4.78	Cinnamic acids and derivatives
21.	29.732	o-Coumaric acid	$C_9H_8O_3$	164.0481	165.0554	4.78	Cinnamic acids and derivatives
22.	30.018	Linoleic acid	$C_{18}H_{32}O_2$	280.45	281.2463	-4.76	Fatty Acyls
23.	31.457	Cinnamic acid	$C_9H_8O_2$	148.0524	149.0596	-0.41	Cinnamic acids and derivatives
24.	34.098	Carnosol	$C_{20}H_{26}O_4$	330.1821	331.1888	-2.97	Prenol lipids
25.	34.627	Coniferyl alcohol	$C_{10}H_{12}O_3$	180.0783	181.0854	-2.16	Phenols
26.	38.073	Oleic acid	$C_{18}H_{34}O_2$	282.2567	283.2643	-4.76	Fatty Acyls
27.	38.810	Linolenic acid	$C_{18}H_{30}O_2$	278.43	279.2365	0.64	Lineolic acids and derivatives

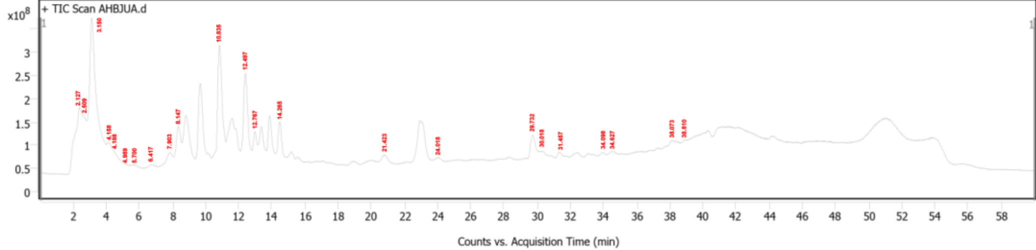


Figure 3.2. Total ion chromatogram of HEAH in the positive ionisation mode

The class of compounds identified includes Benzene and substituted derivatives – vanilloylglycine **(117)**, benzoyl meso-tartaric acid **(118)** and methoxyphenylacetic acid **(119)**; carboxylic acids and derivatives – arginine **(120)**; cinnamic acids and derivatives – ferulic acid **(32)**, m-coumaric acid **(121)**, o-coumaric acid **(122)**, and cinnamic acid **(123)**; coumarins and derivatives – coumarin **(16)** and umbelliferone **(17)**; fatty acyls – linoleic acid **(25)** and oleic acid **(124)**; flavonoids – catechin **(23)**, apigenin 7-o-glucoside **(125)** and apigenin **(10)**; furanoid lignans – dimethylmatairesinol **(126)**; indoles and derivatives – indoleacrylic acid **(127)** and tryptophanol **(128)**; lineolic acids and derivatives – linolenic acid **(24)**; organic sulfuric acids and derivatives – vanillin 4-sulfate **(129)**; phenols – coniferyl alcohol **(31)**; phenylpropanoic acids – 3-hydroxyphenylpropionic acid **(130)** and dihydro-p-coumaric acid **(131)**; prenol lipids – carnosol **(132)**; stilbenes – 3'-hydroxy-3,4,5,4'-tetramethoxystilbene **(133)**; tannins – gomisin M2 **(134)**; and thiosulfinic acid esters – allicin **(61)**.

(117) **(118)** **(119)**

(120) **(121)** **(122)**

(123) (124) (125)

(126) (127) (128)

(129) (130) (131)

(132) (133) (134)

3.3.2. *Network analysis*

3.3.2.1. *Identified targets and disease association network*

The compounds (BA) were screened in BindingDB, and the associated genes (TAR) were identified from the UniProt database. The 27 compounds showing targets were used to create a bioactive-target (BA–TAR) network, as shown in Figure 3.3. The network topology statistics revealed 867 nodes interconnected through 11,485 edges, indicating a dense connectivity between the phytocompounds and their targets. The phytocompound apigenin exhibited the highest connectivity, targeting 302 proteins. Aldose reductase (AKR1B1) was found to be the most targeted protein, targeted by 14 compounds.

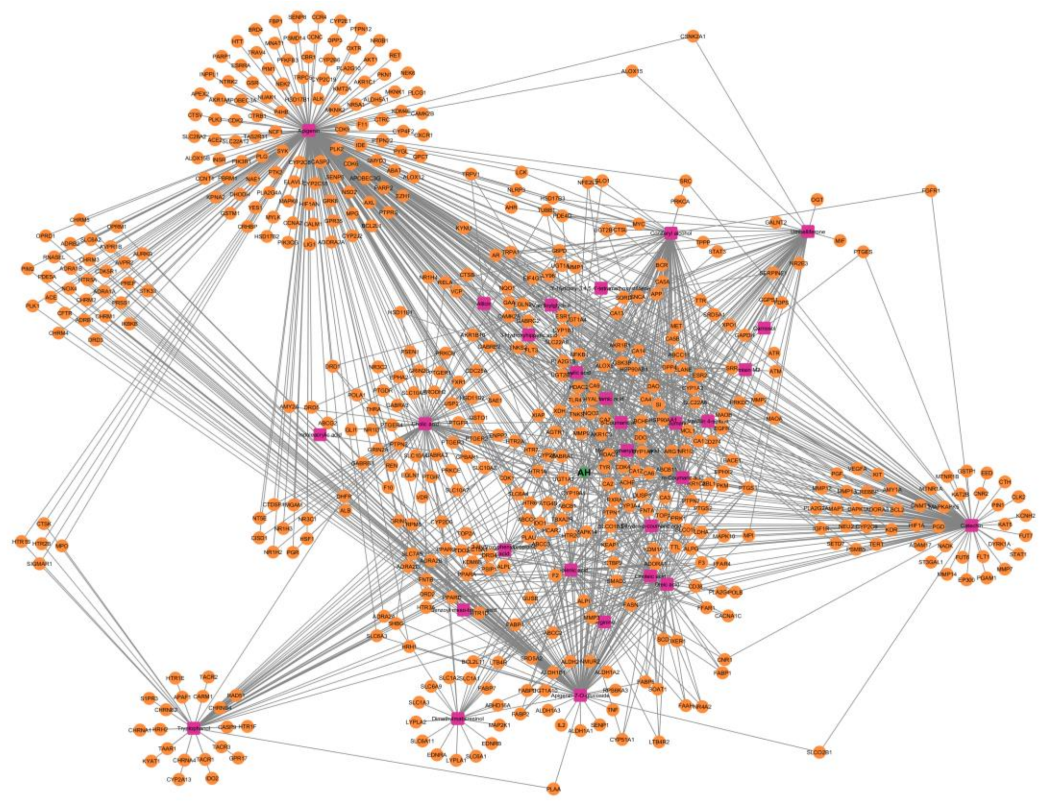


Figure 3.3. The bioactive–target (BA–TAR) network

[*A. hookeri* denoted as AH in green node, identified compounds are in pink squares, and corresponding protein targets are in orange circles]

The disease association was performed in the DisGeNET database, and the common targets for NIDDM, Obesity, Hyperlipidaemia, Hypertriglyceridaemia, and Hyperglycaemia are shown in the Venn diagram (Figure 3.4.).

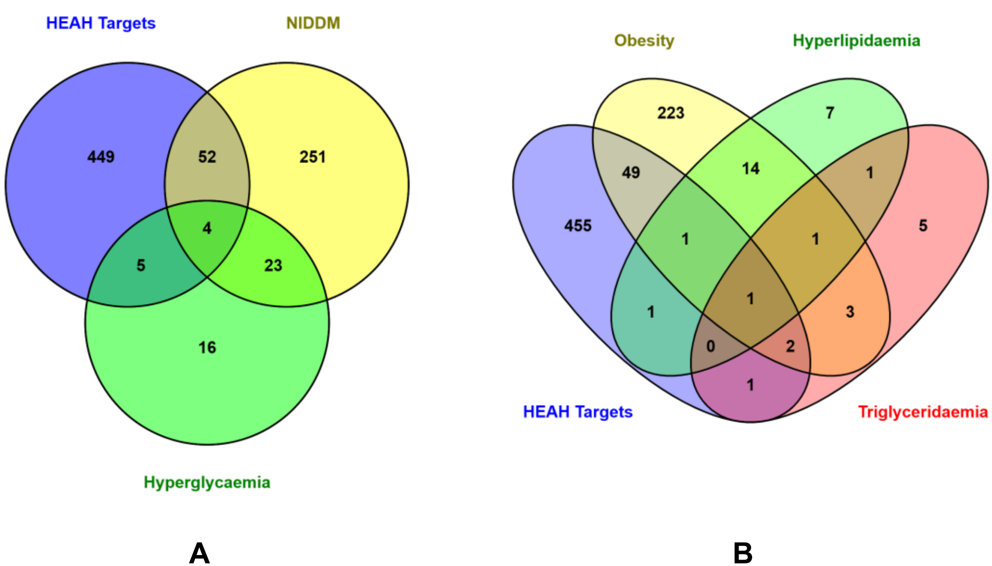


Figure 3.4. Venn diagrams showing the common targets for (A) NIDDM and Hyperglycaemia; (B) Obesity, Hyperlipidaemia and Hypertriglyceridaemia between identified protein targets of HEAH and disease targets in the DisGeNET database.

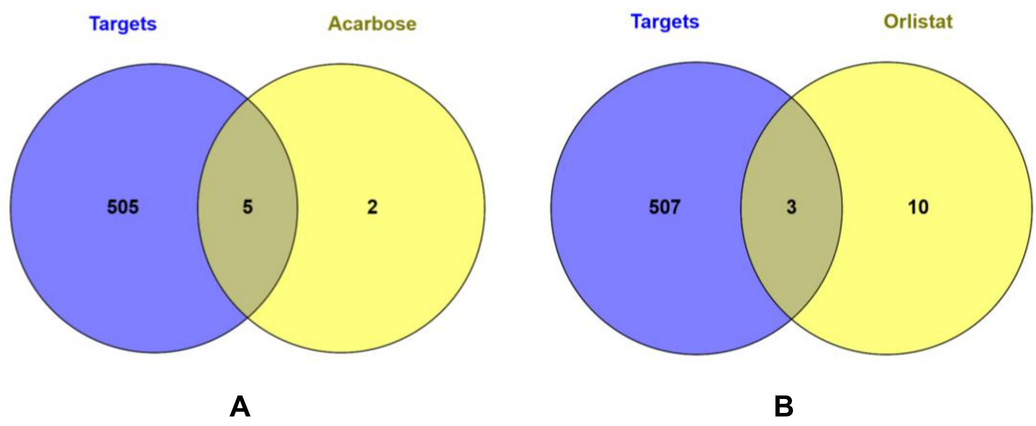
There were 56 common targets for NIDDM, followed by Obesity (53), Hyperglycaemia (9), Triglyceridaemia (4) and Hyperlipidaemia (3) between targets of HEAH and disease targets in the DisGeNET database. The list of common targets is listed in Table 3.2.

Table 3.2. Shared targets between the identified phytocompounds of *A. hookeri* and selected metabolic disorders and reference standards.

Common elements in "Targets" and "selected disorders":		
Disorder	**Number of Common Genes from the obtained targets**	**Common targets**
Hyperlipidaemia	3	ABCB1, ADRB2, PPARA
Triglyceridaemia	4	AKT1, BCHE, MET, PPARA
NIDDM	56	ABCB1, ACE, ADAM17, ADRA2A, ADRA2B, AKR1B1, AKT1, ALDH2, AXL, BCHE, BCL2, BCL2L1, BCL2L11, CASP3, CASP9, CD38, CDK4, CNR1, CYP1A1, CYP1A2, CYP2C9, CYP3A4, DPP4, DRD2, EDNRA, EDNRB, EGFR, ESR1, FABP3, FFAR1, GLO1, GSTM1, GSTP1, HIF1A, HSD11B1, IDE, IKBKB, INPPL1, INSR, MAPK8, MTNR1B, NFKB1, NR1H2, PIK3CG, PIK3R1, PPARA, PPARD, PPARG, PRKCB, PTPN1, SHBG, SLC1A2, SRC, STAT3, TLR4, TNF
Hyperglycaemia	9	CNR2, CYP2C9, HSD11B1, INSR, NFE2L2, NQO1, PRKCB, PTGS2, TERT
Obesity	53	ACHE, ADRA2B, ADRB1, ADRB2, AHR, AKR1C3, AKT1, AR, BCHE, CA3, CDK4, CNR1, CYP1B1, CYP2E1, DRD2, EP300, ESR1, F2, FAAH, FABP2, FASN, GPR17, HSD11B1, HSD11B2, HTR2A, HTR2C, IDO1, INPPL1, KCNH2, MIF, MMP9, NQO1, NR1H2, NR1H3, NR1I2, NR1I3, NR3C1, NTRK2, OPRM1, PARP1,

		PFKFB3, PGR, PPARA, PPARD, PPARG, PTGS2, PTPN1, SCD, SERPINE1, SLC6A3, STAT3, TNF, TRPV1
Common elements in "Targets" and "selected reference standards":		
Atorvastatin	4	HDAC1, HDAC2, SLCO1B1, SLCO1B3
Metformin	0	
Orlistat	3	ABHD16A, FAAH, FASN
Acarbose	5	AMY1A, AMY2A, GAA, MGAM, SI

The common targets for acarbose, Orlistat, Metformin, and Atorvastatin with HEAH were also screened and given in Figure 3.5. While Atorvastatin shared HDAC1, HDAC2, SLCO1B1 and SLCO1B3 as common targets with HEAH, no common targets were found between Metformin and HEAH. Similarly, acarbose shared AMY1A, AMY2A, GAA, MGAM, SI and Orlistat shared ABHD16A, FAAH, FASN as common targets with HEAH. These common targets suggest a mechanistic overlap and potential of HEAH as an alternative therapeutic. The gene-disease association (TAR–DIS) networks were created using Cytoscape. The top 50 hub proteins acting on NIDDM, Obesity, Hyperlipidaemia, Hypertriglyceridaemia, and Hyperglycaemia, calculated by degree and shortest path length using cytoHubba, are shown in Figure 3.5.



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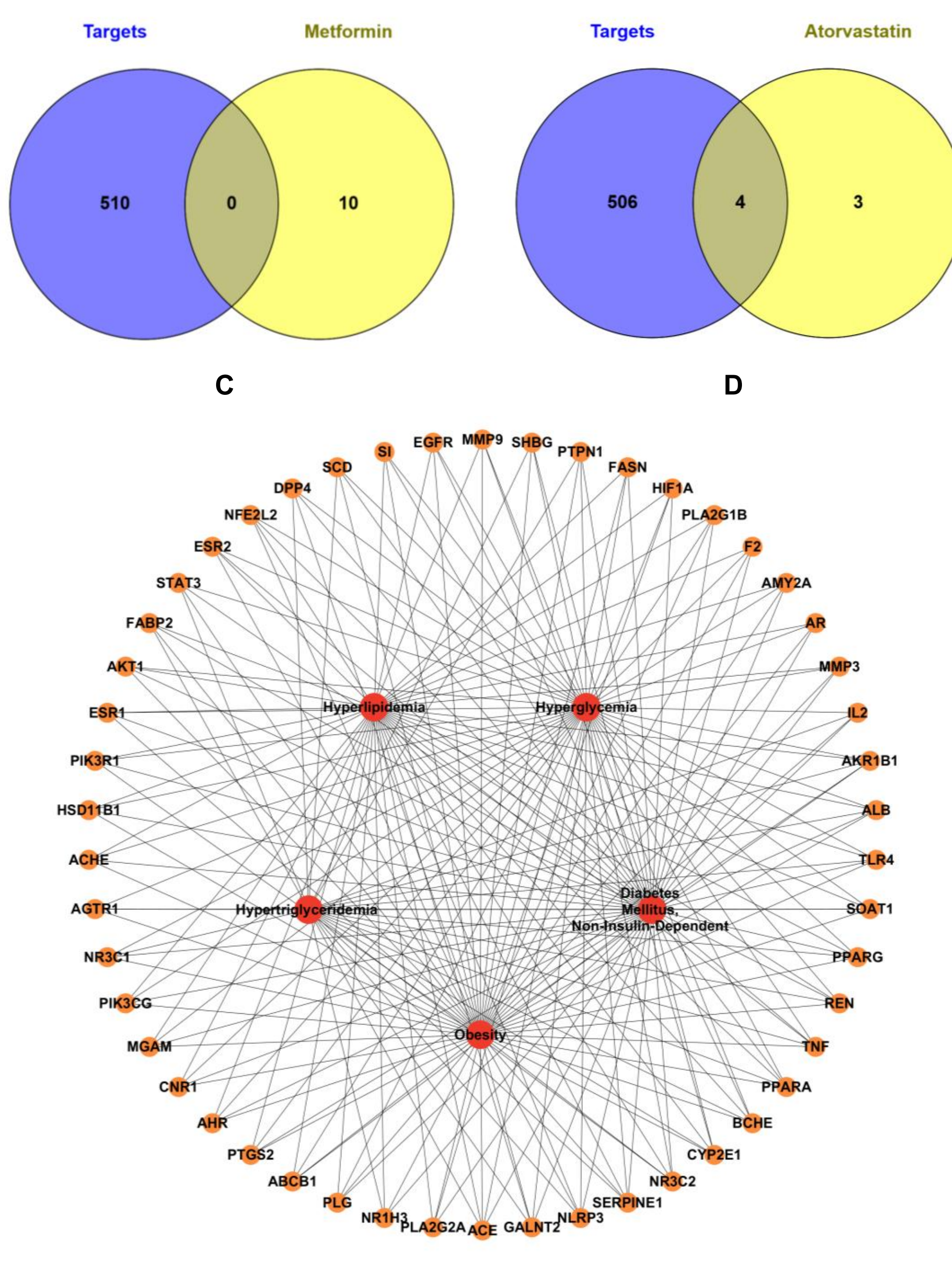


Figure 3.5. The common targets for (A) acarbose, (B) orlistat, (C) metformin and (D) atorvastatin with HEAH. (E) The top 50 hub proteins acting on NIDDM, Obesity, Hyperlipidaemia, Hypertriglyceridaemia and Hyperglycaemia

3.3.2.2. *Analysis of protein-protein interactions and associated pathways*

The target genes were visualised in STRING 12.0, and the PPI enrichment analysis showed that the target proteins have significantly more interactions, indicating that they are at least partially biologically connected as a group. The PPI enrichment analysis exhibited 10073 edges, which is significantly more than the expected number of 4285. The average node degree was found to be 43, suggesting each protein interacts with 43 others (Figure 3.6.).

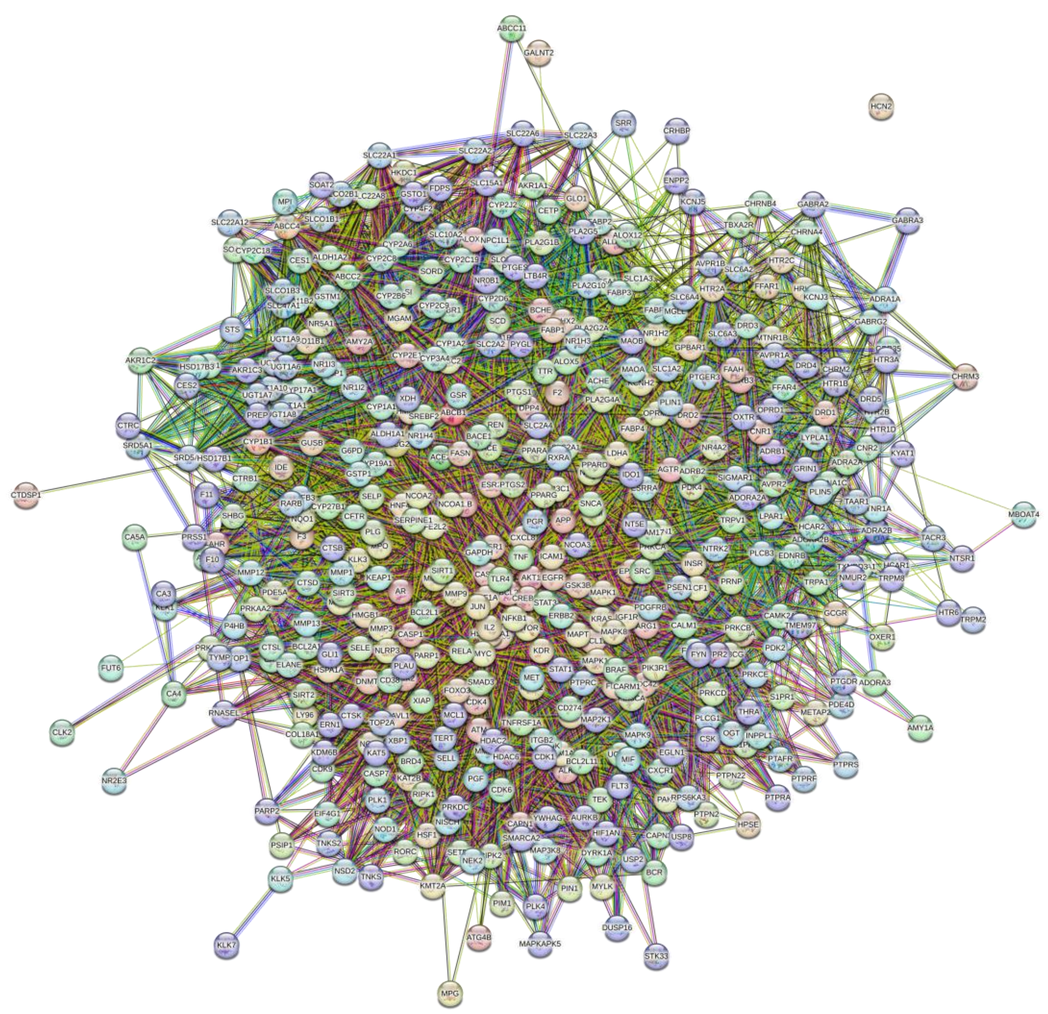


Figure 3.6. Protein-protein interaction network

The PPI enrichment network was filtered by degree and shortest path length using the cytoHubba tool in Cytoscape to calculate the top 10 hub proteins (Figure 3.7.).

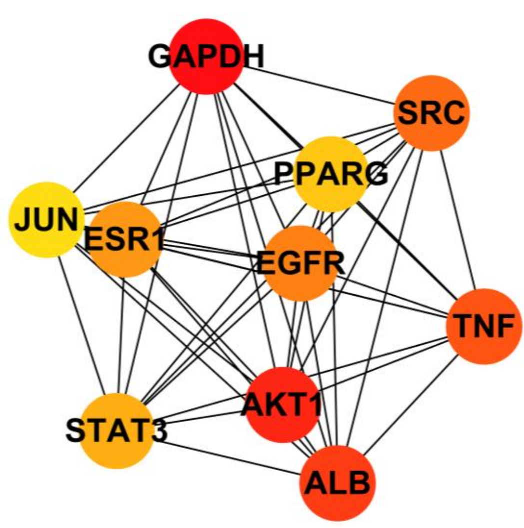


Figure 3.7. PPI enrichment network with the top 10 hub proteins

The pathway enrichment analysis by KEGG demonstrated hsa04910: Insulin signalling pathway, hsa04930: Type II diabetes mellitus, hsa04931: Insulin resistance, hsa04933: AGE-RAGE signalling pathway in diabetic complications, hsa04973: Carbohydrate digestion and absorption, hsa00010: Glycolysis/Gluconeogenesis, and hsa00040: Pentose and glucuronate interconversions to be involved in NIDDM and Hyperglycaemia. In the case of Obesity, Hyperlipidaemia, and Hypertriglyceridaemia, the pathways involved were hsa03320: PPAR signalling pathway, hsa05417: Lipid and atherosclerosis, hsa04152: AMPK signalling pathway, hsa04920: Adipocytokine signalling pathway, and hsa01100: Metabolic pathways. Other critical pathways included hsa04151: PI3K-Akt signalling pathway, hsa04310: Wnt signalling pathway, hsa04972: Pancreatic secretion, and hsa04970: Salivary secretion (Table 3.3.). The hub gene-enriched pathways have been shown in Figure 3.8.

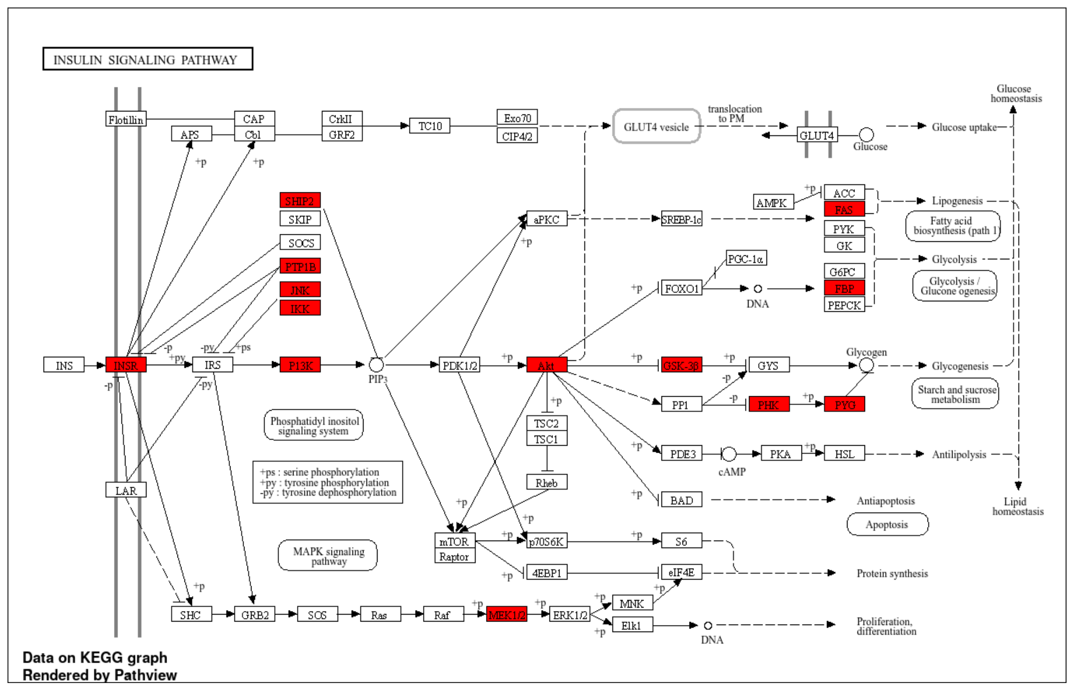
Table 3.3. KEGG enrichment pathways involved in NIDDM, Obesity, Hyperlipidaemia, Hypertriglyceridaemia and Hyperglycaemia.

Enrichment FDR	Pathway	Genes
5.38E-24	Path:hsa01100 Metabolic pathways	AKR1A1 CYP1A1 CYP1A2 CYP2A6 CYP2B6 CYP2C19 CYP2C8 CYP2C9 CYP2C18 CYP2E1 CYP2J2 CYP3A4 CYP19A1 NQO1 DNMT1 EPHX2 ALDH1A1 ALDH2 FASN FBP1 FDPS AKR1B1 ALOX12 ALOX15 ALPL FUT6 G6PD GALNT2 GAPDH GLO1 AMY1A AMY2A GSR GSTM1 GSTP1 GUSB HSD11B1 HSD11B2 HSD17B1 HSD17B3 IDO1 INPPL1 ARG1 MAOA MAOB KMT2A MPI NT5E PDE4D ENPP2 PFKFB3 PIK3CG PKM PLA2G1B PLA2G2A PLA2G4A PLCG1 UGT1A10 UGT1A6 UGT1A4 UGT1A1 PTGS1 PTGS2 PYGL SCD SRR SI SORD SRD5A1 SRD5A2 NSD2 XDH CA2 CA3 CA4 CA5A SETD7 CYP4F2 AKR1C3 PDE5A CBR1 ALDH1A2 GSTO1 CD38 PTGES
5.38E-24	Path:hsa05417 Lipid and atherosclerosis	NLRP3 MAPK14 CYP1A1 CYP2A6 CYP2B6 CYP2C8 CYP2C9 CYP2J2 AKT1 LY96 GSK3B HSP90AA1 IKBKB MMP1 MMP3 MMP9 NFE2L2 NFKB1 PIK3R1 PLCG1 PPARG PRKCA MAPK8 MAPK10 BCL2 RELA BCL2L1 RXRA NCF1 SRC STAT3 TLR4 CALM1 CAMK2A CASP3

1.16E-20	Path:hsa04933 AGE-RAGE signalling pathway in diabetic complications	CDK4 MAPK14 AGTR1 AKT1 F3 SMAD3 MMP2 NFKB1 NOX4 SERPINE1 PIM1 PIK3R1 PLCG1 PRKCA PRKCB PRKCE MAPK8 MAPK10 BCL2 RELA STAT1 STAT3 VEGFA CASP3
8.71E-20	Path:hsa04066 HIF-1 signalling pathway	CREBBP EGFR EP300 AKT1 FLT1 GAPDH HIF1A IGF1R INSR NFKB1 SERPINE1 PFKFB3 PIK3R1 PLCG1 EGLN1 PRKCA PRKCB MAP2K1 BCL2 RELA STAT3 TLR4 VEGFA CAMK2A
1.70E-15	Path:hsa04151 PI3K-Akt signalling pathway	BCL2L11 CDK2 CDK4 CDK6 CHRM2 EGFR AKT1 FGFR1 FLT1 FLT3 GSK3B HSP90AA1 IGF1R IKBKB IL2 INSR KDR MCL1 MET MYC NFKB1 NTRK2 PGF PIK3CG PIK3R1 PRKCA MAP2K1 BCL2 RELA BCL2L1 RXRA TLR4 VEGFA
4.60E-14	Path:hsa04931 Insulin resistance	NR1H3 AKT1 GSK3B IKBKB INSR NFKB1 PIK3R1 PPARA PRKCB PRKCE MAPK8 MAPK10 PTPN1 PYGL RELA RPS6KA3 STAT3 NR1H2 OGT
1.44E-12	Path:hsa04068 FoxO signalling pathway	BCL2L11 CDK2 CREBBP MAPK14 EGFR EP300 AKT1 IGF1R IKBKB INSR SMAD3 ATM PIK3R1 PLK1 MAPK8 MAPK10 MAP2K1 STAT3 SETD7
6.25E-12	Path:hsa04010 MAPK signalling pathway	MAPK14 EGFR AKT1 FGFR1 FLT1 FLT3 IGF1R IKBKB INSR KDR MET MYC NFKB1 NTRK2 PGF PLA2G4A

		PRKCA PRKCB MAPK8 MAPK10 MAP2K1 RELA RPS6KA3 VEGFA CASP3 MAPKAPK5
1.28E-09	Path:hsa04932 Non-alcoholic fatty liver disease	BCL2L11 NR1H3 MAPK14 CYP2E1 AKT1 GSK3B IKBKB INSR NFKB1 PIK3R1 PPARA PPARG MAPK8 MAPK10 RELA RXRA CASP3
6.18E-09	Path:hsa03320 PPAR signalling pathway	NR1H3 FABP4 FABP1 FABP2 FABP3 FABP5 MMP1 PPARA PPARD PPARG RXRA SCD
1.76E-08	Path:hsa04935 Growth hormone synthesis secretion and action	CREBBP MAPK14 EP300 AKT1 GSK3B PIK3R1 PLCG1 PRKCA PRKCB MAPK8 MAPK10 MAP2K1 STAT1 STAT3
5.77E-08	Path:hsa04923 Regulation of lipolysis in adipocytes	ADORA1 ADRB1 ADRB2 AKT1 FABP4 INSR PIK3R1 PTGER3 PTGS1 PTGS2
8.44E-08	Path:hsa04910 Insulin signalling pathway	AKT1 FASN FBP1 GSK3B IKBKB INPPL1 INSR PIK3R1 MAPK8 MAPK10 MAP2K1 PTPN1 PYGL CALM1
9.83E-07	Path:hsa04152 AMPK signalling pathway	CFTR ADRA1A ELAVL1 AKT1 FASN FBP1 IGF1R INSR PFKFB3 PIK3R1 PPARG SCD
2.21E-06	Path:hsa00040 Pentose and glucuronate interconversions	AKR1B1 GUSB UGT1A10 UGT1A6 UGT1A4 UGT1A1 SORD
2.59E-06	Path:hsa04920 Adipocytokine signalling pathway	AKT1 IKBKB NFKB1 PPARA MAPK8 MAPK10 RELA RXRA STAT3
5.58E-06	Path:hsa04310 Wnt	CREBBP EP300 GSK3B SMAD3

	signalling pathway	MMP7 MYC PPARD PRKCA PRKCB MAPK8 MAPK10 PSEN1 CAMK2A
8.86E-06	Path:hsa04972 Pancreatic secretion	CFTR CHRM3 AMY1A AMY2A PLA2G1B PLA2G2A PRKCA PRKCB CA2 CD38
1.31E-05	Path:hsa04930 Type II diabetes mellitus	IKBKB INSR PIK3R1 PKM PRKCE MAPK8 MAPK10
2.32E-05	Path:hsa04970 Salivary secretion	CHRM3 ADRA1A ADRB1 ADRB2 AMY1A AMY2A PRKCA PRKCB CALM1
7.25E-05	Path:hsa04922 Glucagon signalling pathway	CREBBP EP300 AKT1 FBP1 PKM PPARA PYGL CALM1 CAMK2A
0.000146568	Path:hsa04973 Carbohydrate digestion and absorption	AKT1 AMY1A AMY2A PIK3R1 PRKCB SI
0.027636655	Path:hsa00010 Glycolysis/Gluconeogenesis	ALDH2 FBP1 GAPDH PKM



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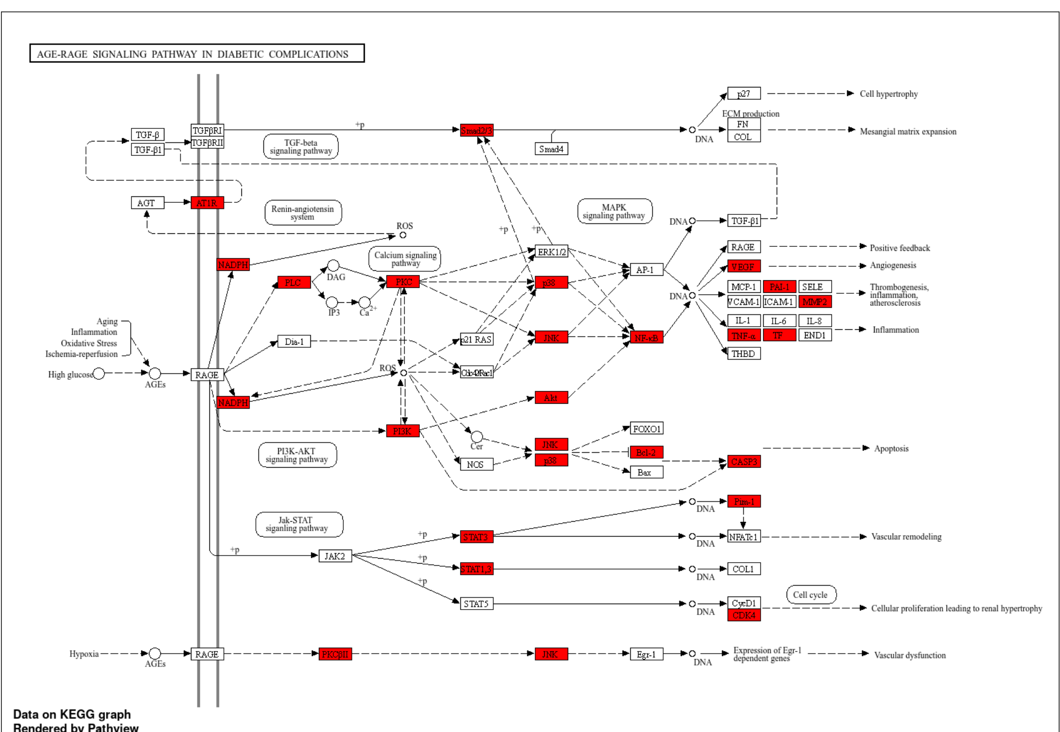

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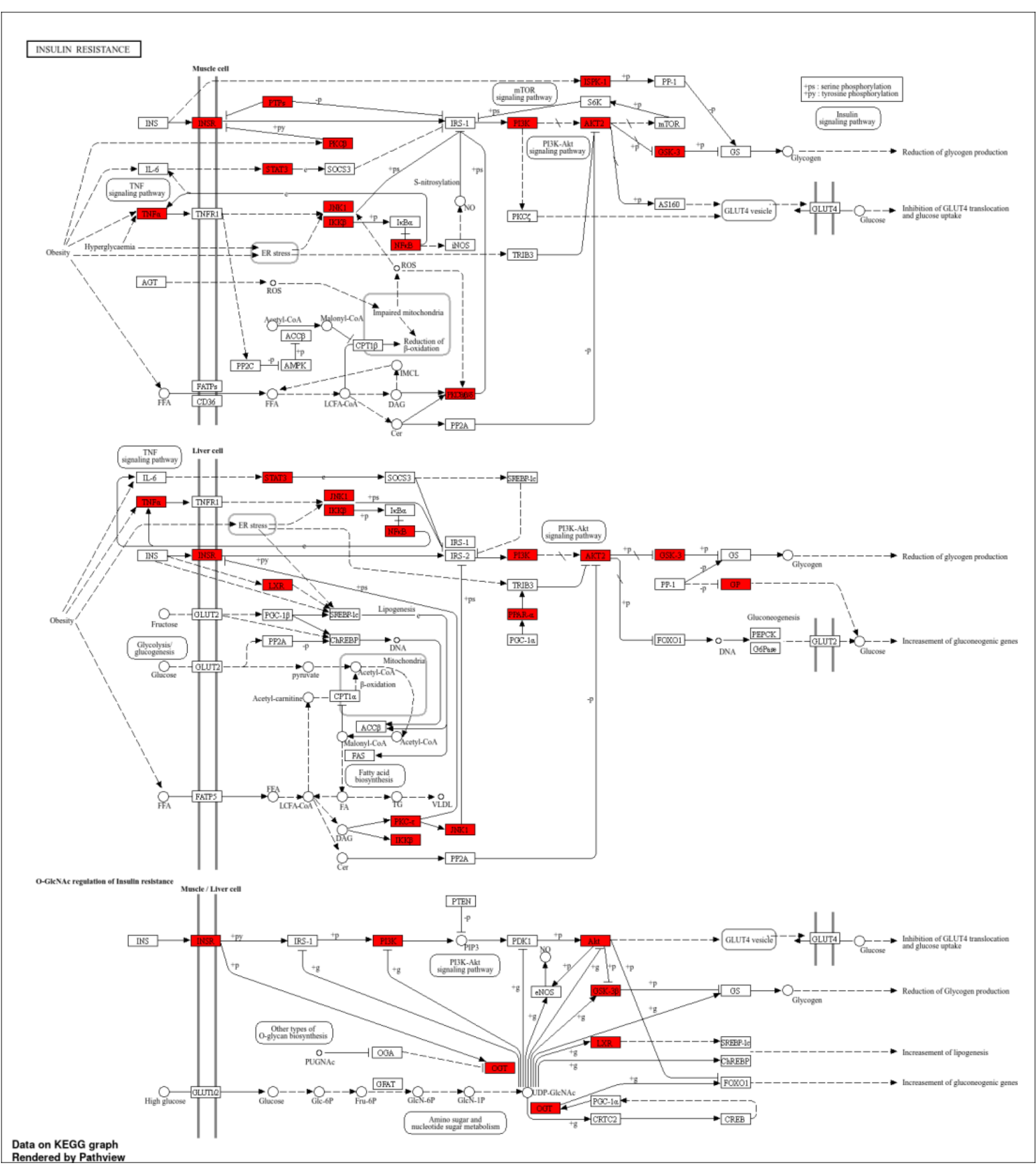

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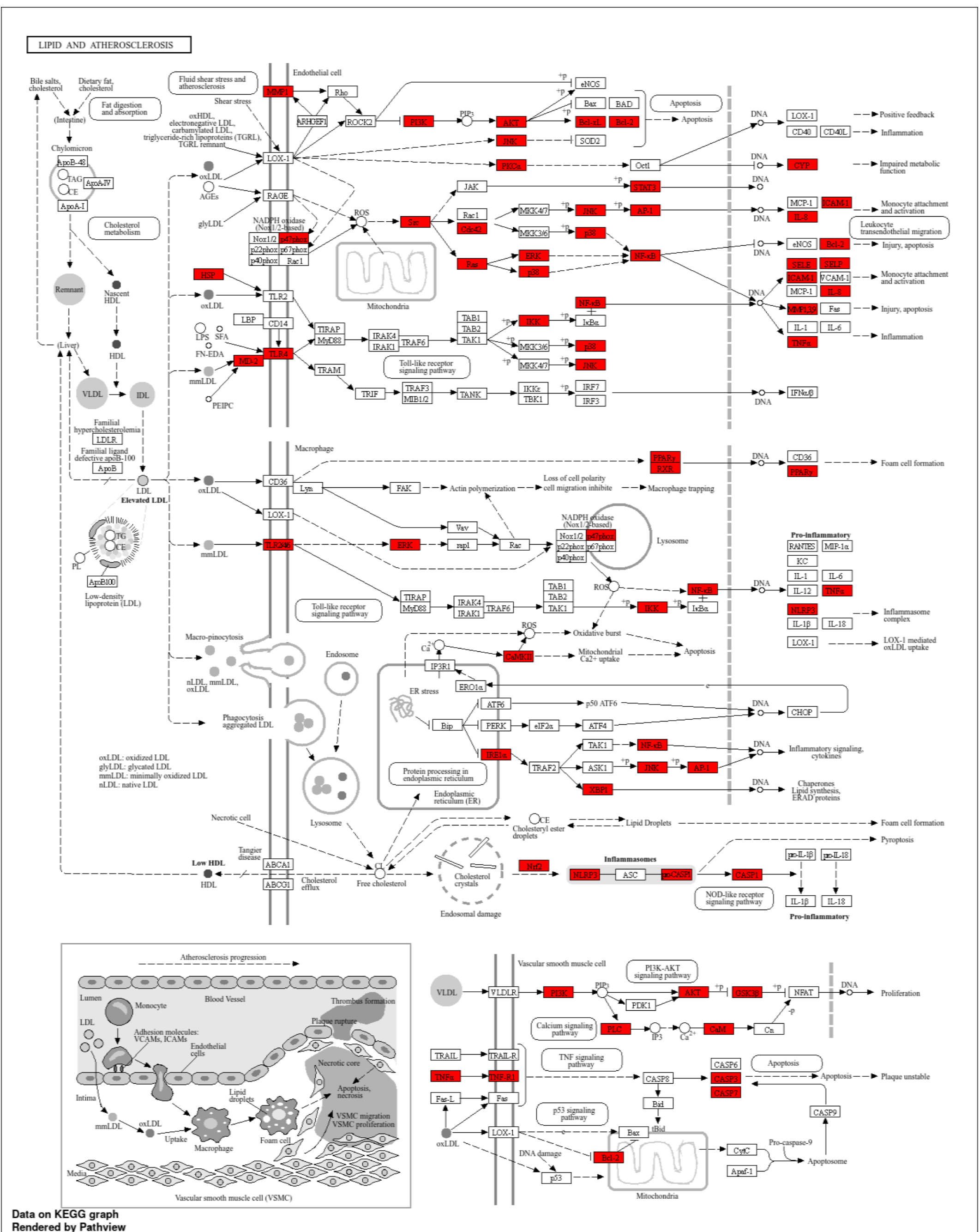

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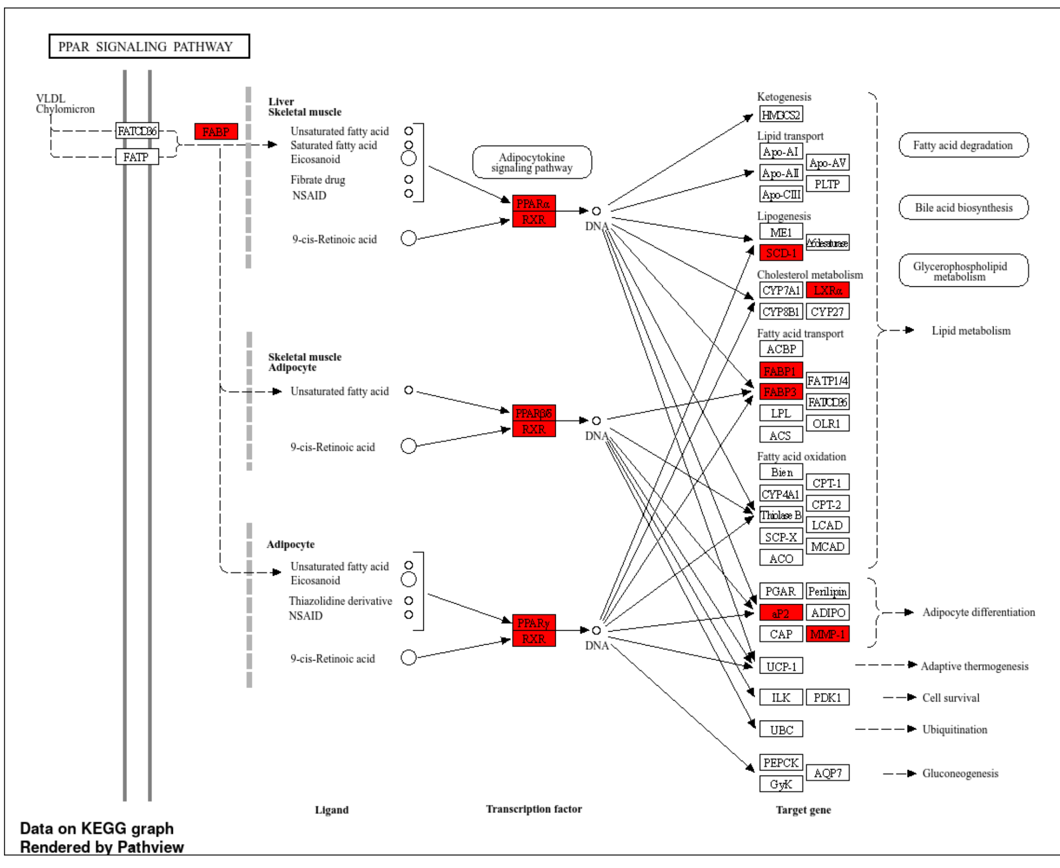


E

Figure 3.8. The hub gene enriched pathways A: Insulin signalling pathway; B: AGE-RAGE signalling pathway in diabetic complications; C: Insulin resistance pathway; D: Lipid and atherosclerosis pathway; E: PPAR signalling pathway

The Gene Ontology (GO) analysis, performed using ShinyGO 0.82, explored the biological processes in which the target genes were involved. At FDR cutoff of 0.05 the enriched biological processes included lipid metabolic process (GO:0006629), cellular lipid metabolic process (GO:0044255), lipid biosynthetic process (GO:0008610), fatty acid metabolic process (GO:0006631), lipid transport (GO:0006869), response to insulin (GO:0032868), cellular response to insulin stimulus (GO:0032869), glucose homeostasis (GO:0042593), glucose metabolic process (GO:0006006), regulation of lipid transport (GO:0032368) and

regulation of glucose transmembrane transport (GO:0010827) (Table 3.4.). The target gene-enriched biological processes are shown in Figure 3.9.

Table 3.4. GO Biological process pathways involved in NIDDM, Obesity, Hyperlipidaemia, Hypertriglyceridaemia and Hyperglycaemia.

Enrichment FDR	Pathway	Genes
1.62E-36	GO:0006629 lipid metabolic process	ALOX5 SOAT1 PLA2G10 PTGS2 PTGS1 SCD CYP2D6 PIK3CG CYP2C18 ALOX12 PPARD PLA2G4A FAAH HSD11B1 EPHX2 CYP2E1 HSD17B3 PPARG ENPP2 CYP2C9 CYP2C8 SRD5A1 PIK3R1 PTGES AKR1C2 FDPS ALOX15 CYP2C19 FASN PLA2G1B CYP4F2 PPARA PLA2G2A AKR1C3 CYP2B6 CYP2A6 SRD5A2 NFKB1 FABP3 CYP1A1 CYP3A4 UGT1A1 NR1H4 GSTP1 HTR2A HSD17B1 ALDH1A2 GSTM1 CYP2J2 HTR2B CYP19A1 CYP1B1 CYP1A2 AKT1 HTR2C FUT6 CBR1 NR0B1 KAT5 F2 TNF MIF UGT1A4 CFTR NR1H3 AKR1B1 ESR1 MAPK14 NR3C1 AKR1A1 TTR CNR1 LYPLA1 PLCG1 THRA NR1H2 PTPN22 CDK4 EDNRB IGF1R APP SNCA FABP2 ATM ADORA1 FABP1 FABP5 ALDH1A1 INPPL1 ALK PRKCE HSD11B2 TRPV1 SRC UGT1A10 ADRA2A FLT1 G6PD HCAR2 FLT3 NR5A1 NR1I2
5.90E-23	GO:0044255 cellular lipid metabolic	ALOX5 SOAT1 PLA2G10 PTGS2 PTGS1 SCD CYP2D6 PIK3CG CYP2C18

	process	ALOX12 PPARD PLA2G4A FAAH EPHX2 CYP2E1 PPARG ENPP2 CYP2C9 CYP2C8 PIK3R1 PTGES AKR1C2 FDPS ALOX15 CYP2C19 FASN PLA2G1B CYP4F2 PPARA PLA2G2A AKR1C3 CYP2B6 CYP2A6 FABP3 UGT1A1 NR1H4 GSTP1 HTR2A ALDH1A2 GSTM1 CYP2J2 HTR2B CYP1B1 CYP1A1 CYP1A2 HTR2C FUT6 CYP3A4 KAT5 MIF NR1H3 AKR1B1 MAPK14 TTR CNR1 LYPLA1 PLCG1 NR1H2 SNCA FABP2 SRD5A1 ATM FABP1 FABP5 ALDH1A1 INPPL1 AKT1 UGT1A10 UGT1A4 CBR1
1.74E-21	GO:0008610 lipid biosynthetic process	ALOX5 PTGS2 PTGS1 SCD PIK3CG ALOX12 HSD17B3 SRD5A1 PIK3R1 PTGES FDPS ALOX15 FASN PLA2G1B SRD5A2 NFKB1 FABP3 NR1H4 GSTP1 HTR2A HSD17B1 PLA2G4A ALDH1A2 GSTM1 HTR2B AKT1 HTR2C FUT6 NR0B1 KAT5 AKR1C3 TNF MIF CFTR PLA2G10 PPARD NR3C1 CDK4 CYP19A1 IGF1R CYP1A1 ATM CYP3A4 FABP5 INPPL1 NR1H3 NR1H2 G6PD PPARA AKR1B1 CYP2D6 CYP2E1 NR5A1 CYP2C9 CYP2C8 CYP1A2 CBR1
3.28E-28	GO:0006631 fatty acid metabolic process	ALOX5 PTGS2 PTGS1 SCD CYP2D6 CYP2C18 ALOX12 PPARD FAAH CYP2E1 PPARG CYP2C9 CYP2C8 PTGES AKR1C2 ALOX15 CYP2C19 FASN PLA2G1B CYP4F2 PPARA

		AKR1C3 CYP2B6 CYP2A6 GSTP1 PLA2G4A GSTM1 CYP2J2 CYP1B1 CYP1A1 MIF PLA2G10 AKR1B1 MAPK14 CNR1 LYPLA1 FABP3 CYP1A2 SNCA FABP2 CYP3A4 FABP1 FABP5 NR1H3 NR1H2 AKT1 UGT1A1 UGT1A10 UGT1A4 CBR1
5.81E-19	GO:0006869 lipid transport	ABCB1 SLCO1B3 FABP3 SLC10A2 SLCO1B1 SLCO2B1 FABP2 FABP1 FABP5 FABP4 NFKB1 PPARG NR1H3 DRD4 PLA2G10 ABCC11 ABCC4 NR1H2 NMUR2 CYP19A1 DRD2 DRD3 ACE AVPR1B CFTR ABCC2 PTGS2 PPARD PLA2G4A ABCG2 REN SIGMAR1 PTGES PLA2G1B PLA2G2A MIF AKT1 CYP4F2 AGTR1 PPARA
1.46E-15	GO:0032868 response to insulin	PKM GSK3B PIK3R1 PPARG KAT2B PARP1 OGT INSR NR1H4 OPRK1 MAPK14 STAT1 CDK4 IGF1R AKT1 SRD5A1 FBP1 INPPL1 ESRRA PTPN2 HSD11B2 PPARA PTPN1 SRC SLC22A12 PRKDC PRKCB PLA2G1B IDE
2.46E-13	GO:0032869 cellular response to insulin stimulus	PKM GSK3B PIK3R1 PPARG KAT2B PARP1 OGT INSR NR1H4 STAT1 CDK4 IGF1R AKT1 SRD5A1 FBP1 ESRRA PTPN2 PTPN1 SRC SLC22A12 PRKDC PRKCB PLA2G1B IDE
1.12E-10	GO:0042593 glucose homeostasis	IGF1R INSR NR1H4 HIF1A CFTR ALOX5 OPRK1 NOX4 PPARD CNR1 FFAR1 AKT1 PIK3R1 ADRA2A ACE FABP5 STAT3 PRKCE KAT5 PTPN2

		PYGL PPARG MTNR1B
1.22E-10	GO:0006006 glucose metabolic process	G6PD FBP1 EP300 INSR PKM GAPDH MAPK14 NR3C1 KAT2B AKT1 OGT FABP5 INPPL1 PTPN2 PPARA SRC TNF CLK2 PPARD GSK3B SORD
3.42E-10	GO:0032368 regulation of lipid transport	NFKB1 PPARG NR1H3 NR1H2 CYP19A1 AVPR1B PLA2G10 PLA2G4A FABP3 REN PTGES MIF AKT1 CYP4F2 AGTR1 PPARA
4.51E-12	GO:0010827 regulation of glucose transmembrane transport	PRKCB INSR SLC1A2 MAPK14 NFE2L2 RNASEL EDNRA ACE TERT FABP5 FFAR4 TNF AKT1 PIK3R1 PLA2G1B

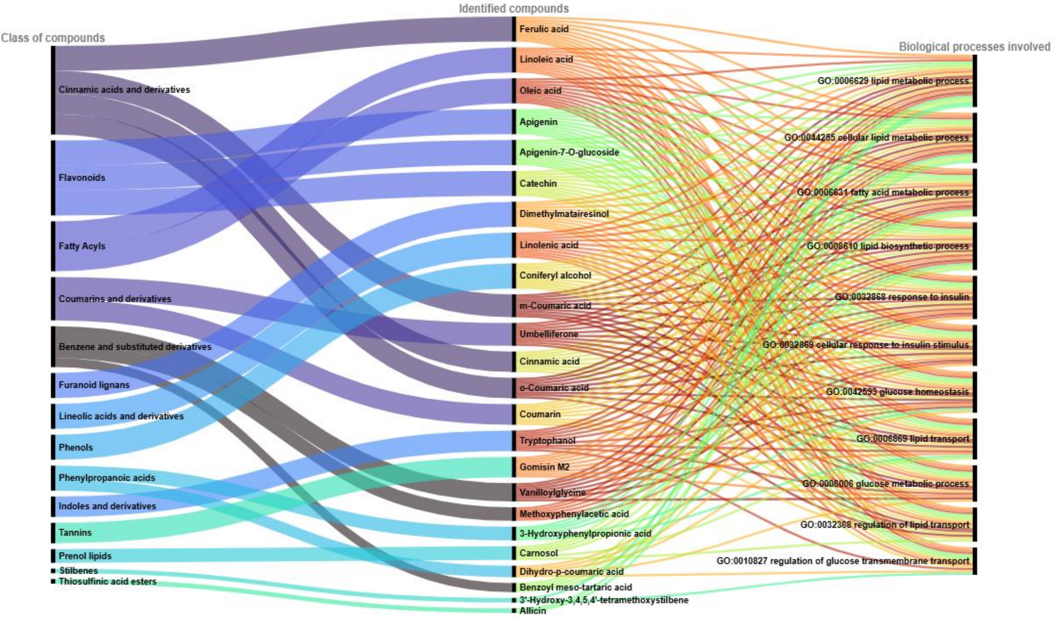
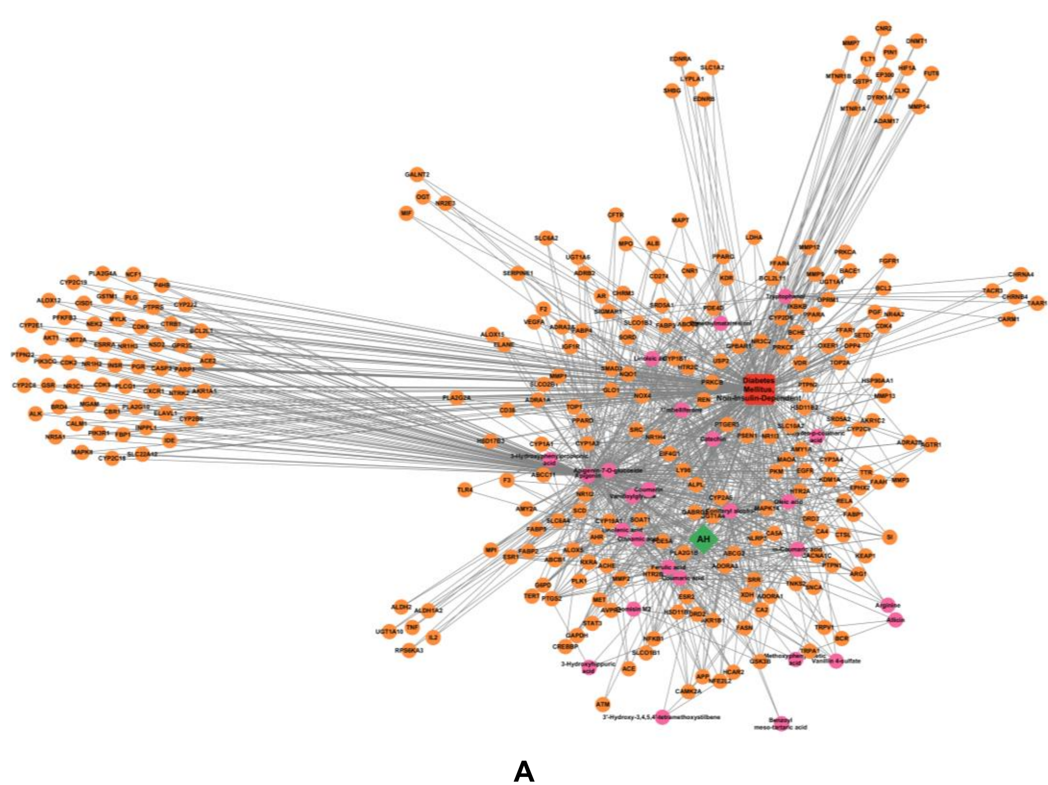


Figure 3.9. Enriched biological process involved in the regulation of NIDDM and Obesity

3.3.2.3. *Combination network analysis*

The union network of BOT–BA–TAR–DIS for NIDDM, Obesity, Hyperlipidaemia, Hypertriglyceridaemia, and Hyperglycaemia was created in Cytoscape and summarised in yfiles organic layout model. Each network was analysed using the "Analyze Network" tool, and in all cases, apigenin showed the highest degree of connectivity, and AKR1B1 was found to be the most targeted protein. All the combination synergy networks have been illustrated in Figure 3.10.



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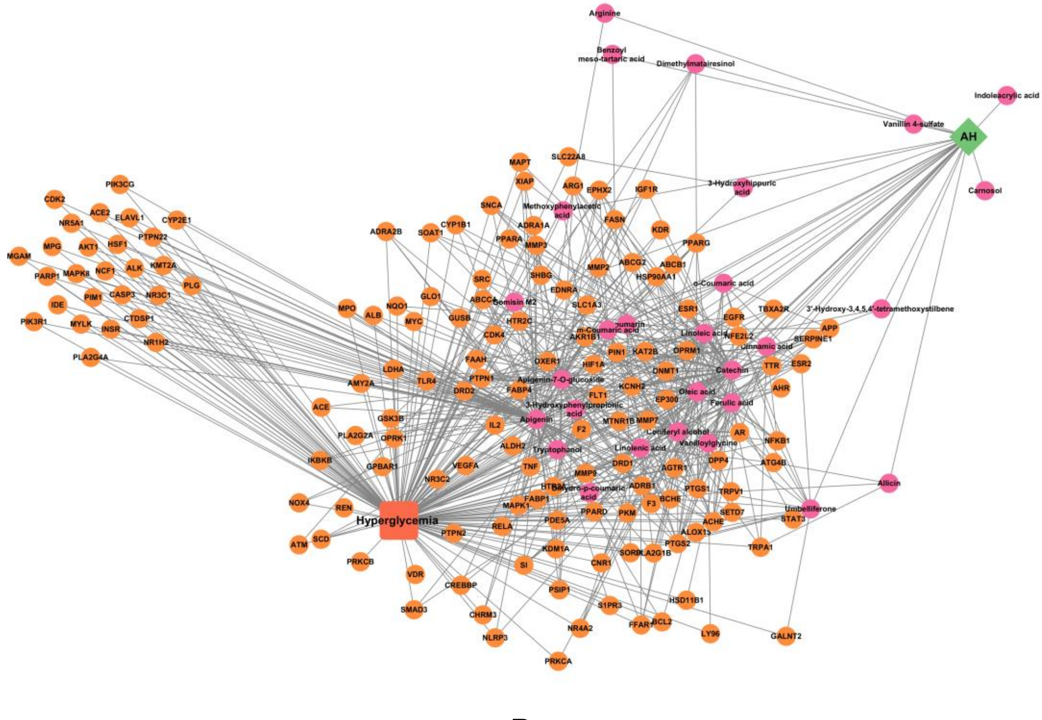

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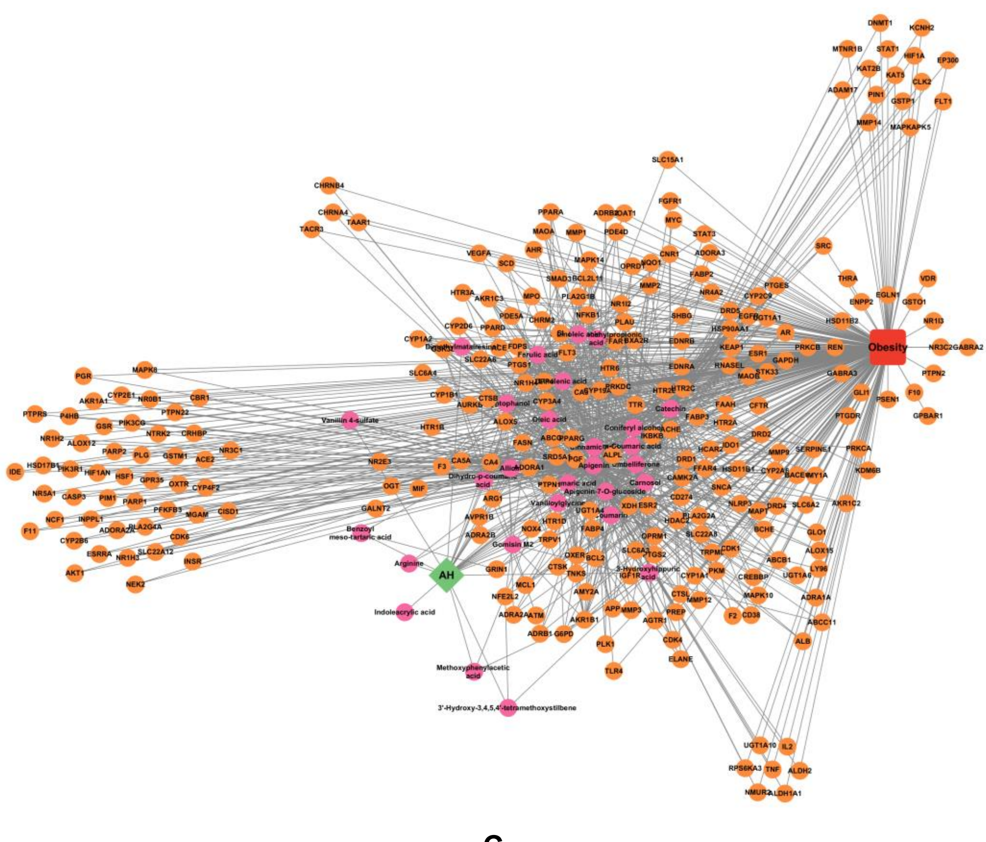

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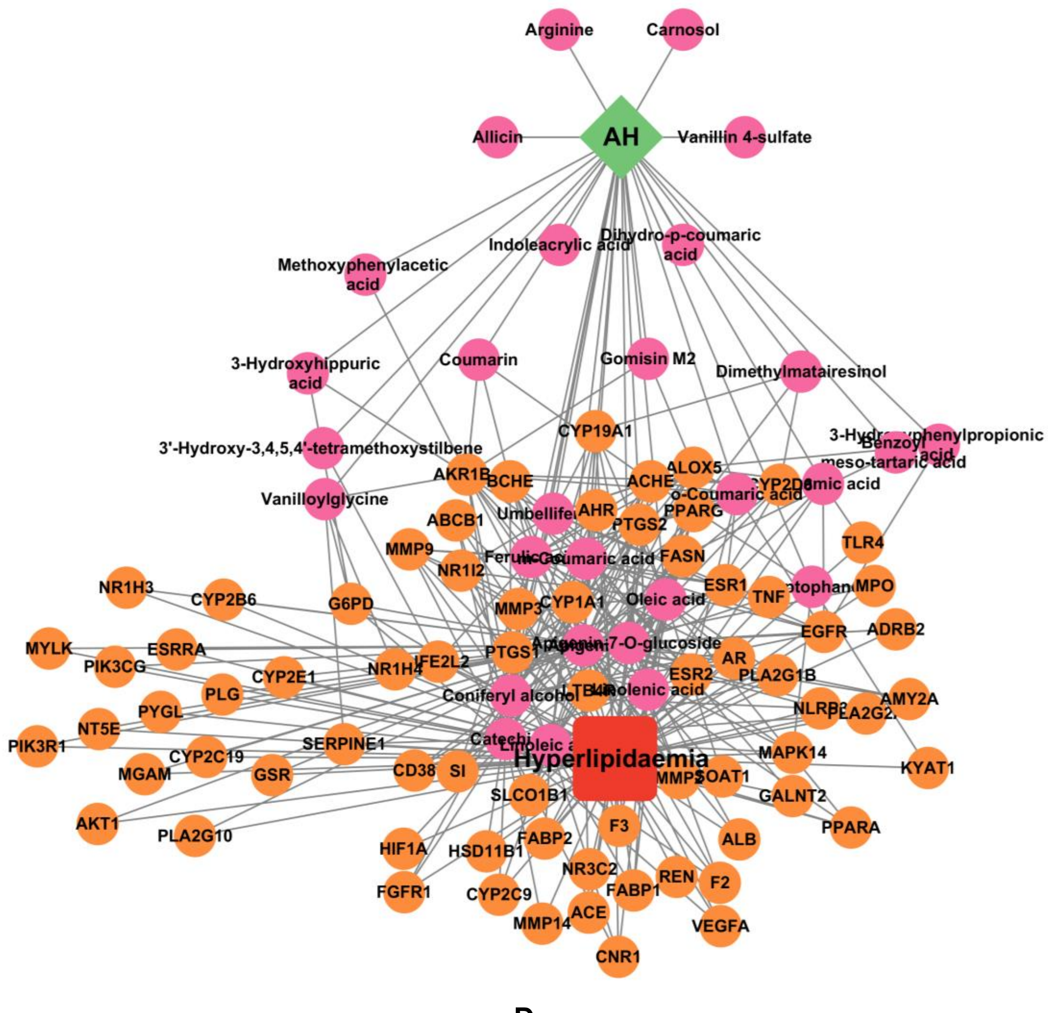

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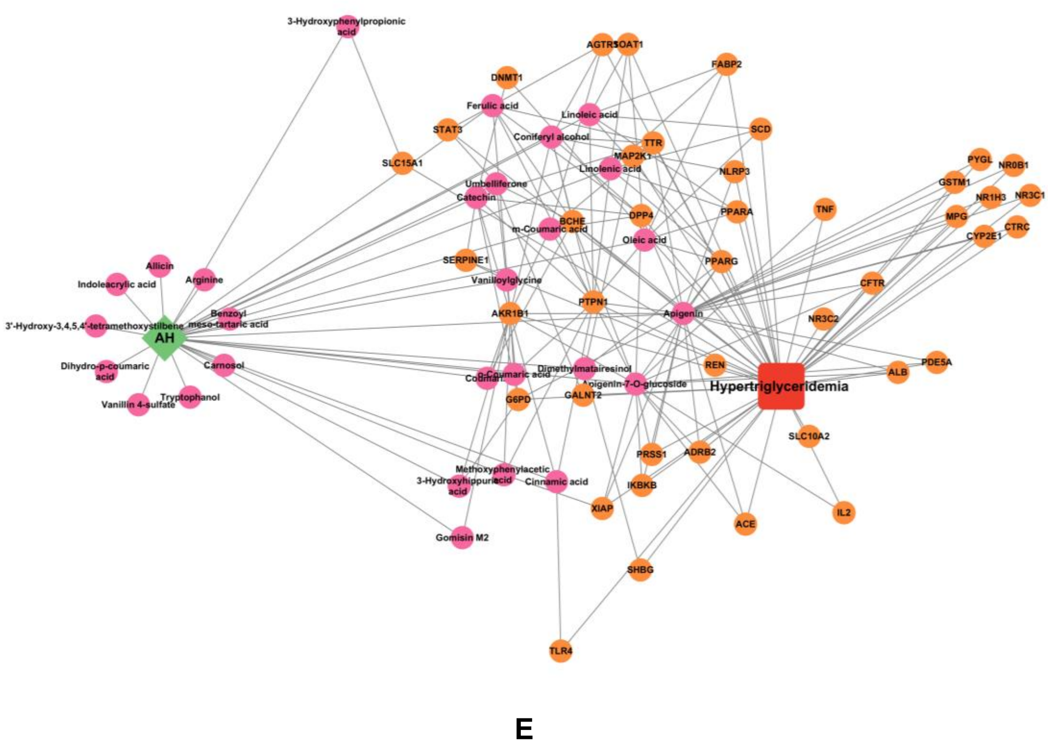
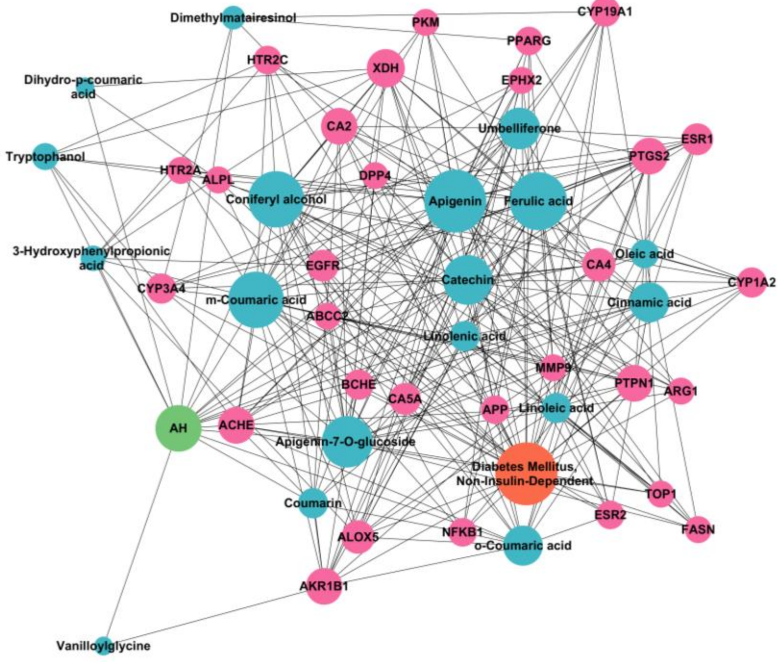


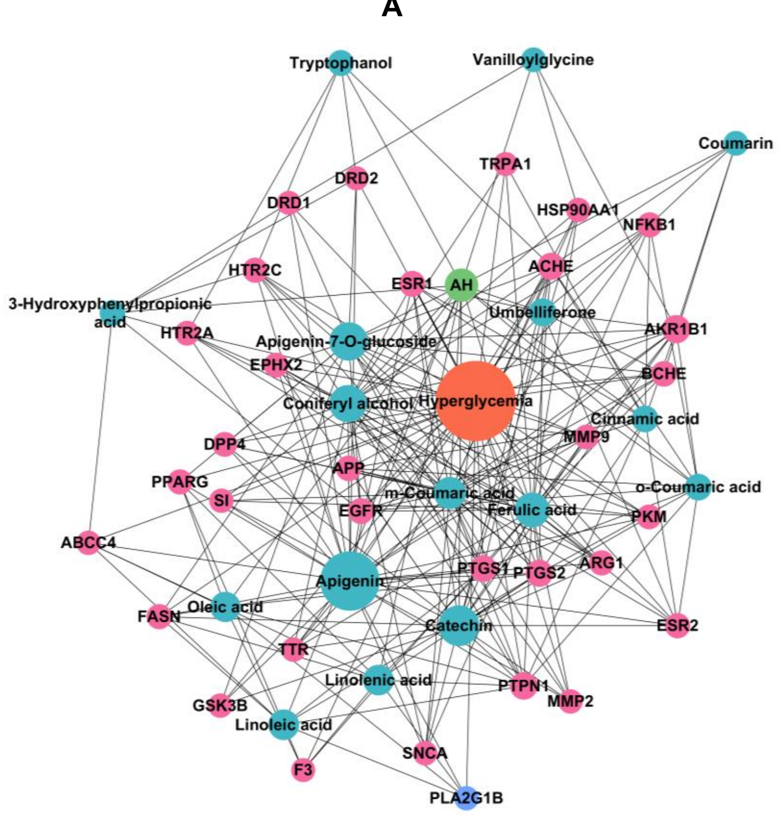
E

Figure 3.10. Combination synergy networks for HEAH against A: NIDDM; B: Hyperglycaemia; C: Obesity; D: Hyperlipidaemia; E: Hypertriglyceridaemia [AH is denoted by the green diamond; Pink circles are the identified phytoconstituents; Orange circles represent human target proteins; Orange squares represent the disorder]

The top 50 hub nodes for each NIDDM, Obesity, Hyperlipidaemia, Hypertriglyceridaemia, and Hyperglycaemia were calculated by degree, and the shortest path length is shown in Figure 3.11.



A



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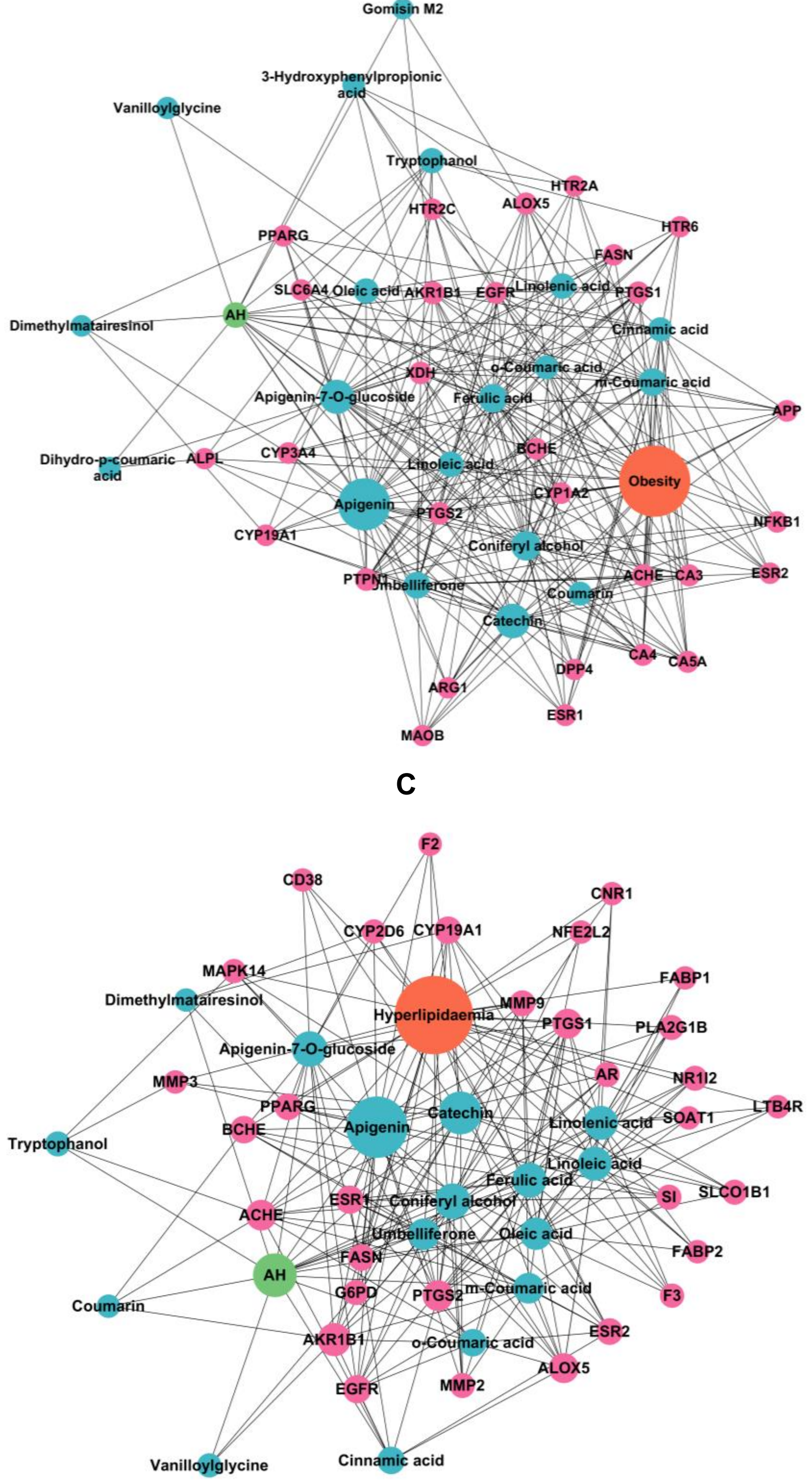

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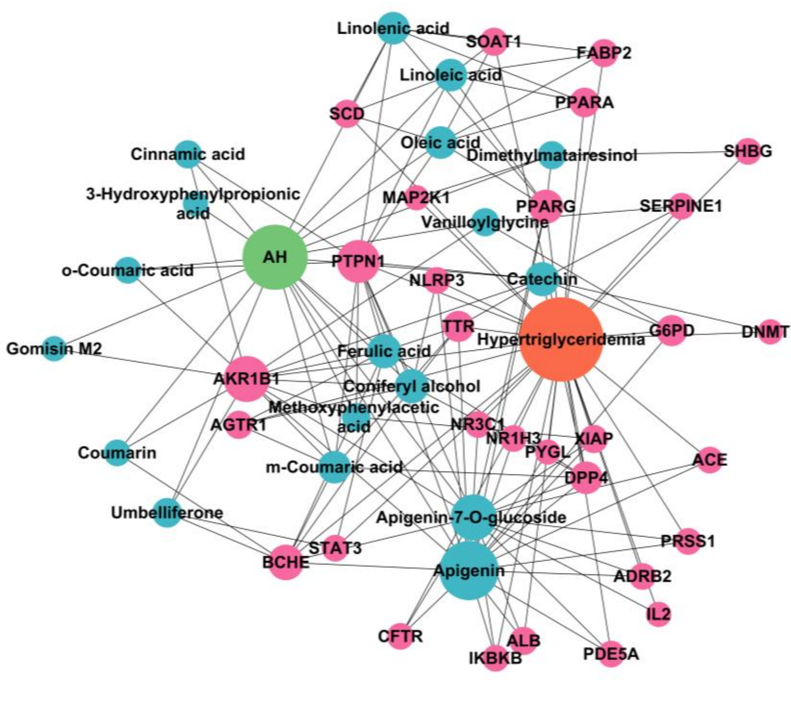


E

Figure 3.11. Combination synergy network analysis of *Allium hookeri* Thwaites (green circle denoted with AH) showing the top 50 hub nodes interacting to exert the pharmacological effect against A: NIDDM; B: Hyperglycaemia; C: Obesity; D: Hyperlipidaemia, and E: Hypertriglyceridaemia. The bigger the size of each node, the more interaction potential there is.

[Blue circles are the identified phytoconstituents; Pink circles represent human target proteins; Orange circles represent the disorder]

3.3.3. *Total flavonoid and phenolic content of A. hookeri*

The percentage yield of HEAH was found to be 19.34% w/w. The phenolic and flavonoid content was determined from the equations of gallic acid ($y = 0.0012x + 0.1132$, $R^2 = 0.9836$) and Rutin ($y = 0.001x + 0.0362$, $R^2 = 0.9919$), respectively. The standard curve is presented in Figure 3.12. HEAH exhibited a TPC of 18.503 ± 0.903 mg gallic acid equivalent/g and TFC of 10.73 ± 0.76 mg Rutin equivalent/g.

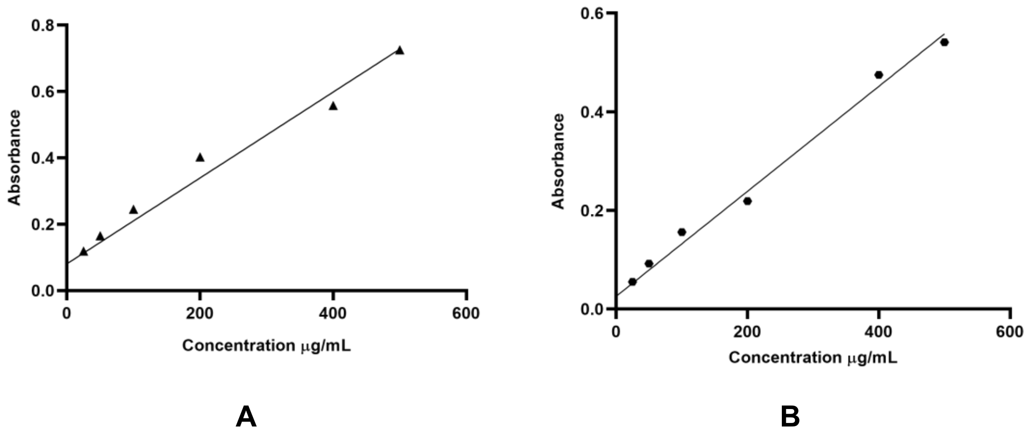
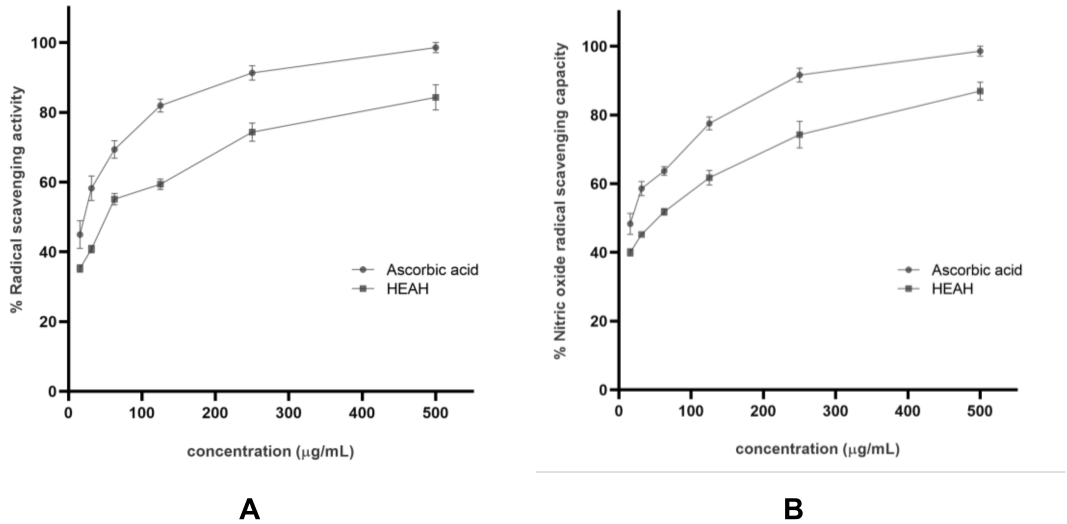
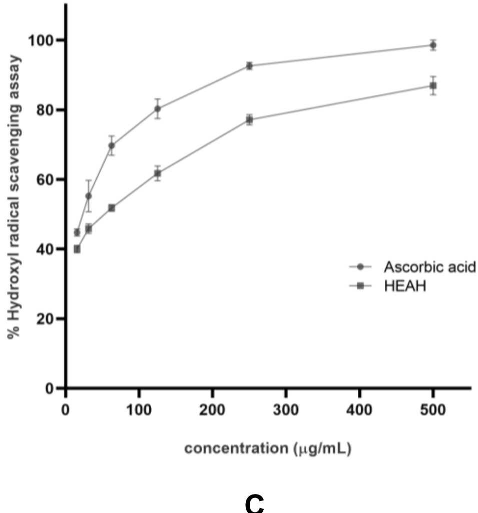


Figure 3.12. Standard curve of (A) Gallic acid and (B) Rutin

3.3.4. *Free radical scavenging potential of A. hookeri*

HEAH exhibited dose-dependent antioxidant potential (Figure 3.13.). In the DPPH radical scavenging potential assay, HEAH showed an IC_{50} value of 128.5 ± 0.031 µg/mL compared to 53.58 ± 0.064 µg/mL by ascorbic acid. The hydroxyl scavenging capacity of HEAH exhibited an IC_{50} value of 210.5 ± 3.4 µg/mL compared to 59.94 ± 0.49 µg/mL by ascorbic acid. HEAH demonstrated significant antioxidant potential, with an IC_{50} of 234 ± 3.5 µg/mL, compared to 120 ± 2.3 µg/mL for ascorbic acid in the nitric oxide scavenging assay.





C

Figure 3.13. Antioxidant potential of *A. hookeri* (A) DPPH free radical scavenging potential (B) Nitric oxide (NO) scavenging potential, and (C) Hydroxyl (OH) radical scavenging potential

3.3.5. *Enzyme inhibitory potential of A. hookeri*

In the α-glucosidase inhibition assay, the half-maximal inhibitory concentration (IC_{50}) of HEAH was found to be 0.517 ± 0.07 mg/mL compared to 0.294 ± 0.08 mg/mL for acarbose. In the α-amylase inhibition assay, the IC_{50} of HEAH was found to be 1.138 ± 0.57 mg/mL, which is comparable to an IC_{50} of 0.532 ± 0.04 mg/mL for acarbose. In the case of pancreatic lipase, HEAH exhibited an inhibition potential of 0.809 ± 0.06 mg/mL compared to 0.739 ± 0.09 for Orlistat. The dose–response curve of HEAH and acarbose against α-glucosidase and α-amylase inhibition, and Orlistat against pancreatic lipase inhibition is shown in Figure 3.14. Multiple comparisons of the IC_{50} values with two-way ANOVA between HEAH and acarbose for both enzymes, α-glucosidase and α-amylase, and Orlistat for pancreatic lipase, followed by Dunnett's test, showed significant results ($p < 0.0001$), suggesting that HEAH has inhibited all three enzymes in a strong manner comparable to the standard inhibitors.

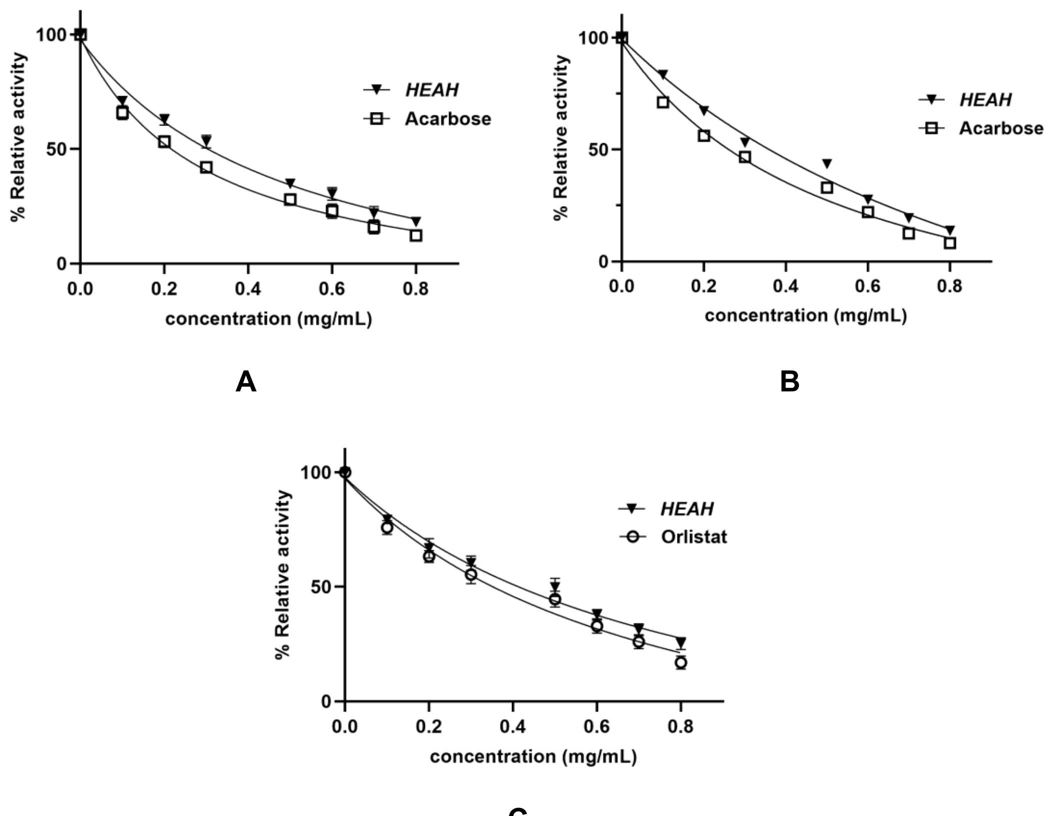
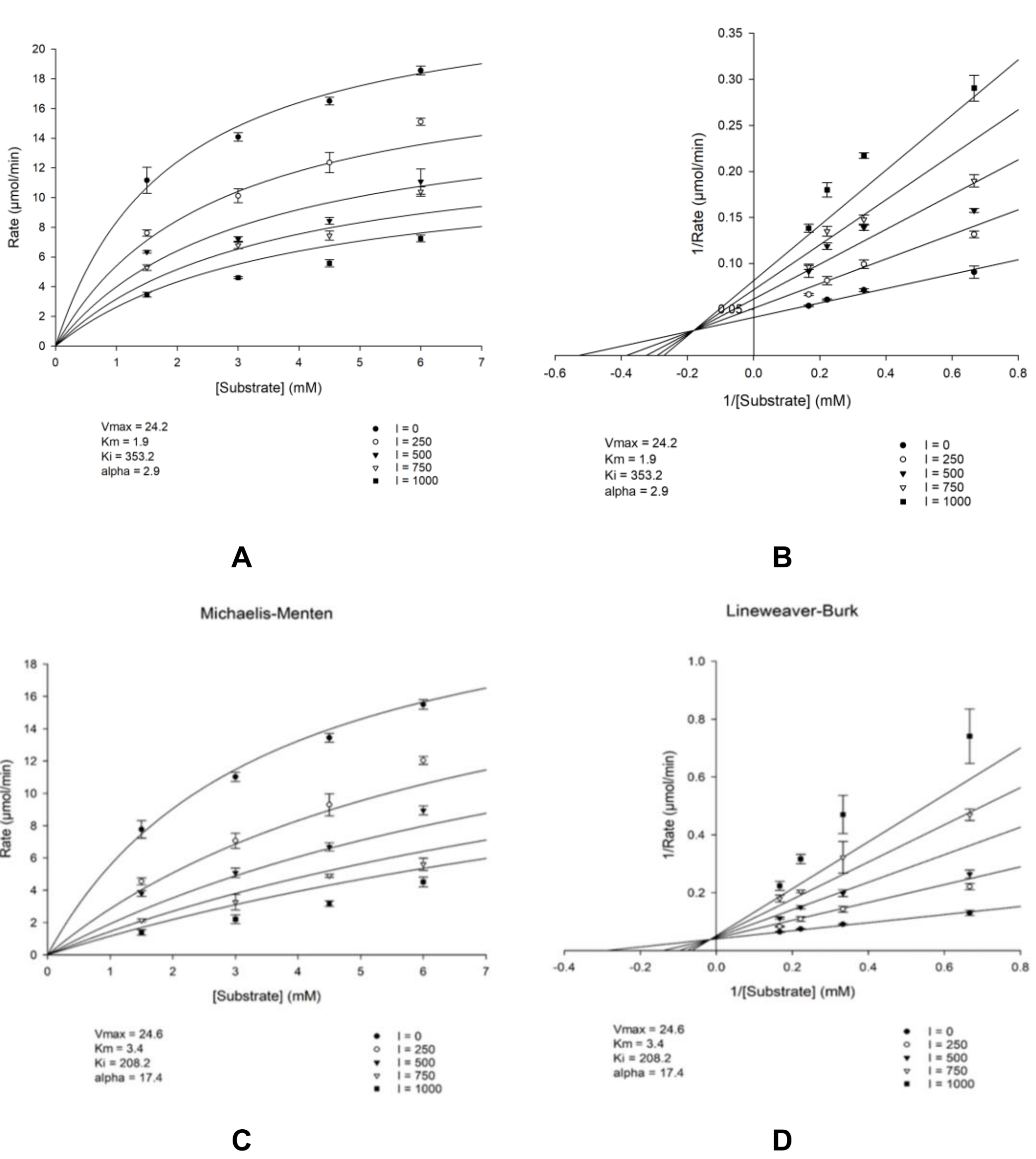


Figure 3.14. Dose–response curve of HEAH and acarbose against (A) α-glucosidase and (B) α-amylase inhibition, and orlistat against (C) Pancreatic lipase inhibition.
(-□-) acarbose (-○-) orlistat and (—▼—) HEAH; data are presented as mean ± SD (n = 3).

3.3.6. *Kinetic parameters and inhibition mechanism of A. hookeri*

Reaction velocity (v) vs enzyme concentration (I = 0–1000 µg/mL) showed reversible inhibition of α-glucosidase, α-amylase, and pancreatic lipase by HEAH, with an inversely decreasing slope at higher inhibitor concentrations (I). The α-glucosidase inhibition kinetics yielded a V_{max} of 24.2 ± 1.49 mM/min, a K_m of 1.9 ± 0.34 mM, a K_i of 353.2, and an α of 2.9. The α-amylase inhibition kinetics yielded a V_{max} of 24.6 ± 2.33 mM/min, a K_m of 3.4 ± 0.55 mM, a K_i of 208.2, and an α of 17.4. The pancreatic lipase inhibition kinetics yielded a V_{max} of 149.8 ± 34.71

mM/min and a K_m of 2.7 ± 0.93 mM, a K_i of 197.4, and an alpha of 3.7 (Figure 3.15).



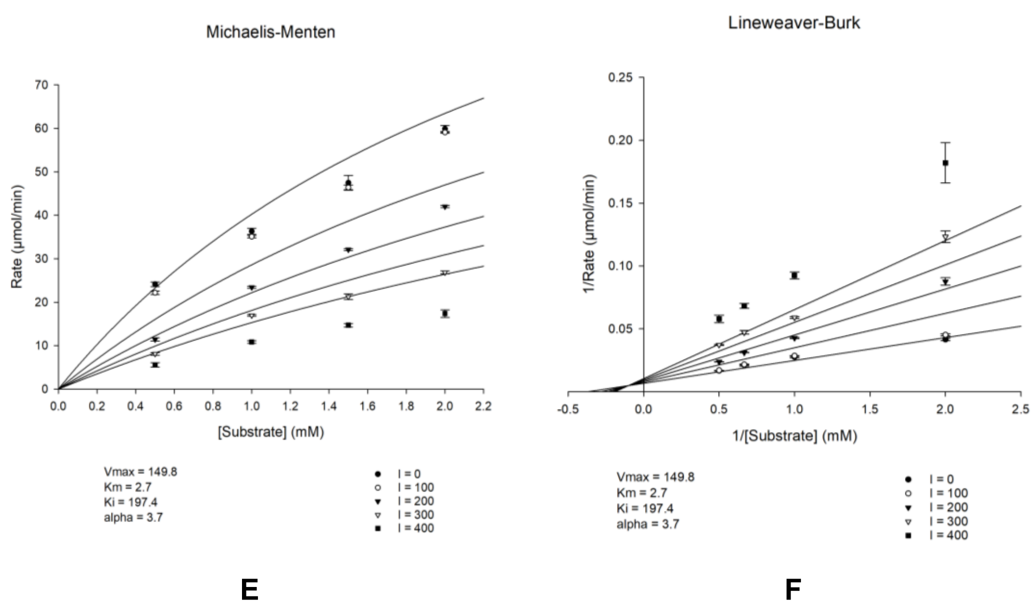


Figure 3.15. Enzyme inhibition kinetics of HEAH (● I=0 µg/ml; ○ I=250 µg/ml; ▼ I=500 µg/ml; ▽ I=750 µg/ml; ■ I=1000 µg/ml).

Michaelis–Menten plot against (A) α-glucosidase, (C) α-amylase and (E) pancreatic lipase inhibitory activity; Lineweaver–Burk plot against (B) α-glucosidase, (D) α-amylase and (F) pancreatic lipase inhibitory activity.

3.3.7. *Correlation Analysis of TPC, TFC, and antioxidant potential with enzyme inhibitory potential*

Pearson correlation analysis between the α-glucosidase and α-amylase inhibitory potential and the TPC & TFC values was evaluated and is shown in Table 3.5. The IC_{50} value of enzymes in mg/mL was correlated with the IC_{50} value (mg/mL) of the antioxidant potential (DPPH, NO, OH) and mg/g of TPC and TFC. It was observed that TPC and TFC of HEAH showed a perfect positive linear relationship with α-amylase, α-glucosidase, and pancreatic lipase, which was reflected in the Pearson r value at $p<0.05$. The enzyme inhibitory activity of HEAH was found to be related to TPC and TFC, indicating that the phenolic and flavonoid compounds present in *A. hookeri* leaves play a significant role in inhibitory activity.

Similarly, Pearson correlation analysis exhibited a very strong correlation between the antioxidant potential and enzyme inhibitory activity of HEAH. The

Pearson correlation coefficient, along with the level of significance, has been summarised in Table 3.5. The strong correlation suggests that the antioxidant potential of HEAH was beneficial in inhibiting all three enzymes.

Table 3.5. Pearson correlation analysis of enzyme inhibitor potential of HEAH with total phenolic and flavonoid content and antioxidant potential [$p < 0.05$].

Pearson correlation coefficient (r) for α-amylase inhibition potential				
DPPH	**NO**	**OH**	**GAE**	**RE**
0.99^{*}	0.99^{*}	0.99^{ns}	1.00^{***}	1.00^{***}
Pearson correlation coefficient (r) for α-glucosidase inhibition potential				
DPPH	**NO**	**OH**	**GAE**	**RE**
0.99^{*}	0.99^{*}	0.99^{ns}	1.00^{***}	1.00^{***}
Pearson correlation coefficient (r) for pancreatic lipase inhibition potential				
DPPH	**NO**	**OH**	**GAE**	**RE**
0.99^{*}	0.99^{*}	0.99^{ns}	1.00^{***}	1.00^{***}

3.4. Discussion

The concept of "food as medicine" gives a substantial advantage in the development of herbal drugs with negligible adverse effects (Mukherjee et al., 2015). This type of food, enriched with medicinal value, is a mainstay in the northeast Indian diet, contributing to the management of various metabolic disorders. The development of evidence-based and value-added alternative therapeutics from food plants in the northeastern region of India may expand the regional bioeconomy (Das et al., 2024). Irrespective of the ethnopharmacological evidence and recent research, no efforts have been made to study the exact mechanistic pathways by which *A. hookeri* exhibits therapeutic activity. This thought led to the development of HEAH, intended for the prevention of metabolic disorders.

Microwave-assisted extraction was selected for its superior effectiveness in extracting phytometabolites, requiring reduced extraction time and minimal solvent volume (Das Gupta et al., 2025a). The metabolite profiling of HEAH

revealed 27 compounds, including flavonoids such as Apigenin and Apigenin 7-O-glucoside, tannins like Gomisin M2, phenolic acids, and fatty acids. These class of compounds have been previously reported to possess therapeutic potential against metabolic disorders, such as NIDDM and Obesity (Das Gupta et al., 2025b; Singha et al., 2024; Banerjee et al., 2023). Previous studies have reported that hub compounds identified in HEAH exert an antidiabetic and antiobesity effect. Apigenin was found to activate the PI3K/Akt/Glut-4 signalling pathway (Miao et al., 2023). Catechin improves insulin resistance, alleviates oxidative stress, regulates mitochondrial function, mitigates endoplasmic reticulum stress, exhibits anti-inflammatory effects, and modulates intestinal function to reduce glucose absorption (Wen et al., 2022).

A study reported that Cinnamic acid was found to reduce blood glucose levels in diabetic rats by improving glucose tolerance (Hafizur et al., 2015). Coumaric acid, particularly m-coumaric acid and dihydro-p-coumaric acid, significantly reduced blood glucose levels, HbA1c, and formation of advanced glycation end products (AGEs) in diabetic rats (Mani et al., 2022; Moselhy et al., 2018). The PPI enrichment analysis demonstrated that the proteins exhibit a high degree of interconnectivity, with interactions occurring 2.4 times more than expected and a very low p-value, indicating their involvement in shared biological pathways. The phytocompounds were found to be linked to human gene targets INSR, PI3K, AKT1, PPARG, PTPN1, and DPP4 (Bao et al., 2020), as well as EGFR, which have therapeutic implications in hyperglycaemia and hyperlipidaemia (Banerjee et al., 2019).

The network pharmacology analysis identified key pathways, including the insulin signalling pathway, insulin resistance pathway, Type 2 Diabetes Mellitus pathway, PI3K-AKT signalling pathway, regulation of lipolysis in adipocytes, and PPAR signalling pathway. Prior research indicates that these pathways are crucial at the cellular level, as they initiate an insulin response, facilitate glucose uptake, and enhance lipogenesis (Banerjee et al., 2023). The insulin signalling pathway and the PI3K-AKT signalling pathway are crucial in regulating glucose and lipid homeostasis, making them essential for the management of diabetes and

obesity. The pathway exhibited enrichment in INSR, PI3K, AKT, and GSK3B. INSR activates the PI3K-AKT pathway, facilitating glucose uptake through the translocation of GLUT4, inhibiting gluconeogenesis via the suppression of FOXO, and enhancing glycogen synthesis by inhibiting GSK3B (Wen et al., 2022).

FABP4 regulates lipid concentrations within blood vessels. The Regulation of Lipolysis in Adipocytes pathway was identified as decreasing lipolysis through the downregulation of FABP4 (Floresta et al., 2022). The PPAR signalling pathway, enriched with PPARA, facilitates cholesterol metabolism through LXRα in the liver and skeletal muscle, leading to an improved lipid profile and a reduction in steatosis (Moller and Berger, 2003). TNFα, a significant mediator in adipose tissues, plays a role in the development of insulin resistance and impedes glucose uptake in various tissues. Inhibiting TNFα may improve the progression of NIDDM (Akash et al., 2018).

The hydroalcoholic extract of *A. hookeri* exhibited significant inhibition potential against α-glucosidase, α-amylase, and pancreatic lipase enzymes, in accordance with previous studies (Deka et al., 2021). All three enzymes exhibited mixed-type inhibition by HEAH, as both the apparent V_{max} and K_m values altered with increasing inhibitor concentration. The alterations in V_{max} and K_m values indicate that HEAH does not just compete with the substrate for the active site. Instead, it may interact with both the free enzyme and the enzyme–substrate complex. The Pearson correlation analysis revealed a positive correlation between the antioxidant potential and the phenolic and flavonoid content of *A. hookeri*, suggesting a multi-target pharmacological mechanism that may involve reducing ROS and promoting enzyme inhibition (Singha et al., 2024).

3.5. Publications and conference presentations

3.5.1. *Paper communicated*

- Das Gupta, B., Gayen, S., Chowdhury, S., Chatterjee, T., Kar, A., Duangyod, T., Charoensup, R., Haldar, P. K., & Mukherjee, P. K. Integrative metabolomics and network pharmacology - Exploring the

antidiabetic and antiobesity potential of *Allium hookeri* Thwaites in C57BL/6J mice. Phytomedicine. Elsevier. [Communicated].

3.5.2. *Paper presented*

- Das Gupta, B., Gayen, S., Haldar, P. K., Sharma, N., Mukherjee, P. K., & Kar, A. Metabolomics integrated network pharmacology analysis for combination synergy-based approach for exploring traditionally used medicinal plants of NER for the management of diabetes and obesity. In the 11th Convention of the Society for Ethnopharmacology & International Conference (SECON 2024) at BRIC-Institute of Bioresources and Sustainable Development, Gangtok, Sikkim, India, during November 15-16, 2024.
- Das Gupta, B., Kar, A., Haldar, P. K., Sharma, N., & Mukherjee, P. K. UHPLC-QTOF-MS/MS analysis coupled with validated network pharmacology approach to reveal synergy-based mechanism of action of medicinal food plants from NER in the treatment of diabetes and related disorders. In P. K. Haldar (Ed.), Proceedings of the International Conference on Indian Medicinal Plants in Drug Discovery: Tradition, Science & Innovation (ISBN: 978-93-48215-95-6). School of Natural Product Studies, Jadavpur University, Kolkata, India, during January 21–22, 2025.

Chapter 4

Benincasa hispida (Thunb.) Cogn. – Metabolomics integrated network pharmacology and *in vitro* evaluation

4.1. *Benincasa hispida* (Thunb.) Cogn. – A profile

4.2. Metabolite profiling by UHPLC-QTOF-MS analysis

4.3. Results

4.4. Discussion

4.5. Publications and conference presentations

4.1. *Benincasa hispida* (Thunb.) Cogn. – A profile

Scientific classification

Kingdom: Plantae

Division: Pteridobiotina

Class: Angiosperms

Order: Cucurbitales

Family: Cucurbitaceae

Genus: *Benincasa*

Species: *Benincasa hispida* Cogn.

Vernacular names

English: Wax gourd

Hindi: Petha

Sanskrit: Kooshmaanda

Mizo: Manipat

Manipuri: Torobot

Assamese: Komora

Goa: Kuvalo

4.1.1. *Plant description*

Benincasa hispida (Thunb.) Cogn., a member of the Cucurbitaceae family, is found in Java and Japan and is extensively cultivated in India (Singh et al., 2024). It is grown throughout the plains and hill areas of India, up to an altitude of 1200 m (Haldar et al., 2024). The climber possesses hairy stems, petioles, leaves, petals, immature fruits, and yellow flowers. Typically, the female flowers are capitate, whereas the male flowers may occur solitarily or in slender pedunculate racemes. The fruits function as a vegetable and can be ingested fresh or cooked, as well as in pickles, stews, preserves, and several other dishes (Singh et al., 2024). The fruits and leaves of *B. hispida* have been demonstrated in Figure 4.1.

A

B

Figure 4.1. A: Fruits and leaves of *Benincasa hispida* (Thunb.) Cogn. B: Herbarium

4.1.2. *Traditional uses of B. hispida*

The bark of *B. hispida* has been found to have a strong diuretic effect, and the fruits are used in traditional Chinese medicine to treat cough and urinary diseases (Islam et al., 2021). Ayurveda reports that dried fruits can be used to treat urinary retention (Mutraghata), diabetes and related metabolic disorders (Prameha), dysuria or urinary tract infection (Mutrakṛcchra), urolithiasis (Aśmarī), polydipsia (Tṛṣṇā), mental disorders (Mānasa Vikāra), constipation (Malabandha) (Government of India, 2004). The fruits have also been utilised for the treatment of jaundice, dyspepsia, and diabetes (Singh et al., 2024).

4.1.3. *Phytochemical profile of B. hispida*

B. hispida is enriched with bioactive phytoconstituents, alkaloids, glycosides, tannins, flavonoids and triterpenes. A study indicated the existence of D-(+)-malic acid **(71)**, atractylenolide Iii **(72)**, 10-hydroxydecanoate **(73)**, sebacic acid **(74)**, (E)-9,12,13-trihydroxyoctadec-10-enoic acid **(75)**, (9E,11E)-13-hydroxyoctadeca-9,11-dienoic acid **(76)** and 16-hydroxypalmitic acid **(77)** in the hydroalcoholic extract of *B. hispida* fruit pulp (Patil et al., 2025). The fruits were identified to contain lupeol **(43)**, β-sitosterol **(26)**, cucurbitin **(78)**, rhamnose **(79)**, mannitol **(80)**, triacontanol **(81)**, and trigonelline **(82)**, while the root was found to contain bryonolic acid **(83)** (Singh et al., 2024). A separate study revealed the phytochemical composition of the fermented fruit pulp extract of *B. hispida*, identifying 2-furoic acid **(84)**, 2,3-dihydroxybenzoic acid **(85)**, and rubinaphthin A **(86)**, which were absent in detectable quantities in the hydroalcoholic extract (Choi et al., 2024).

O OH OH HO O

H_3C CH_2 O O OH CH_3

HO O OH

(71) **(72)** **(73)**

(74) (75) (76)

(77) (78) (79)

(80) (81) (82)

(83) (84) (85)

(86)

4.1.4. *Pharmacological activities of B. hispida*

The anti-inflammatory efficacy of *B. hispida* seeds was evaluated in rats exhibiting carrageenan-induced paw oedema. The methanolic extract of the seeds, administered at a dosage of 300 mg/kg body weight, demonstrated an inhibitory effect on oedema comparable to that of diclofenac (Gill et al., 2010). The antidiabetic efficacy of *B. hispida* seeds was investigated in alloxan-induced diabetic rats. The methanolic seed extract demonstrated a dose-dependent decrease in serum glucose levels, as well as reductions in cholesterol and triglyceride levels (Patil et al., 2011). The neuroprotective effect of *B. hispida* fruits was studied *in vivo* in rats with aluminium chloride-induced Alzheimer's disease. The ethanolic extract improved memory and spatial learning in a dose-dependent manner, as revealed by behavioural tests. The extract reduced Amyloid-β42 levels and increased antioxidant enzymes, such as SOD and GSH, thereby reducing oxidative stress (Rapaka et al., 2021).

The hydroalcoholic extract of *B. hispida* fruit pulp was evaluated for its anti-inflammatory characteristics and its efficacy in alleviating irritable bowel syndrome in Wistar rats produced by monosodium glutamate and microplastics. The extract demonstrated a reduction in body weight and serum lipid levels. Normalised SGPT, SGOT, triglyceride, and cholesterol levels showed a hepatoprotective effect. The regulation of mTOR/PI3K-Akt signalling was explored as the mechanism of action (Patil et al., 2025). The anti-ulcer efficacy of *B. hispida* was assessed *in vivo* by providing fresh fruit juice and alcoholic extract to rats subjected to ulcer induced by aspirin restriction, indomethacin + histamine and serotonin models. At a dosage of 2 mL per animal, the juice conferred ulcer

protection across all models, whereas the alcoholic extract demonstrated a dose-dependent decrease in the ulcer index in the aspirin + restraint and swimming stress models (Grover et al., 2001).

4.2. Metabolite profiling by UHPLC-QTOF-MS analysis

UHPLC–QTOF–MS/MS analysis with Agilent 1260 Infinity II LC System and Agilent 6530 LC/Q-TOF (Agilent, Santa Clara, CA, United States) was executed to identify metabolites in HEBH. The chromatographic method and conditions were optimised as per the previously developed method, with slight modifications to the mobile phase (Gupta et al., 2023). The mobile phase consisted of acetonitrile (A) & water (B) both containing 0.1% formic acid in gradient elution of 5% A (0–1 min), 17% A (1–6 min), 100% A (6–35 min), and 5% A (from 35–45 min), operating at 0.5 mL/min. A 100 µg/mL sample was injected, and phytocompounds were identified based on mass, score (0–100), and theoretical formula. The column temperature was set at 25°C with a 5 µL injection volume. Data interpretation was done using MassHunter B.06.01 software (Agilent Technologies, CA, USA). The compound identification was achieved by uploading a custom library; the phenolic compounds were obtained from the Phenol-Explorer database (Neveu et al., 2010).

4.3. Results

4.3.1. *Metabolite profile of B. hispida*

The percentage yield of HEBH was found to be 21.2% w/w. The UHPLC–QTOF–MS revealed a variety of compounds, which are detailed in Table 4.1. The positive ion chromatogram is depicted in Figure 4.2., displaying the total ion chromatogram. The analysis identified 17 distinct compounds; the details of these compounds, including their chemical formula, theoretical mass (g/mol), observed mass-to-charge ratio (m/z), retention time (RT), mass error (Dif (Tgt, ppm)), and compound class, are presented in Table 4.1. The results confirmed the existence of several compounds known for their therapeutic properties in addressing metabolic disorders, such as diabetes and obesity.

Table 4.1. Compounds identified in the hydroalcoholic extract of *B. hispida* fruits by UHPLC-QTOF-MS.

Sl. No.	RT	Name	Formula	Mass (g/mol)	m/z	Error (ppm)	Class of compounds
1.	10.903	Catechin	$C_{15}H_{14}O_6$	290.27	290.0790	0.00	Flavonoid
2.	15.281	Friedoolean-8-en-3-one	$C_{30}H_{48}O$	424.7	424.3702	-0.70	Friedooleanane-type Triterpenes
3.	16.4	Cucurbitacin E	$C_{32}H_{44}O_8$	556.7	556.3030	-1.07	Triterpenes
4.	17.220	Naringenin	$C_{15}H_{12}O_5$	272.25	272.0687	1.10	Flavonoid
5.	17.673	Butanoic acid, 2,3-dihydroxypropyl ester	$C_{21}H_{40}O_4$	162.18	356.2929	0.84	Glycerolipids
6.	19.3	β-sitosterol	$C_{29}H_{50}O$	414.7	414.3860	0.84	Stigmastanes and derivatives
7.	22.844	Linolenic acid	$C_{18}H_{30}O_2$	278.43	278.2250	1.79	Lineolic acids and derivatives
8.	23.174	Linoleic acid	$C_{18}H_{32}O_2$	280.45	280.2402	0.00	Fatty acyls
9.	25.002	Stigmasterol	$C_{29}H_{48}O$	412.7	412.3705	0.00	Stigmastanes and derivatives
10.	25.222	Palmitic acid	$C_{16}H_{32}O_2$	256.42	256.2401	-0.39	Long-chain fatty acids
11.	25.655	Stearic acid	$C_{18}H_{36}O_2$	284.5	284.2717	0.70	Long-chain fatty acids
12.	26.792	Oleic acid	$C_{18}H_{34}O_2$	282.2567	282.2555	-1.06	Fatty acyls
13.	26.803	9,12-Octadecadenoic acid	C18H32O2	280.4	280.2404	0.71	Fatty acyls

14.	27.181	Esculetin	$C_9H_6O_4$	178.14	178.0265	-0.56	6,7-Dihydroxycoumarins
15.	28.946	Eicosenoic acid	$C_{20}H_{38}O_2$	310.5	310.2875	1.28	Monounsaturated long-chain fatty acid
16.	29.292	Kaemperol	$C_{27}H_{30}O_{15}$	286.24	594.1589	0.84	Tetrahydroxyflavone
17.	29.723	Kaempferol 3-O-L-rhamnopyranoside	$C_{21}H_{19}O_{10}$	432.11	431.0974	-0.92	Flavonoid glycoside

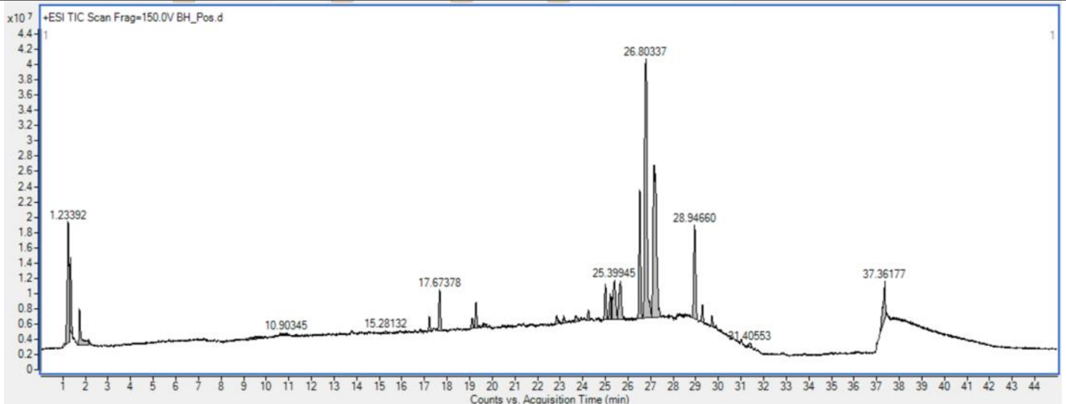


Figure 4.2. Total ion chromatogram of HEBH in the positive ionisation mode

The class of compounds identified includes Flavonoid – catechin **(23)**, naringenin **(11)**; Friedooleanane-type triterpenes – friedoolean-8-en-3-one **(135)**; Triterpenes – cucurbitacin E **(38)**; Glycerolipids – butanoic acid, 2,3-dihydroxypropyl ester **(136)**; Stigmastanes and derivatives – β-sitosterol **(26)**, stigmasterol **(28)**; Lineolic acids and derivatives – linolenic acid **(24)**; Fatty acyls – linoleic acid **(25)**, oleic acid **(124)**, 9,12-octadecadenoic acid **(137)**; Long-chain fatty acids – palmitic acid **(138)**, stearic acid **(139)**; 6,7-Dihydroxycoumarins – esculetin **(18)**; Monounsaturated long-chain fatty acid – eicosenoic acid **(140)**; Tetrahydroxyflavone – kaempferol **(3)**; Flavonoid glycoside – kaempferol 3-O-L-rhamnopyranoside **(141)**.

(135) (136) (137)

(138) (139) (140)

(141)

4.3.2. *Network analysis*

4.3.2.1. *Identified targets and disease association network*

The compounds (BA) were screened in BindingDB, and the associated genes (TAR) were identified from the UniProt database. The 16 compounds showing target activity were used to create a bioactive–target (BA–TAR) network, as shown in Figure 4.3. The network topology statistics revealed 145 nodes interconnected through 269 edges, indicating a dense connectivity between the phytocompounds and their targets. The phytocompound Apigenin exhibited the

highest connectivity by targeting 54 proteins, and aldose reductase (ESR2) was found to be the most targeted protein, targeted by 7 compounds.

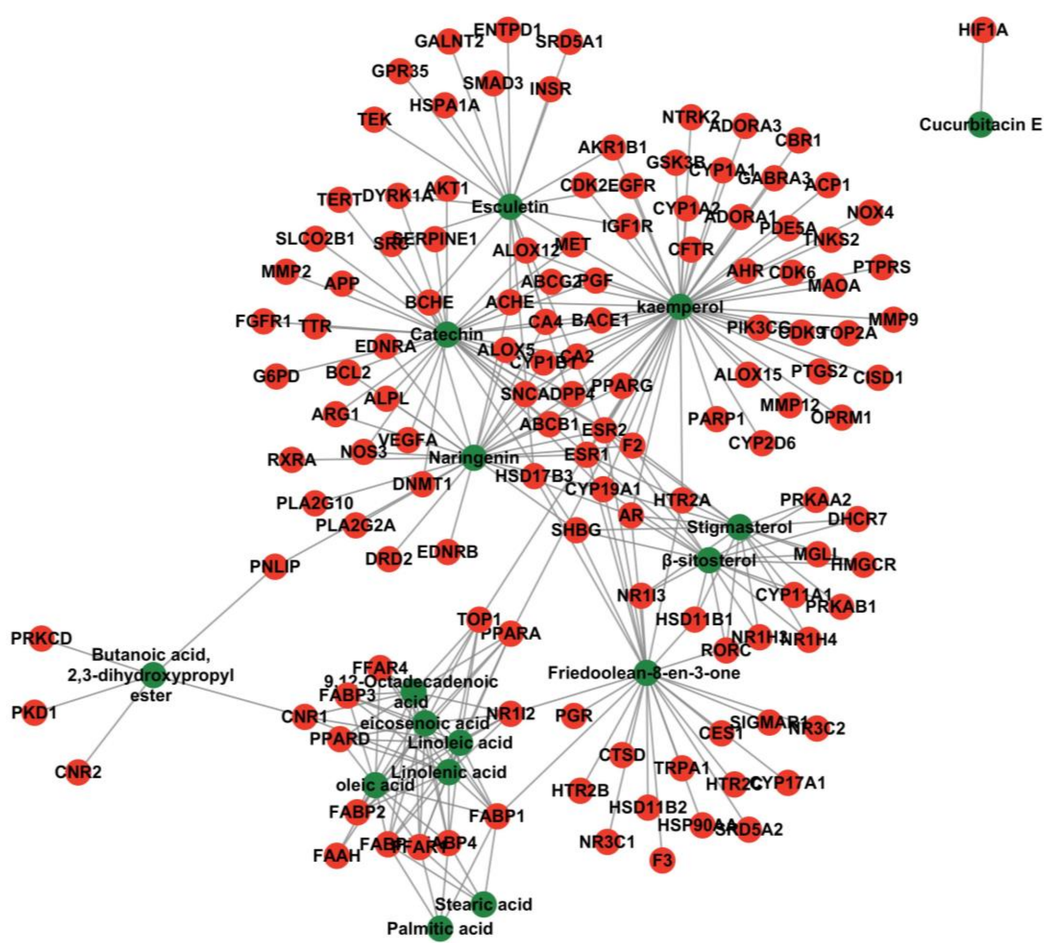
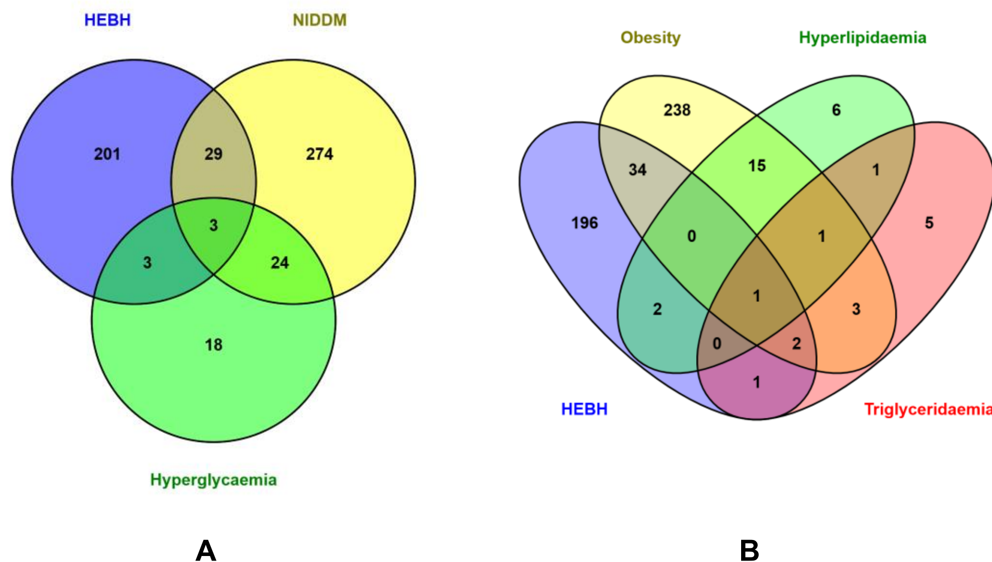


Figure 4.3. The bioactive–target (BA–TAR) network
[Phytocompounds are shown in green circles, and corresponding protein targets are in red circles]

The disease association was performed using the DisGeNET database, and the common targets for Non-Insulin-Dependent Diabetes Mellitus (NIDDM), Obesity, Hyperlipidaemia, Hypertriglyceridaemia, and Hyperglycaemia are shown in the Venn diagram (Figure 4.4).



A **B**

Figure 4.4. Venn diagrams showing the common targets for (A) NIDDM and Hyperglycaemia; (B) Obesity, Hyperlipidaemia and Hypertriglyceridaemia between identified protein targets of HEBH and disease targets in the DisGeNET database.

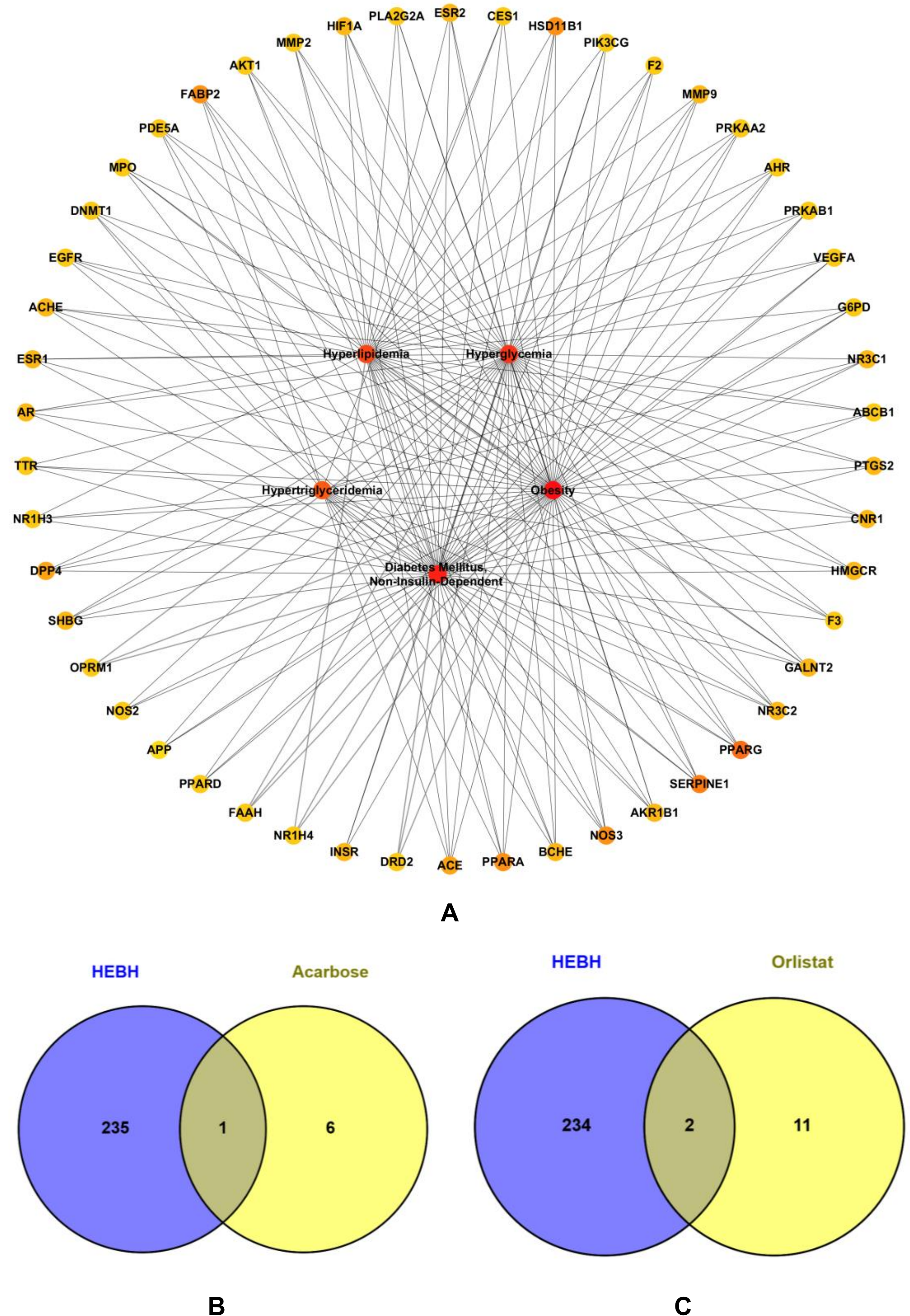
There were 29 common targets for NIDDM, followed by Obesity (34), Hyperglycaemia (3), Triglyceridaemia (1) and Hyperlipidaemia (2) between targets of HEBH and disease targets in the DisGeNET database. The list of common targets is listed in Table 4.2.

Table 4.2. Shared targets between the identified phytocompounds of *B. hispida* and selected metabolic disorders and reference standards.

Common elements in "Targets" and "selected disorders":		
Disorder	**Number of Common Genes from the obtained targets**	**Common targets**
Hyperlipidaemia	2	ABCB1, NOS3
Triglyceridaemia	1	MET

NIDDM	29	ABCB1, ACE, ACP1, AKR1B1, AKT1, BCHE, BCL2, CNR1, CYP1A1, CYP1A2, DPP4, DRD2, ECE1, EDNRA EDNRB, EGFR, ESR1, FABP3, FFAR1, HIF1A, HMGCR NOS2, PIK3CG, PPARA, PPARD, PPARG, PRKAA2, SHBG, SRC
Hyperglycaemia	3	CNR2, PTGS2, TERT
Obesity	34	ACHE, ACP1, AHR, AKR1C3, AR, CES1, CNR1, CYP1B1, DRD2, ESR1, F2, FAAH, FABP2, GRIA4, HSD11B1, HSD11B2, HTR2A, HTR2C, IDO1, MMP9, NR1H3, NR1I2, NR1I3, NR3C1, NTRK2, OPRM1, PARP1, PGR, PPARD, PPARG, PRKCH, PTGS2, SERPINE1, SLC6A3
Common elements in “Targets” and “selected reference standards”:		
Atorvastatin	1	HMGCR
Metformin	1	PRKAB1
Orlistat	2	FAAH, PNLIP
Acarbose	1	GAA

The common targets for acarbose, Orlistat, Metformin, and Atorvastatin with HEBH were also screened and given in Figure 4.5. While Atorvastatin shared HMGCR as a common target with HEBH, Metformin shared PRKAB1 as the common target with HEBH. Similarly, acarbose shared GAA, and Orlistat shared FAAH and PNLIP as common targets with HEBH. The gene-disease association (TAR–DIS) networks were created using Cytoscape. The top 50 hub proteins acting on NIDDM, Obesity, Hyperlipidaemia, Hypertriglyceridaemia, and Hyperglycaemia, calculated by degree and shortest path length using cytoHubba, are shown in Figure 4.5.


HIF1A
PLA2G2A
ESR2
CES1
HSD11B1
PIK3CG
F2
MMP9
PRKAA2
AHR
PRKAB1
VEGFA
G6PD
NR3C1
ABCB1
PTGS2
CNR1
HMGCR
F3
GALNT2
NR3C2
PPARG
SERPINE1
AKR1B1
NOS3
BCHE
PPARA
ACE
DRD2
INSR
NR1H4
FAAH
PPARD
APP
NOS2
OPRM1
SHBG
DPP4
NR1H3
TTR
AR
ESR1
ACHE
EGFR
DNMT1
MPO
PDE5A
FABP2
AKT1
MMP2
Hyperlipidemia
Hyperglycemia
Hypertriglyceridemia
Obesity
Diabetes Mellitus, Non-Insulin-Dependent
A
HEBH
Acarbose
235
1
6
B
HEBH
Orlistat
234
2
11
C

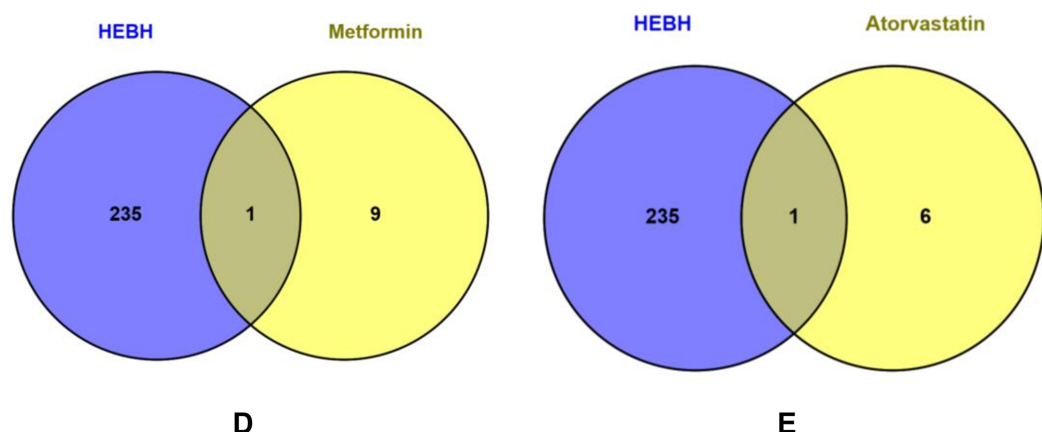


Figure 4.5. The common targets for (A) The top 50 hub proteins acting on NIDDM, Obesity, Hyperlipidaemia, Hypertriglyceridaemia and Hyperglycaemia (B) acarbose, (C) orlistat, (D) metformin and (E) atorvastatin with HEBH.

4.3.2.2. *Analysis of protein-protein interactions and associated pathways*

The target genes were visualised in STRING 12.0, and the PPI enrichment analysis showed that the target proteins have significantly more interactions, indicating that they are at least partially biologically connected as a group. The PPI enrichment analysis exhibited 1750 edges, which is significantly more than the expected number of 600. The average node degree was found to be 19.7, suggesting each protein interacts with 19 others (Figure 4.6.).

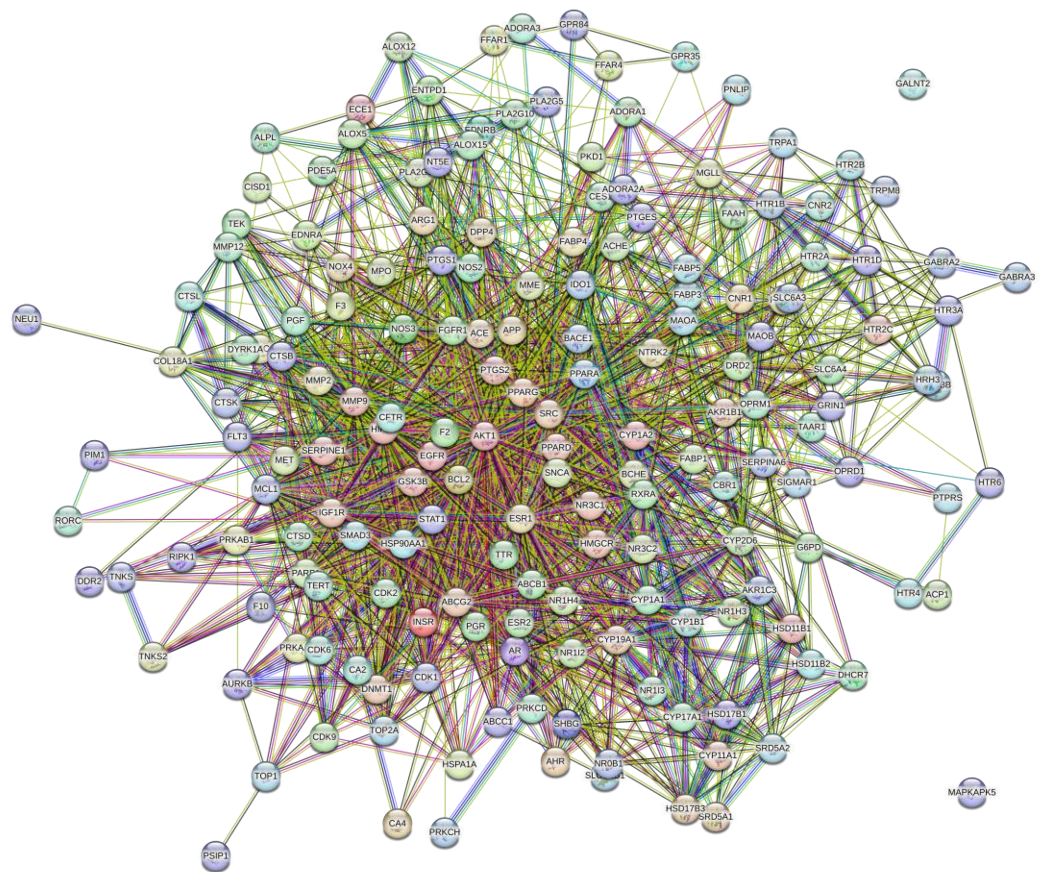


Figure 4.6. Protein-protein interaction network

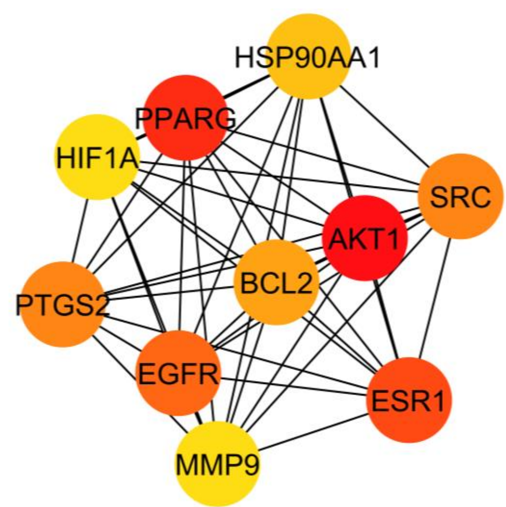


Figure 4.7. PPI enrichment network with the top 10 hub proteins

The PPI enrichment network was filtered by degree and shortest path length using the cytoHubba tool in Cytoscape to calculate the top 10 hub proteins (Figure 4.7.).

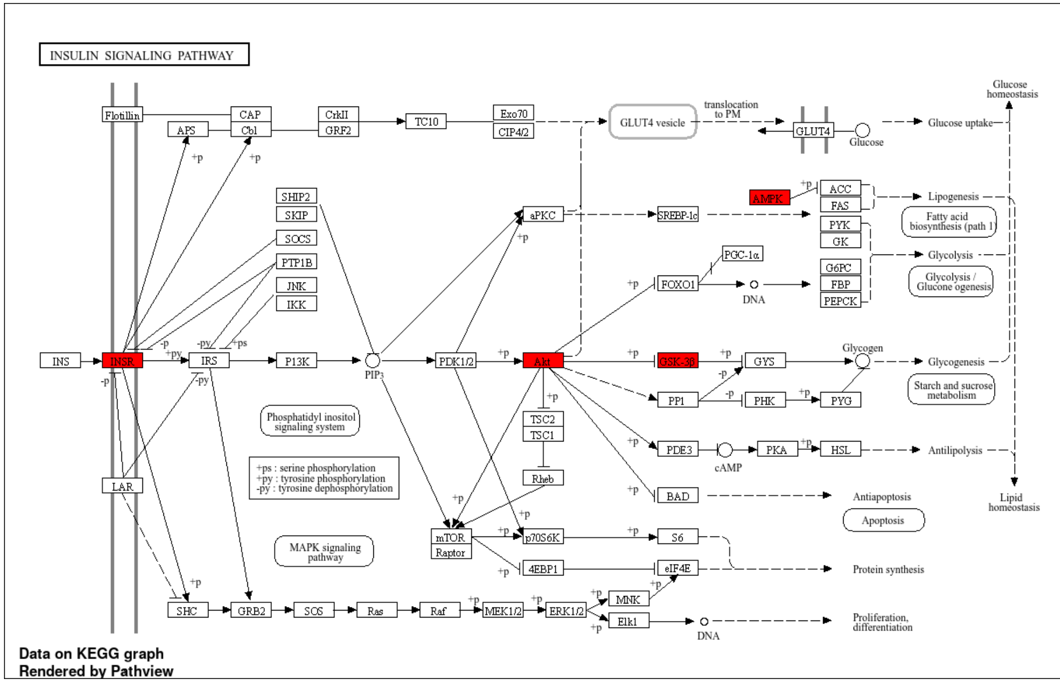
The pathway enrichment analysis by KEGG demonstrated that hsa04910: Insulin signalling pathway, hsa04930: Type II diabetes mellitus, hsa04931: Insulin resistance, and hsa04933: AGE-RAGE signalling pathway in diabetic complications are involved in NIDDM and Hyperglycaemia. In the case of Obesity, Hyperlipidaemia, and Hypertriglyceridaemia, the pathways involved were hsa03320: PPAR signalling pathway, hsa05417: Lipid and atherosclerosis, hsa04152: AMPK signalling pathway, hsa04920: Adipocytokine signalling pathway, and hsa01100: Metabolic pathways. Other important pathways included hsa04151: PI3K-Akt signalling pathway, hsa04310: Wnt signalling pathway, and hsa04972: Pancreatic secretion (Table 4.3.). The hub gene-enriched pathways are shown in Figure 4.8.

Table 4.3. KEGG enrichment pathways involved in NIDDM, Obesity, Hyperlipidaemia, Hypertriglyceridaemia and Hyperglycaemia.

Enrichment FDR	Pathway	Genes
3.00E-11	Path:hsa04151 PI3K-Akt signalling pathway	CDK2 CDK6 EGFR AKT1 FGFR1 FLT3 GSK3B HSP90AA1 IGF1R INSR MCL1 MET NOS3 NTRK2 PGF PIK3CG PRKAA2 BCL2 RXRA TEK VEGFA
9.12E-11	Path:hsa01100 Metabolic pathways	MGLL CYP1A1 CYP1A2 CYP17A1 CYP19A1 DHCR7 DNMT1 AKR1B1 ALOX12 ALOX15 ALPL G6PD GALNT2 HMGCR HSD11B1 HSD11B2 HSD17B1 HSD17B3 IDO1 ARG1 MAOA MAOB NOS2 NOS3 NT5E ACP1 PIK3CG PLA2G2A PLA2G5 PNLIP PTGS1 PTGS2 SRD5A1 SRD5A2 CA2 CA4

		AKR1C3 PDE5A CBR1 ENTPD1 PTGES
5.38E-10	Path:hsa04933 AGE-RAGE signalling pathway in diabetic complications	AKT1 F3 SMAD3 MMP2 NOS3 NOX4 SERPINE1 PIM1 PRKCD BCL2 STAT1 VEGFA
5.95E-09	Path:hsa03320 PPAR signalling pathway	NR1H3 FABP4 FABP1 FABP2 FABP3 FABP5 PPARA PPARD PPARG RXRA
9.56E-08	Path:hsa05415 Diabetic cardiomyopathy	PARP1 CTSD ACE AKT1 G6PD GSK3B INSR SMAD3 MMP2 MMP9 NOS3 PPARA PRKCD
1.58E-06	Path:hsa04931 Insulin resistance	NR1H3 AKT1 GSK3B INSR NOS3 PPARA PRKAA2 PRKAB1 PRKCD
2.71E-06	Path:hsa04923 regulation of lipolysis in adipocytes	MGLL ADORA1 AKT1 FABP4 INSR PTGS1 PTGS2
2.29E-05	Path:hsa04932 Non-alcoholic fatty liver disease	NR1H3 AKT1 GSK3B INSR PPARA PPARG PRKAA2 PRKAB1 RXRA
3.01E-05	Path:hsa04152 AMPK signalling pathway	CFTR AKT1 HMGCR IGF1R INSR PPARG PRKAA2 PRKAB1
4.34E-05	Path:hsa05417 Lipid and atherosclerosis	CYP1A1 AKT1 GSK3B HSP90AA1 MMP9 NOS3 PPARG BCL2 RXRA SRC
5.05E-05	Path:hsa04068 FoxO signalling pathway	CDK2 EGFR AKT1 IGF1R INSR SMAD3 PRKAA2 PRKAB1
0.000100732	Path:hsa04975 Fat digestion and	FABP1 FABP2 PLA2G2A PLA2G5 PNLIP

	absorption	
0.000267705	Path:hsa04976 Bile secretion	CFTR HMGCR ABCB1 RXRA CA2 ABCG2
0.000703987	Path:hsa04920 Adipocytokine signalling pathway	AKT1 PPARA PRKAA2 PRKAB1 RXRA
0.003465095	Path:hsa04972 Pancreatic secretion	CFTR PLA2G2A PLA2G5 PNLIP CA2
0.010168869	Path:hsa04910 Insulin signalling pathway	AKT1 GSK3B INSR PRKAA2 PRKAB1
0.015298167	Path:hsa04150 mTOR signalling pathway	AKT1 GSK3B IGF1R INSR PRKAA2
0.018789159	Path:hsa04922 Glucagon signalling pathway	AKT1 PPARA PRKAA2 PRKAB1
0.019141979	Path:hsa04714 Thermogenesis	MGLL CNR1 FGFR1 PPARG PRKAA2 PRKAB1
0.023485497	Path:hsa00561 Glycerolipid metabolism	MGLL AKR1B1 PNLIP
0.077894868	Path:hsa04930 Type II diabetes mellitus	INSR PRKCD
0.194008906	Path:hsa04310 Wnt signalling pathway	GSK3B SMAD3 PPARD



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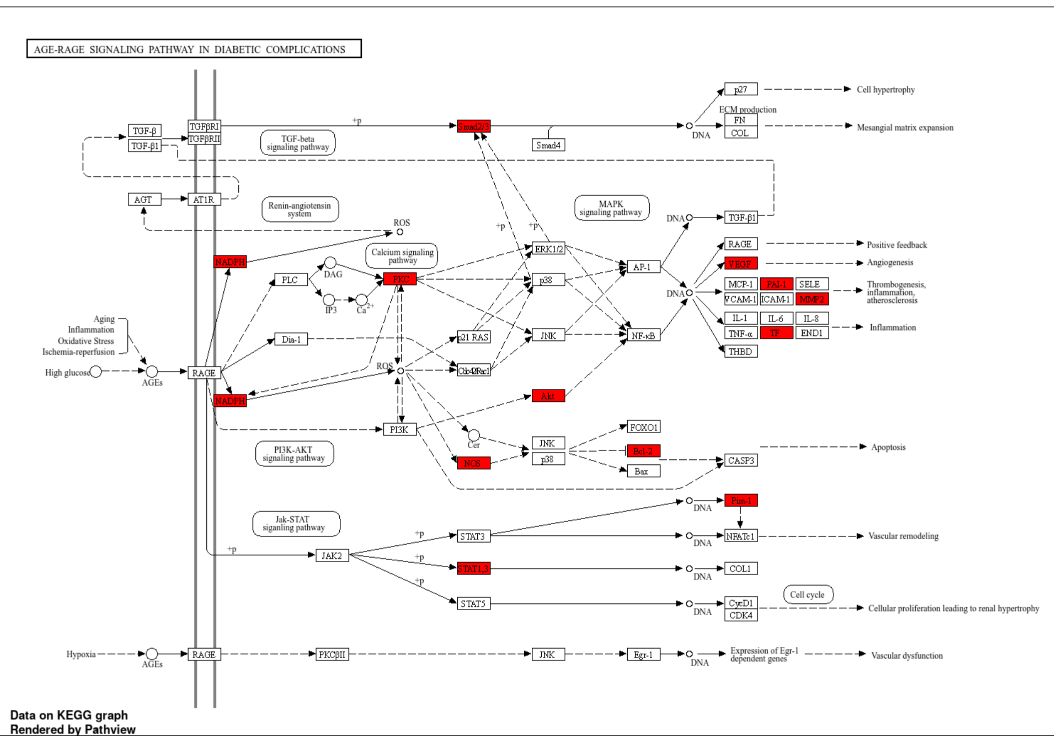

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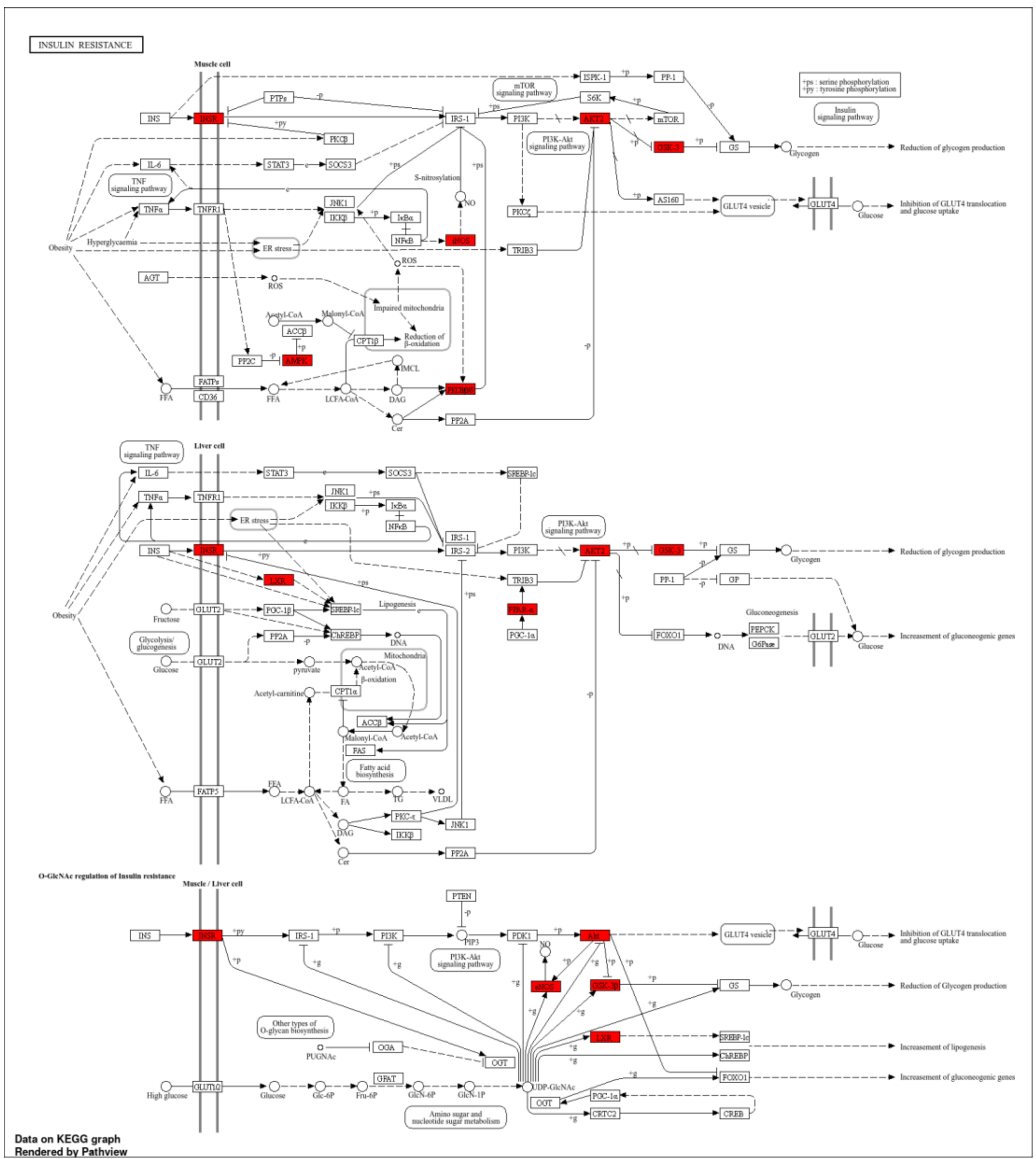

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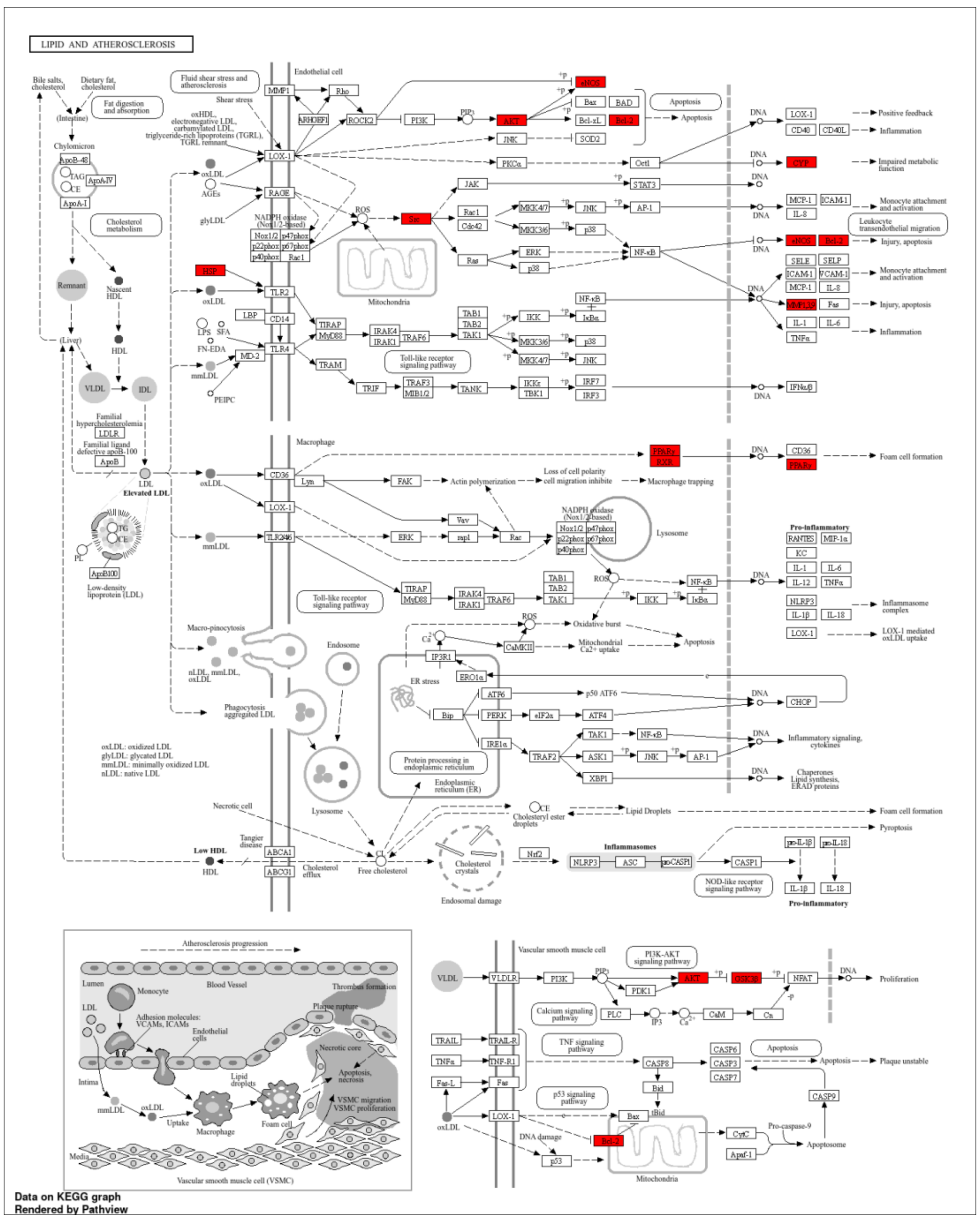

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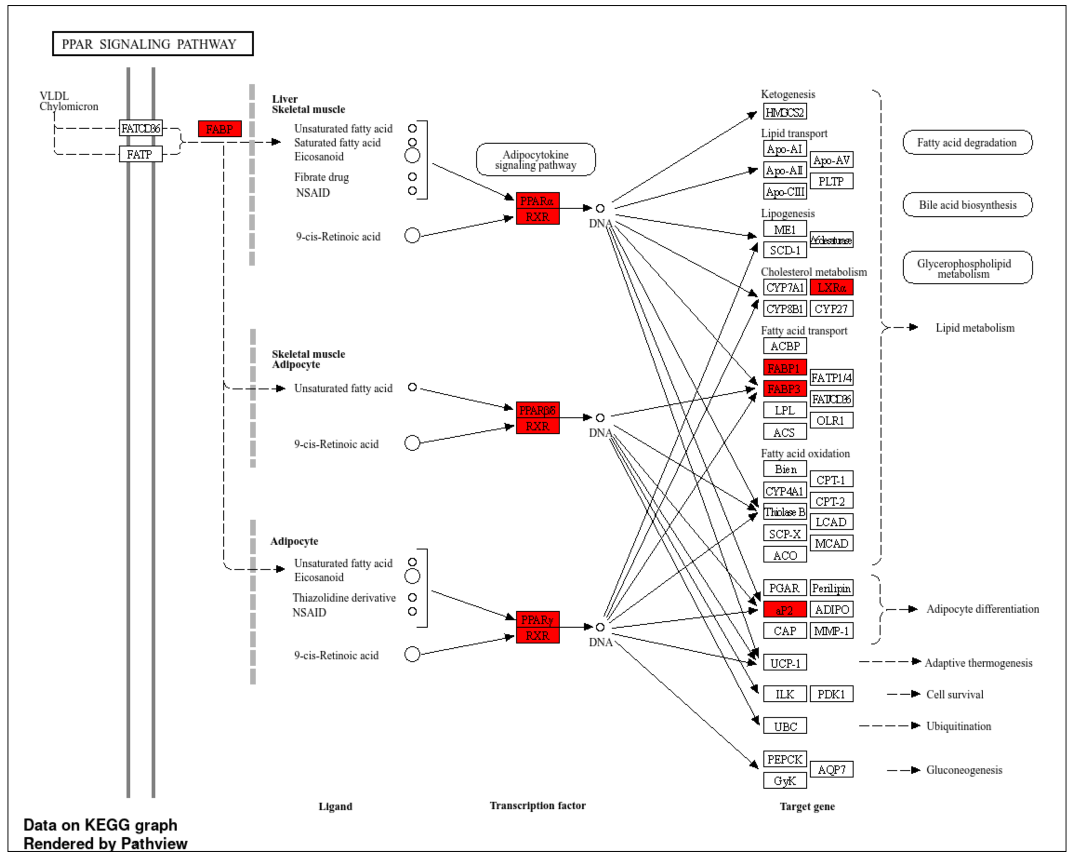


E

Figure 4.8. The hub gene enriched pathways A: Insulin signalling pathway; B: AGE-RAGE signalling pathway in diabetic complications; C: Insulin resistance pathway; D: Lipid and atherosclerosis pathway; E: PPAR signalling pathway

The Gene Ontology (GO) analysis, performed using ShinyGO 0.82, explored the biological processes in which the target genes were involved. At FDR cutoff of 0.05 the enriched biological processes included Lipid metabolic process (GO:0006629), cellular lipid metabolic process (GO:0044255), lipid biosynthetic process (GO:0008610), fatty acid metabolic process (GO:0006631), lipid transport (GO:0006869), response to insulin (GO:0032868), cellular response to insulin stimulus (GO:0032869), glucose homeostasis (GO:0042593), glucose metabolic process (GO:0006006), regulation of lipid transport (GO:0032368) and

regulation of glucose transmembrane transport (GO:0010827) (Table 4.4.). The target gene-enriched biological processes are shown in Figure 4.9.

Table 4.4. GO Biological process pathways involved in NIDDM, Obesity, Hyperlipidaemia, Hypertriglyceridaemia and Hyperglycaemia.

Enrichment FDR	Pathway	Genes
8.74E-32	GO:0006629 lipid metabolic process	ALOX5 PLA2G10 PTGS2 PTGS1 CYP2D6 PIK3CG ALOX12 PPARD HMGCR FAAH HSD11B1 PLA2G5 HSD17B3 PPARG CYP11A1 SRD5A1 PTGES CYP17A1 ALOX15 DHCR7 PNLIP PPARA PLA2G2A AKR1C3 CES1 NEU1 SRD5A2 FABP3 CYP1A1 NR1H4 MGLL HTR2A HSD17B1 HTR2B CYP19A1 CYP1B1 CYP1A2 AKT1 HTR2C CBR1 PRKCD NR0B1 F2 CFTR NR1H3 AKR1B1 ESR1 PRKAB1 NR3C1 TTR CNR1 EDNRB IGF1R APP SNCA FABP2 PRKAA2 ADORA1 FABP1 FABP5 SERPINA6 HSD11B2 SRC TEK G6PD RORC FLT3 NR1I2
1.11E-26	GO:0009725 response to hormone	NOS2 PGR GSK3B ESR1 PPARD NR3C1 STAT1 PPARG ESR2 CYP11A1 NR3C2 NOS3 AR PPARA SRC NR1H3 CA2 PARP1 PRKCD NR0B1 INSR RXRA AKR1C3 NR1H4 MAOB PTGS2 HSP90AA1 MMP2 BCHE ARG1 PGF FLT3 HTR1B EDNRB CYP1B1 IGF1R AKT1 SRD5A1 EDNRA ACE PRKAA2 ALPL DDR2 BCL2 HSD11B2 MME SRD5A2 CTSL CTSB HSPA1A
6.49E-26	GO:0010817	ECE1 CYP11A1 CYP17A1 DHCR7 FFAR4

	regulation of hormone levels	AKR1C3 ACE NR1H4 CYP2D6 HIF1A HSD17B1 CYP19A1 CYP1B1 CYP1A1 CYP1A2 CTSK MME SRD5A2 CFTR ALOX5 AKR1B1 ESR1 PPARD NR3C1 TTR CNR1 FFAR1 PPARG EDNRB IGF1R SRD5A1 HTR2C DRD2 ADORA1 CTSB PNLIP NOS2 CTSL BCHE HSD17B3 DPP4
1.81E-24	GO:0050727 regulation of inflammatory response	NR1H4 NR1H3 PPARD PPARG PPARA PRKCD ALOX5 SNCA ALOX15 PLA2G10 PTGS2 ESR1 ABCC1 PIK3CG CNR1 ADORA2A IDO1 NT5E EDNRB RIPK1 CYP19A1 PTGES ACE ADORA1 SMAD3 FABP4 FFAR4 CNR2 SERPINE1 APP MGLL MMP9 TEK PLA2G2A SRC
1.10E-23	GO:0042592 homeostatic process	HTR2A CA2 HTR2B IGF1R HTR2C DRD2 INSR GPR35 SRC HIF1A NR1H4 NR1H3 TRPA1 OPRM1 FFAR1 SNCA EDNRA ALPL SMAD3 BCL2 GRIN1 F2 FFAR4 CFTR PKD1 ALOX5 PLA2G10 PTGS2 AKR1B1 NOX4 ACHE PIK3CG ALOX12 PRKAB1 PPARD CNR1 FABP3 ADORA2A HTR1B NT5E EDNRB APP AKT1 CTSK TRPM8 PTGES ACE PRKAA2 ADORA1 FABP5 NOS3 FABP4 CDK6 MET VEGFA STAT1 PPARG G6PD CES1 HSPA1A ESR1 FLT3 PLA2G2A MCL1 NOS2
7.01E-23	GO:0042445 hormone metabolic process	ECE1 CYP11A1 CYP17A1 DHCR7 AKR1C3 ACE CYP2D6 HIF1A HSD17B1 CYP19A1 CYP1B1 CYP1A1 CYP1A2 CTSK MME SRD5A2 AKR1B1 ESR1

		NR3C1 TTR EDNRB IGF1R SRD5A1 CTSB PNLIP CTSL BCHE HSD17B3 DPP4
1.07E-22	GO:0008610 lipid biosynthetic process	ALOX5 PTGS2 PTGS1 PIK3CG ALOX12 HMGCR HSD17B3 CYP11A1 SRD5A1 PTGES ALOX15 DHCR7 SRD5A2 FABP3 NR1H4 HTR2A HSD17B1 HTR2B AKT1 HTR2C PRKCD NR0B1 AKR1C3 CES1 CFTR PLA2G10 MGLL PRKAB1 PPARD NR3C1 CYP19A1 IGF1R CYP1A1 CYP17A1 PRKAA2 FABP5 NR1H3 G6PD PPARA AKR1B1 CYP2D6 CYP1A2 CBR1
4.02E-21	GO:0006979 response to oxidative stress	SNCA HIF1A MMP9 TRPA1 FABP1 PRKCD NOS3 AKR1C3 MPO ALOX5 PTGS2 MMP2 PTGS1 STAT1 ARG1 RIPK1 CYP1B1 APP MCL1 PARP1 SIGMAR1 EDNRA PRKAA2 DDR2 PSIP1 CDK1 BCL2 SRC MET AKT1 EGFR G6PD ABCC1 HSPA1A
6.59E-21	GO:0034599 cellular response to oxidative stress	SNCA HIF1A MMP9 TRPA1 FABP1 PRKCD NOS3 AKR1C3 MPO ALOX5 MMP2 ARG1 RIPK1 CYP1B1 MCL1 PARP1 SIGMAR1 EDNRA PRKAA2 DDR2 CDK1 BCL2 SRC MET AKT1 EGFR G6PD ABCC1 HSPA1A
8.07E-20	GO:0006631 fatty acid metabolic process	ALOX5 PTGS2 PTGS1 CYP2D6 ALOX12 PPARD FAAH PPARG PTGES ALOX15 PPARA AKR1C3 CYP1B1 CYP1A1 CES1 PLA2G10 MGLL AKR1B1 PRKAB1 CNR1 FABP3 PLA2G5 CYP1A2 SNCA FABP2 PRKAA2 FABP1 FABP5 NR1H3 AKT1 CBR1

2.31E-19	GO:0019216 regulation of Lipid metabolic process	PPARD PPARG PPARA FABP3 NR1H4 HTR2A HTR2B AKT1 HTR2C PRKCD NR0B1 F2 AKR1C3 PTGS2 PIK3CG NR3C1 CNR1 IGF1R SNCA ADORA1 FABP1 FABP5 DHCR7 SRC NR1H3 TEK CES1 RORC FLT3
2.53E-19	GO:0032870 cellular response to hormone stimulus	PGR GSK3B ESR1 PPARD NR3C1 PPARG ESR2 CYP11A1 NR3C2 AR PPARA SRC NR1H3 CA2 PARP1 PRKCD NR0B1 INSR RXRA AKR1C3 NR1H4 STAT1 ARG1 PGF FLT3 CYP1B1 IGF1R AKT1 SRD5A1 EDNRA ACE PRKAA2 DDR2 CTSL CTSB HSPA1A
3.69E-17	GO:0044255 cellular lipid metabolic process	ALOX5 PLA2G10 PTGS2 PTGS1 CYP2D6 PIK3CG ALOX12 PPARD HMGCR FAAH PLA2G5 PPARG PTGES ALOX15 PPARA PLA2G2A AKR1C3 NEU1 FABP3 NR1H4 MGLL HTR2A HTR2B CYP1B1 CYP1A1 CYP1A2 HTR2C PRKCD CES1 NR1H3 AKR1B1 PRKAB1 TTR CNR1 SNCA FABP2 SRD5A1 PRKAA2 FABP1 FABP5 PNLIP AKT1 CBR1
6.51E-17	GO:0045834 positive regulation of Lipid metabolic process	PPARD PPARG PPARA FABP3 NR1H4 HTR2A HTR2B AKT1 HTR2C F2 PTGS2 IGF1R ADORA1 FABP1 SRC NR1H3 TEK PRKCD CES1 FLT3
1.06E-16	GO:0006869 lipid transport	ABCB1 FABP3 PLA2G5 SLCO2B1 FABP2 FABP1 FABP5 FABP4 PPARG NOS2 NR1H3 PLA2G10 CYP19A1 DRD2 ACE CFTR PTGS2 ABCC1 PPARD ABCG2 SIGMAR1 PTGES PNLIP PLA2G2A AKT1 PRKCD CES1 PPARA

1.51E-16	GO:0042327 positive regulation of phosphorylation	FGFR1 MET VEGFA PGF TEK FLT3 IGF1R EGFR NTRK2 DDR2 INSR HIF1A AURKB SRC PKD1 HSP90AA1 PIK3CG OPRD1 HTR2B APP AKT1 SNCA PRKCD F2 PTGS2 NOX4 HTR2A EDNRB RIPK1 PDE5A DRD2 ACE ADORA1 BCL2 PPARG MMP9 AR PRKAA2
4.06E-13	GO:0016042 lipid catabolic process	FAAH HSD11B1 PNLIP CES1 NEU1 PLA2G10 MGLL PLA2G5 CYP19A1 AKR1C3 PIK3CG PPARD CNR1 CYP1B1 SRD5A1 ADORA1 FABP1 PLA2G2A SRD5A2 CYP1A2 AKT1 PRKCD PPARA
1.40E-12	GO:0046890 regulation of Lipid biosynthetic process	FABP3 NR1H4 HTR2A HTR2B AKT1 HTR2C PRKCD NR0B1 AKR1C3 PTGS2 NR3C1 IGF1R FABP5 DHCR7 NR1H3 PPARA CES1
2.48E-12	GO:0043491 protein kinase B signalling	MET TEK IGF1R NTRK2 INSR PIK3CG VEGFA F10 AKR1C3 HSP90AA1 NOX4 AKT1 DRD2 FGFR1 F3 EGFR PPARA SRC
2.29E-11	GO:0014065 phosphatidylinositol 3-kinase signalling	PIK3CG TEK IGF1R NTRK2 INSR HTR2A HTR2B PPARD F2 VEGFA AKT1 EGFR FGFR1 FLT3 SRC
3.32E-11	GO:0043434 response to peptide hormone	GSK3B STAT1 CYP11A1 PPARG CA2 PARP1 PRKCD INSR NR1H4 PTGS2 ARG1 EDNRB CYP1B1 IGF1R AKT1 SRD5A1 EDNRA DDR2 HSD11B2 PPARA SRC SRD5A2
9.01E-09	GO:0043467 regulation of generation of	HIF1A INSR PPARA HTR2A CISD1 APP AKT1 CDK1 PRKAA2 NOS2 GSK3B SNCA

	precursor metabolites and energy	
1.69E-08	GO:0008203 cholesterol metabolic process	CYP11A1 DHCR7 HMGCR CES1 CFTR CYP2D6 CYP1A2 APP PRKAA2 G6PD NR1H4 PPARD
1.83E-08	GO:0042593 glucose homeostasis	IGF1R INSR NR1H4 HIF1A CFTR ALOX5 NOX4 PPARD CNR1 FFAR1 AKT1 ACE PRKAA2 FABP5 PPARG
2.61E-07	GO:0043068 positive regulation of programmed cell death	CTSD TOP2A RIPK1 MCL1 AKR1C3 PTGS2 MMP2 MMP9 NR3C1 CNR1 ADORA2A IDO1 PPARG CYP1B1 ACE PRKCD SRC BACE1 NOS2 SNCA
3.69E-07	GO:0034440 lipid oxidation	ALOX5 ALOX12 ALOX15 PPARD CNR1 FABP3 PPARG FABP1 PPARA AKT1
1.17E-06	GO:0032868 response to insulin	GSK3B PPARG PARP1 INSR NR1H4 STAT1 IGF1R AKT1 SRD5A1 PRKCD HSD11B2 PPARA SRC
2.63E-06	GO:0032368 regulation of lipid transport	PPARG NR1H3 CYP19A1 PLA2G10 FABP3 PTGES AKT1 PRKCD PPARA
5.15E-06	GO:0032869 cellular response to insulin stimulus	GSK3B PPARG PARP1 INSR NR1H4 STAT1 IGF1R AKT1 SRD5A1 PRKCD SRC
1.15E-05	GO:0010827 regulation of glucose transmembrane transport	INSR EDNRA ACE TERT FABP5 FFAR4 AKT1
3.32E-05	GO:0006006 glucose metabolic process	G6PD INSR NR3C1 AKT1 FABP5 PPARA SRC RORC PPARD GSK3B

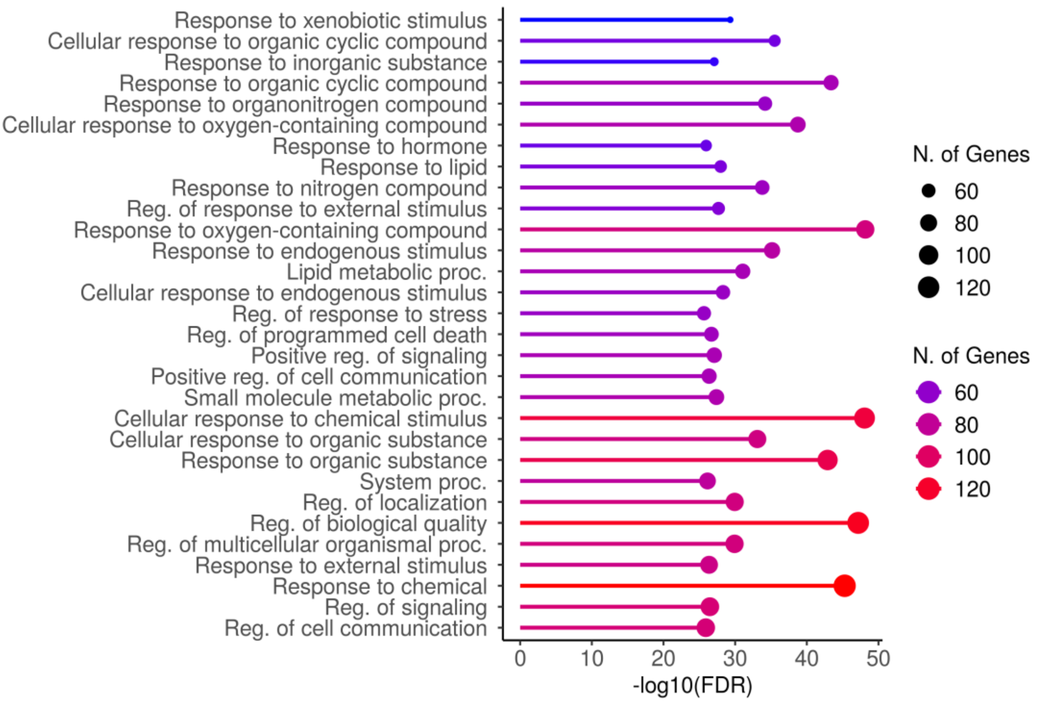
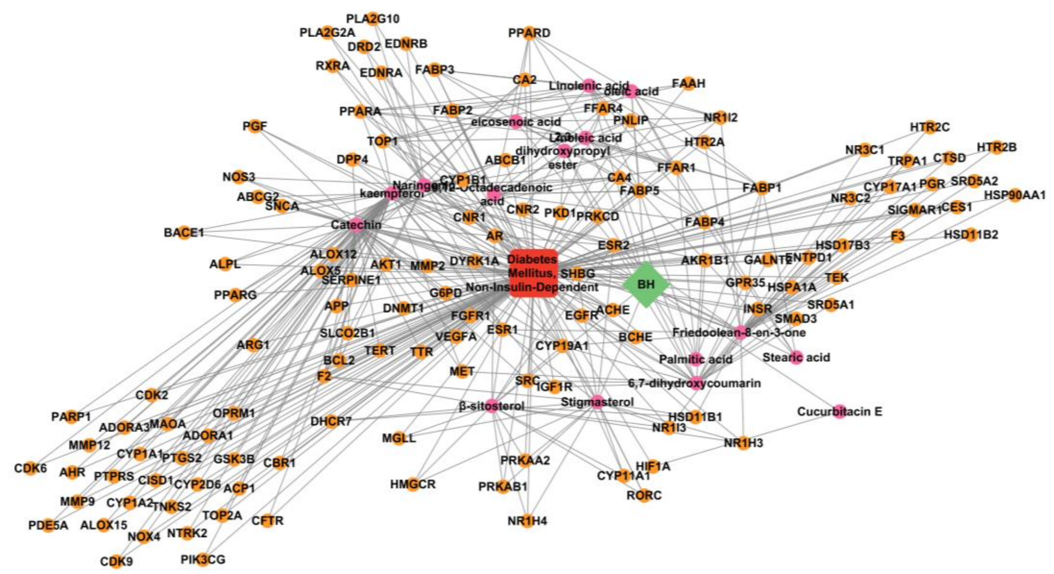


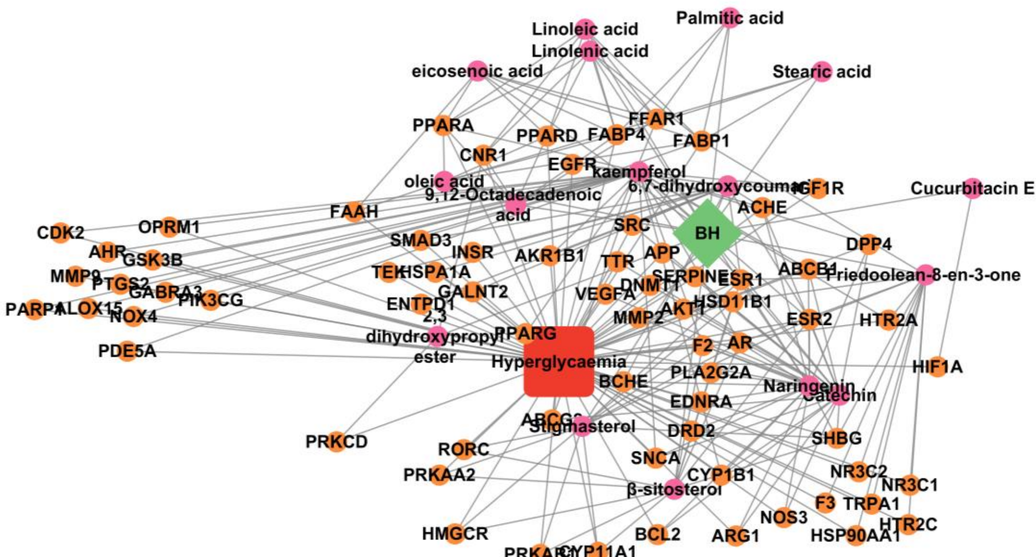
Figure 4.9. Top 30 enriched biological processes involved in the regulation of NIDDM, Obesity, Hyperlipidaemia, Hypertriglyceridaemia and Hyperglycaemia.

4.3.2.3. *Combination network analysis*

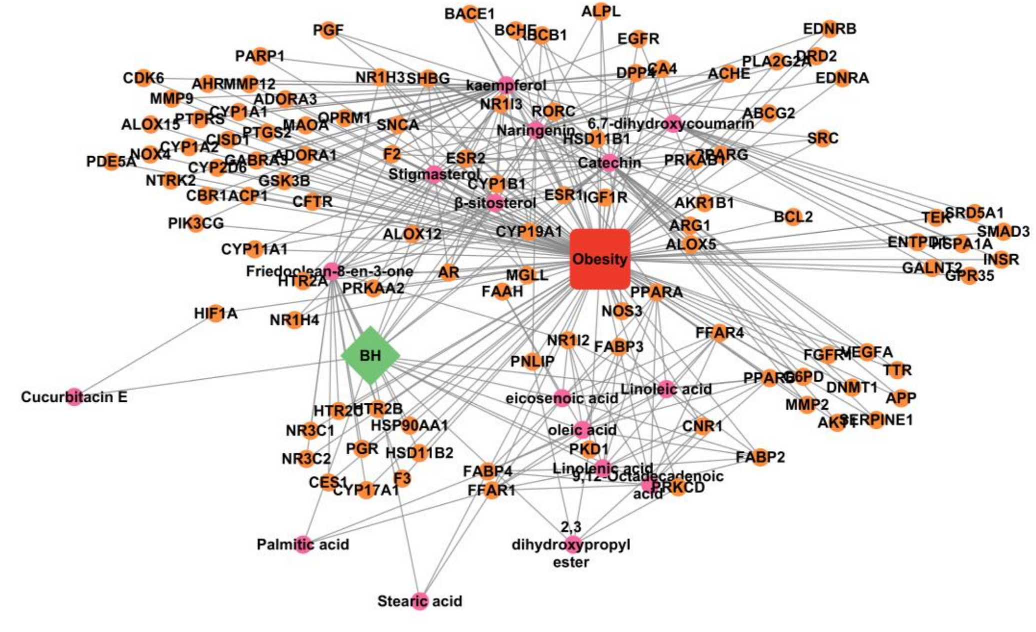
The union network of BOT–BA–TAR–DIS for NIDDM, Obesity, Hyperlipidaemia, Hypertriglyceridaemia, and Hyperglycaemia was created in Cytoscape and summarised in a yFiles organic layout model. Each network was analysed using the “Analyze Network” tool. In NIDDM, hyperglycaemia, obesity, and hyperlipidaemia, kaempferol exhibited the highest level of connection. While FABP1 was identified as the main targeted protein in NIDDM, hyperglycaemia, and hyperlipidaemia, ESR1 was identified as the most targeted protein in obesity. In Hypertriglyceridaemia, Catechin had the strongest connection, while PPARA emerged as the most targeted protein. All the combination synergy networks have been illustrated in Figure 4.10.



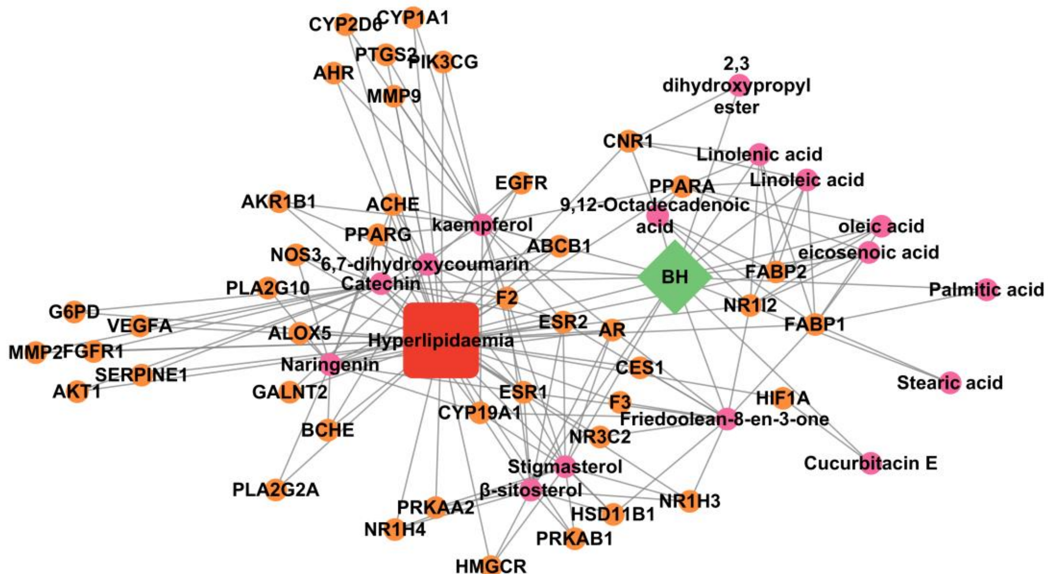
A



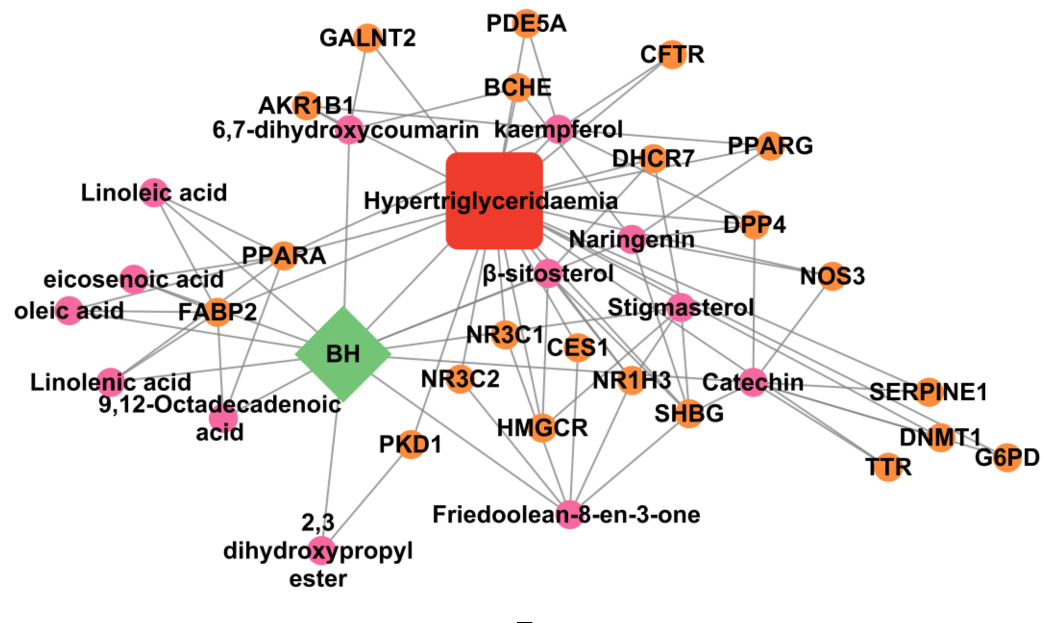
B



C



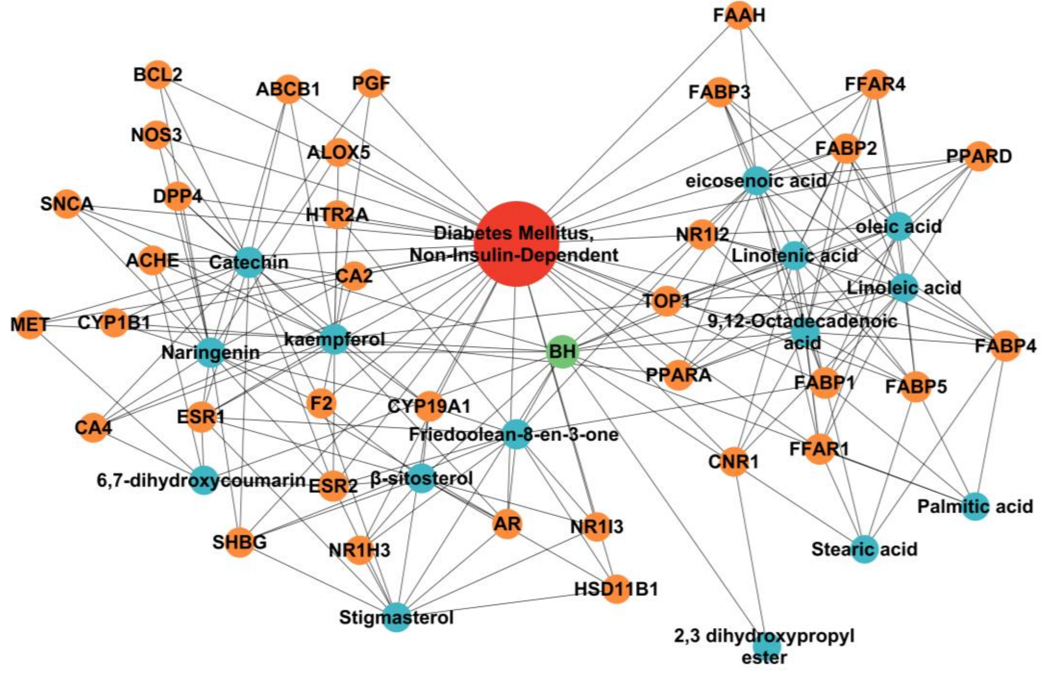
D



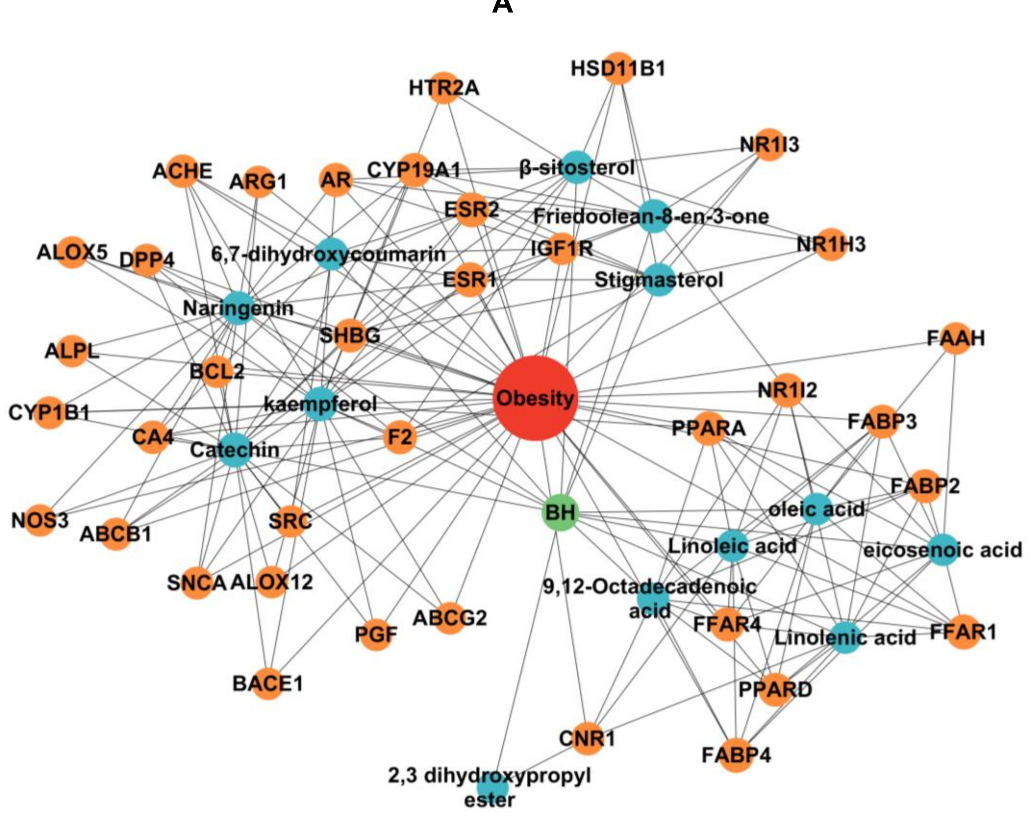
E

Figure 4.10. Combination synergy networks for HEBH against A: NIDDM; B: Hyperglycaemia; C: Obesity; D: Hyperlipidaemia; E: Hypertriglyceridaemia [AH is denoted by the green diamond; Pink circles are the identified phytoconstituents; Orange circles represent human target proteins; Red rounded square represents the disorder]

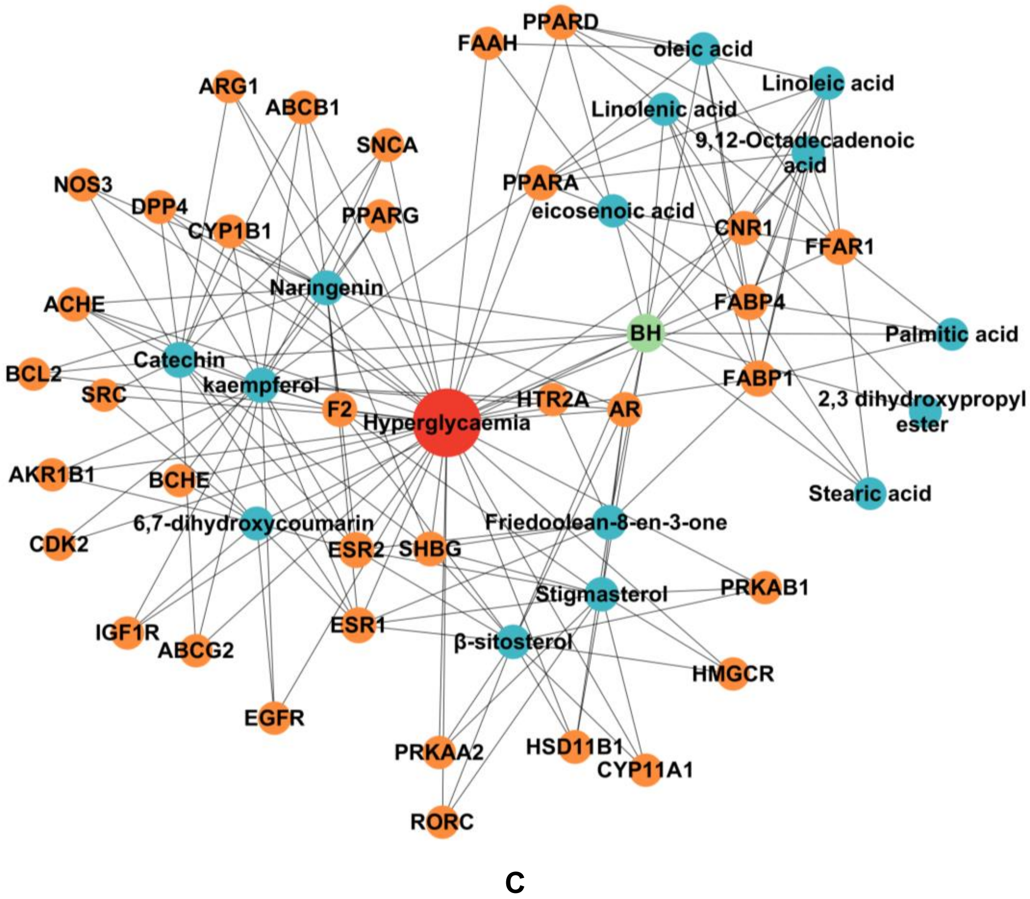
The top 50 hub nodes for NIDDM, Obesity, Hyperlipidaemia, and Hyperglycaemia, together with the 35 nodes for Hypertriglyceridaemia, were identified based on degree, and the shortest path length is illustrated in Figure 4.11.



A



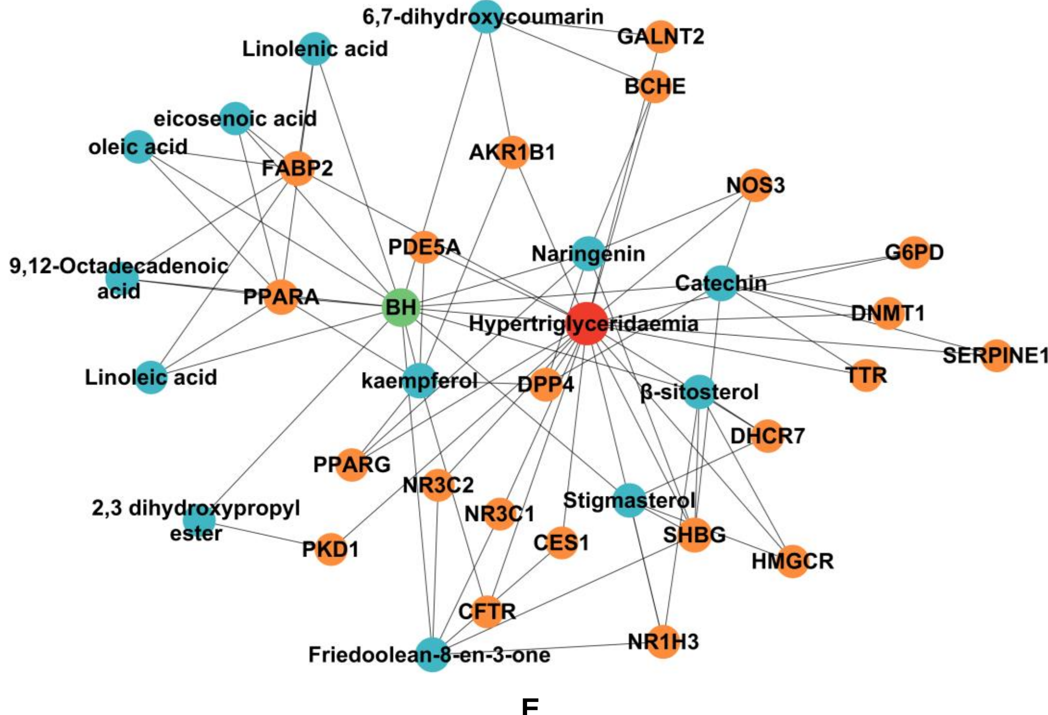
B



C

EGFR
AHR
BCHE
Stearic acid
ABCB1
ALOX5
PPARG
CYP1A1
eicosenoic acid
kaempferol
ACHE
6,7-dihydroxycoumarin
CYP19A1
ESR1
PPARA
FABP1
oleic acid
AKR1B1
Naringenin
CES1
Palmitic acid
GALNT2
Catechin
Hyperlipidaemia
F3
BH
9,12-Octadecadenoic acid
NOS3
ESR2
FABP2
NR1I2
NR3C2
Friedoolean-8-en-3-one
Linoleic acid
PLA2G2A
VEGFA
F2
AR
Linolenic acid
β-sitosterol
CNR1
Stigmasterol
NR1H3
2,3 dihydroxypropyl ester
SERPINE1
PRKAA2
HSD11B1
PRKAB1
NR1H4
Cucurbitacin E
HIF1A
HMGCR

D



E

Figure 4.11. Combination synergy network analysis of *Allium hookeri* Thwaites (green circle denoted with AH) showing top 50 hub nodes interacting to exert the pharmacological effect against A: NIDDM; B: Obesity; C: Hyperglycaemia; D: Hyperlipidaemia, and the top 35 hub nodes against E: Hypertriglyceridaemia. The bigger the size of each node, the more interaction potential.

[The green circle represents BH; Blue circles are the identified phytoconstituents; Pink circles represent human target proteins, and Red circles represent the disorder]

4.3.3. *Total flavonoid and phenolic content of B. hispida*

The phenolic and flavonoid content was determined from the equations of gallic acid (y = 0.001x + 0.0299, R^2 = 0.9913) and Rutin (y = 0.0008x + 0.0367, R^2 = 0.9848), respectively. The standard curve is presented in Figure 4.12. HEBH exhibited a TPC of 15.353 ± 1.019 mg gallic acid equivalent/g and TFC of 12.42 ± 0.97 mg Rutin equivalent/g.

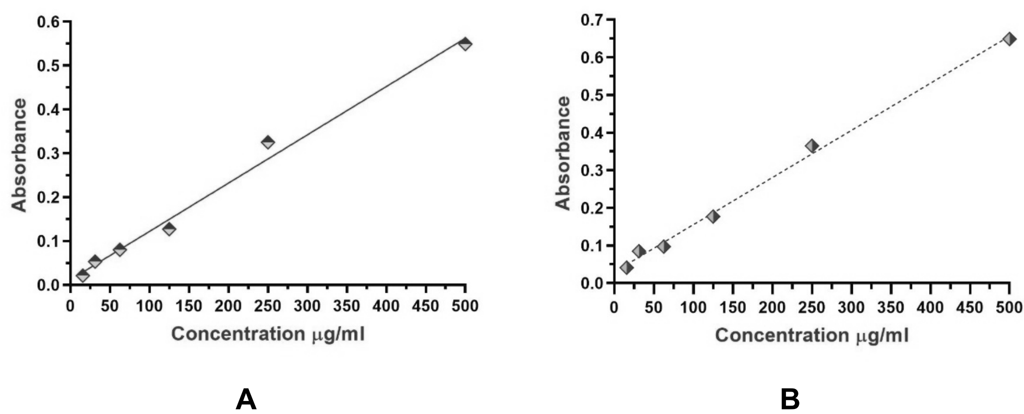
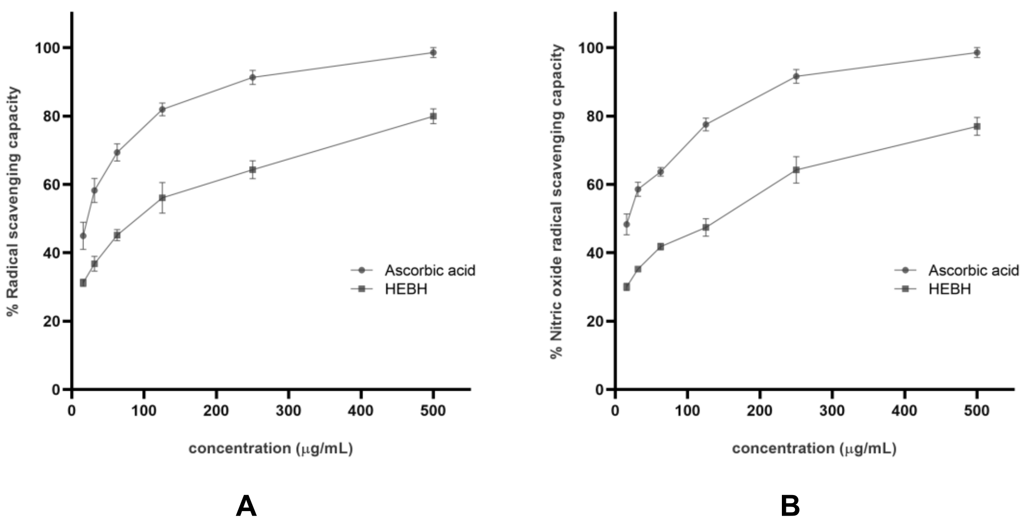
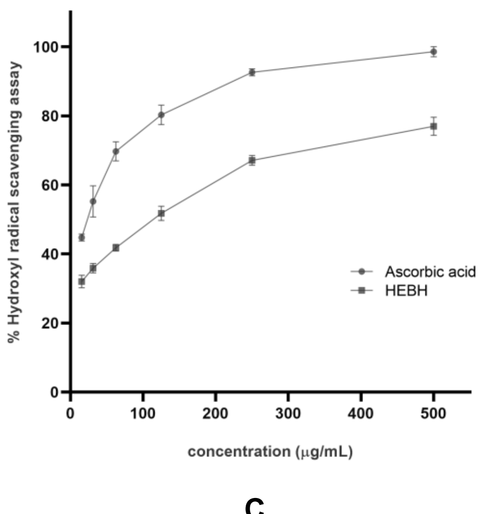


Figure 4.12. Standard curve of (A) Gallic acid and (B) Rutin

4.3.4. *Radical scavenging potential of B. hispida*

HEBH exhibited dose-dependent antioxidant potential (Figure 4.13.). In the DPPH radical scavenging potential assay, HEBH showed an IC_{50} value of 199.6 ± 0.57 µg/mL compared to 53.58 ± 0.064 µg/mL by ascorbic acid. The hydroxyl scavenging capacity of HEBH exhibited an IC_{50} value of 239.2 ± 0.24 µg/mL compared to 59.94 ± 0.49 µg/mL by ascorbic acid. HEBH demonstrated significant antioxidant potential, with an IC_{50} of 339.5 ± 0.65 µg/mL, compared to 120 ± 0.23 µg/mL for ascorbic acid in the nitric oxide scavenging assay.





C

Figure 4.13. Antioxidant potential of *B. hispida* (A) DPPH free radical scavenging potential (B) Nitric oxide (NO) scavenging potential, and (C) Hydroxyl (OH) radical scavenging potential

4.3.5. *Enzyme inhibitory potential of B. hispida*

In the α-glucosidase inhibition assay, the half-maximal inhibitory concentration (IC_{50}) of HEBH was found to be 1.394 ± 0.16 mg/mL compared to 0.294 ± 0.08 mg/mL for acarbose. In the α-amylase inhibition assay, the IC_{50} of HEBH was found to be 1.905 ± 0.21 mg/mL, which is comparable to an IC_{50} of 0.532 ± 0.04 mg/mL for acarbose. In the case of pancreatic lipase, HEBH exhibited an inhibition potential of 2.564 ± 0.08 mg/mL compared to 0.739 ± 0.09 for Orlistat. The dose–response curve of HEBH and acarbose against α-glucosidase and α-amylase inhibition, and Orlistat against pancreatic lipase inhibition is shown in Figure 4.14. Multiple comparisons of the IC_{50} values with two-way ANOVA between HEBH and acarbose for both enzymes, α-glucosidase and α-amylase, and Orlistat for pancreatic lipase, followed by Dunnett's test, showed significant results ($p < 0.0001$), suggesting that HEBH has inhibited all three enzymes in a strong manner comparable to the standard inhibitors.

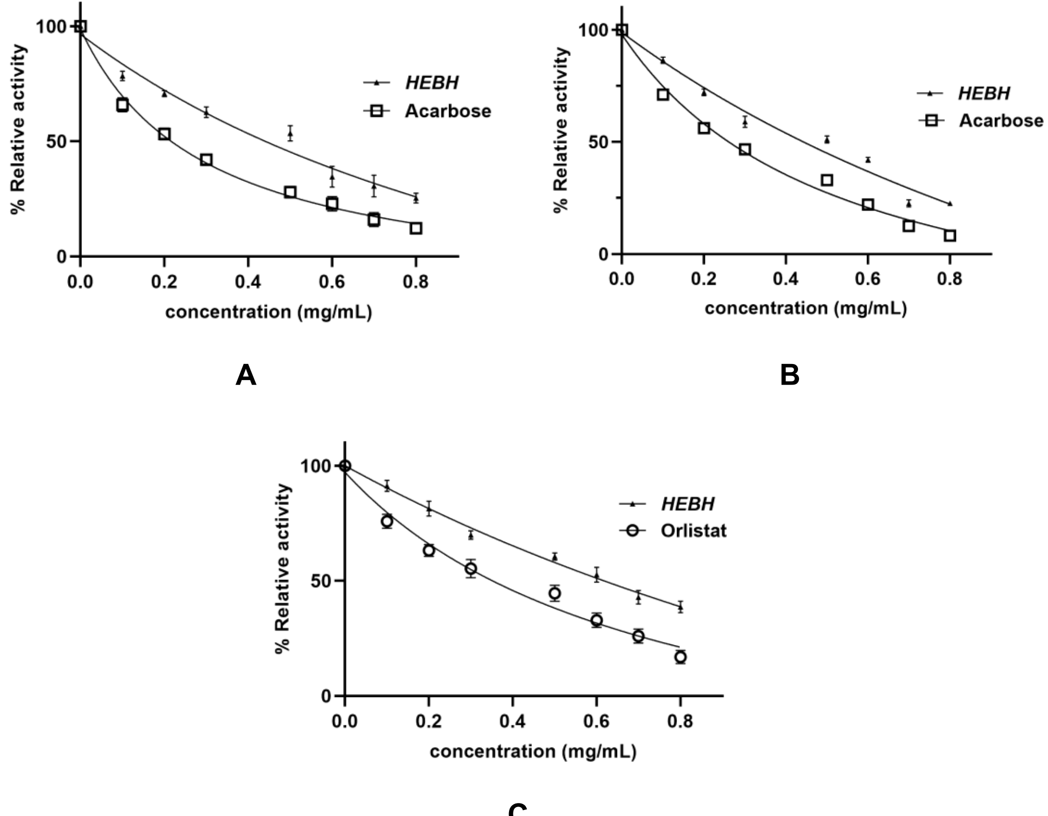
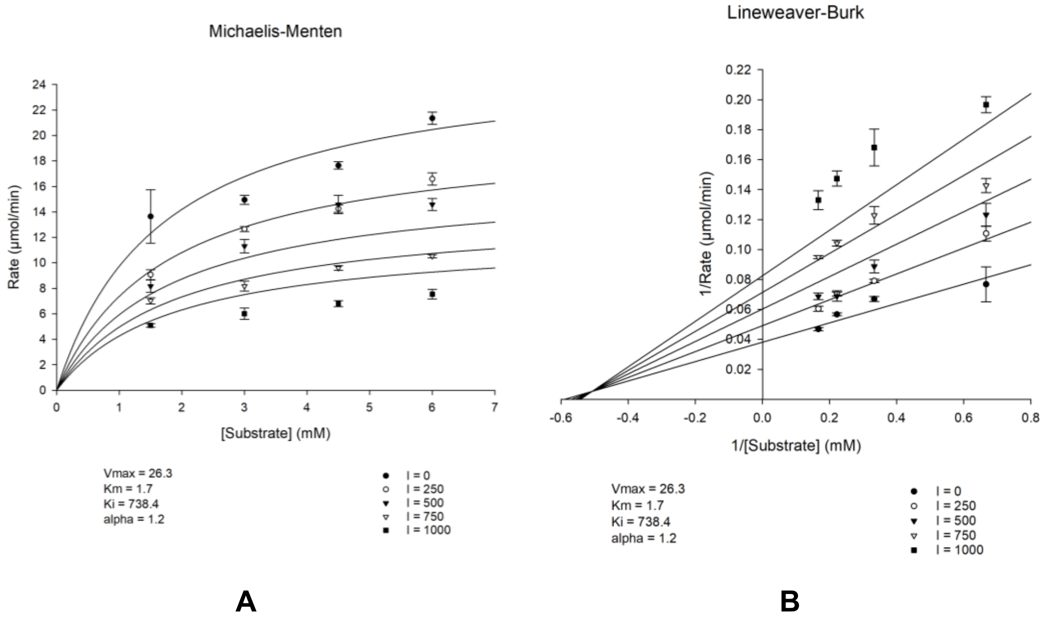


Figure 4.14. Dose–response curve of HEBH and acarbose against (A) α-glucosidase and (B) α-amylase inhibition, and orlistat against (C) Pancreatic lipase inhibition.

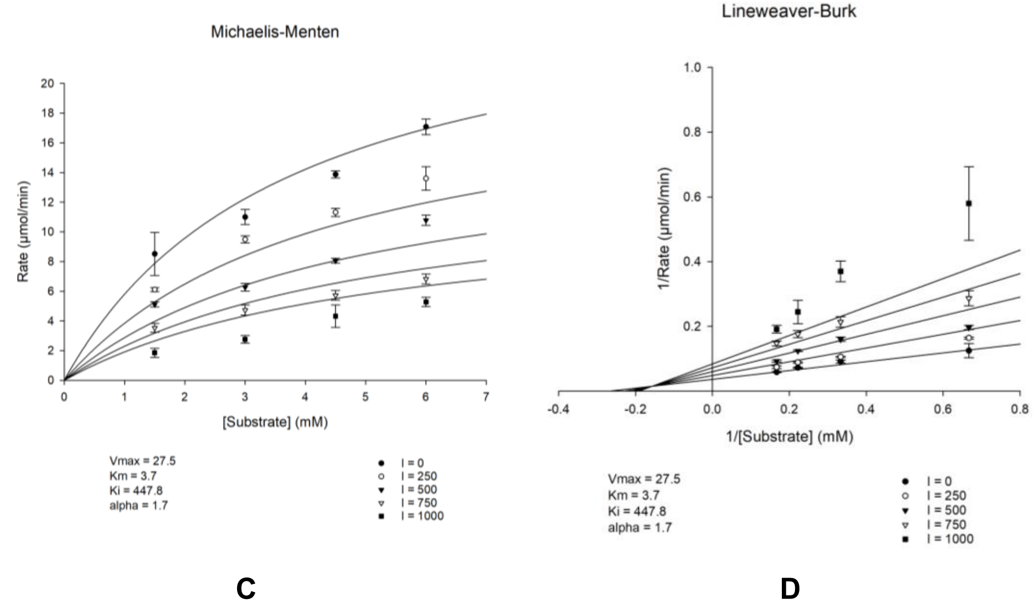
(-□-) acarbose (-○-) orlistat and (—▲—) HEBH; data are presented as mean ± SD (n = 3).

4.3.6. *Kinetic parameters and inhibition mechanism of B. hispida*

Reaction velocity (v) vs enzyme concentration (I = 0–1000 µg/mL) showed reversible inhibition of α-glucosidase, α-amylase, and pancreatic lipase by HEBH, with an inversely decreasing slope at higher inhibitor concentrations (I). The α-glucosidase inhibition kinetics yielded a V_{max} of 26.3 ± 0.54 mM/min, a K_m of 1.7 ± 0.44 mM, a K_i of 738.4, and an α of 1.2. The α-amylase inhibition kinetics yielded a V_{max} of 27.5 ± 1.16 mM/min, a K_m of 3.7 ± 0.91 mM, a K_i of 447.8, and an α of 1.7. The pancreatic lipase inhibition kinetics yielded a V_{max} of 152.1 ± 4.26 mM/min and a K_m of 2.8 ± 1.24 mM, a K_i of 173.5, and an α of 13 (Figure 4.15).



A **B**



C **D**

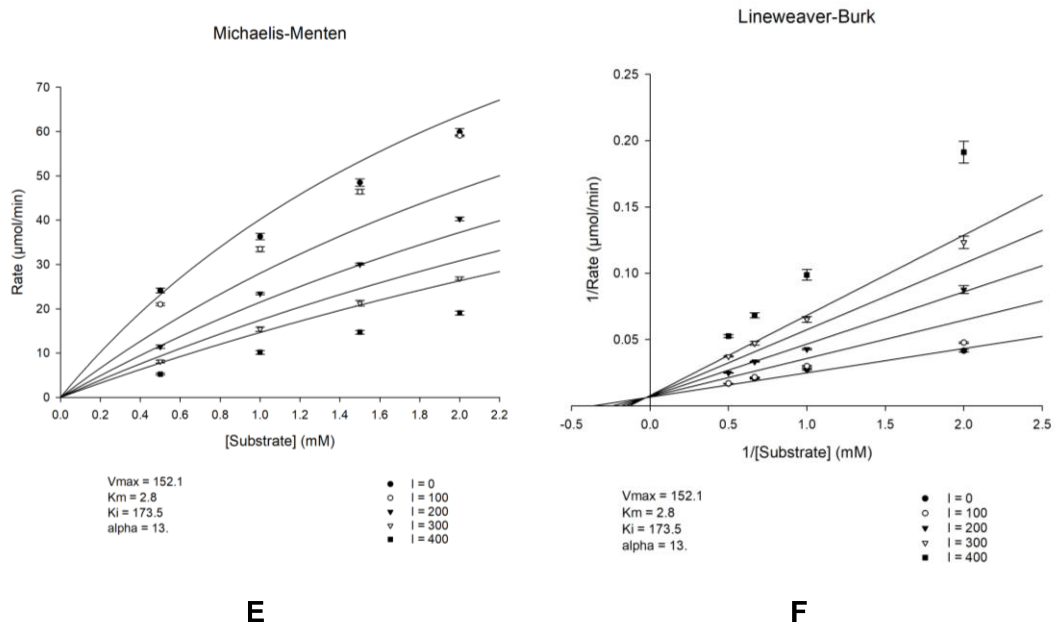


Figure 4.15. Enzyme inhibition kinetics of HEBH (● I=0 µg/ml; ○ I=250 µg/ml; ▼ I=500 µg/ml; ▽ I=750 µg/ml; ■ I=1000 µg/ml).

Michaelis–Menten plot against (A) α-glucosidase, (C) α-amylase, and (E) pancreatic lipase inhibitory activity; Lineweaver–Burk plot against (B) α-glucosidase, (D) α-amylase, and (F) pancreatic lipase inhibitory activity.

4.3.7. *Correlation Analysis of TPC, TFC, and antioxidant potential with enzyme inhibitory potential*

Pearson correlation analysis between the α-glucosidase and α-amylase inhibitory potential and the TPC & TFC values was evaluated and is shown in Table 4.5. The IC_{50} value of enzymes in mg/mL was correlated with the IC_{50} value (mg/mL) of the antioxidant potential (DPPH, NO, OH) and mg/g of TPC and TFC. It was observed that TPC and TFC of HEBH showed a positive linear relationship with α-amylase, α-glucosidase, and pancreatic lipase, which was reflected in the Pearson r value at $p<0.05$. The enzyme inhibitory activity of HEBH was found to be related to TPC and TFC, indicating that phenolic and flavonoid compounds present in *B. hispida* fruits play a significant role in inhibitory activity, albeit not as significant as those in *A. hookeri*.

Similarly, Pearson correlation analysis exhibited a strong correlation between the antioxidant potential and enzyme inhibitory activity of HEBH. The Pearson correlation coefficient, along with the level of significance, has been summarised in Table 4.5. The strong correlation suggests that the antioxidant potential of HEBH was beneficial in inhibiting all three enzymes, albeit to a lesser extent, compared to *A. hookeri*.

Table 4.5. Pearson correlation analysis of enzyme inhibitor potential of HEBH with total phenolic and flavonoid content and antioxidant potential [$p < 0.05$].

Pearson correlation coefficient (r) for α-amylase inhibition potential				
DPPH	**NO**	**OH**	**GAE**	**RE**
0.98^{ns}	0.93^{ns}	0.88^{ns}	0.90^{ns}	0.99*
Pearson correlation coefficient (r) for α-glucosidase inhibition potential				
DPPH	**NO**	**OH**	**GAE**	**RE**
0.98^{ns}	0.93^{ns}	0.88^{ns}	0.90^{ns}	0.99*
Pearson correlation coefficient (r) for pancreatic lipase inhibition potential				
DPPH	**NO**	**OH**	**GAE**	**RE**
0.98^{ns}	0.93^{ns}	0.88^{ns}	0.90^{ns}	0.99*

4.4. Discussion

Polyphenols, flavonoids, phenols, triterpenes, flavanones, stigmastanes and amino acids were identified as significant compounds through metabolite profiling. The identified bioactive compounds like catechin, naringenin, kaempferol, kaempferol 3-O-L-rhamnopyranoside, cucurbitacin E, butanoic acid, linolenic acid, linoleic acid, palmitic acid, stearic acid, oleic acid, eicosenoic acid, β-sitosterol, stigmasterol, Friedoolean-8-en-3-one and Esculetin were all previously reported to possess therapeutic potential against metabolic disorders (Maity et al., 2022; Shahwan et al., 2022; Wang et al., 2022; Yang et al., 2022; Behl et al., 2021; Hussain et al., 2020; Alkhalidy et al., 2018).

The phytocompound target interaction network exhibited a total of 269 predicted interactions. The Venn diagram analysis suggested B. hispida shared a

mechanistic overlap with the reference standards atorvastatin, metformin, Orlistat and acarbose. The PPI enrichment analysis demonstrated that the proteins exhibit a high degree of interconnectivity, with interactions occurring 2.9 times more than expected and a very low p-value, indicating their involvement in shared biological pathways. The phytocompounds were found to be linked to human gene targets AKT1 and PPARG (Bao et al., 2020), as well as EGFR, which have therapeutic implications in hyperglycaemia and hyperlipidaemia (Banerjee et al., 2019). The pathway enrichment analysis revealed that HEBH modulated the insulin signalling pathway through INSR, AKT, GSK3B, and AMPK. Upregulation of INSR phosphorylates insulin receptor substrates, which further activate AKT in the PI3K/AKT pathway. This activation leads to the regulation of glucose and Lipid (Lee et al., 2023). Inhibition or downregulation of GSK3B was found to enhance glycogen synthesis (Ullah et al., 2023). Activation of AMPK triggers catabolic pathways that produce ATP, such as glucose uptake and fatty acid oxidation, in skeletal muscles (Long and Zierath, 2006). The compounds were also found to modulate PPARα, PPARβ and PPARγ in the PPAR signalling pathway. PPAR was found to metabolise cholesterol and improve lipid profile (Moller and Berger, 2003). The biological processes identified through enrichment analysis normalise lipid and glucose metabolism, restore insulin sensitivity, reduce fat accumulation in tissues, and balance blood sugar and lipid levels.

The combination network analysis revealed that kaempferol and catechin were the most biologically active phytocompounds. Previous studies report that kaempferol and catechin act through the PPAR signalling pathway via fatty acid-binding protein (FABP), regulating fatty acid metabolism, oxidation, and Lipid storage through PPARα, PPARβ, and PPARγ (Ebrahimi et al., 2015). The potential of kaempferol to improve insulin sensitivity and protect against β-cell dysfunction by upregulating glucose transport-4 (GLUT4) and AMP-dependent protein kinase (AMPK) expression in a high-fat diet animal model has also been reported previously (Banerjee et al., 2023). A recent study revealed that estrogen receptor 1 (ESR1) upregulates VEGFA in adipose tissue, thereby regulating obesity through angiogenesis and decreasing inflammation (Fatima et al., 2017).

The enzyme inhibition analysis results indicate that *B. hispida* effectively inhibited both α-glucosidase, α-amylase, and lipase to a degree comparable to that of conventional inhibitors. Although the correlation study demonstrated a positive correlation between enzyme inhibition potential and antioxidant and phenolic content, the correlations were largely found to be non-significant except for the correlation with flavonoid content at $p < 0.05$. Thus, flavonoids were identified as the principal bioactive molecules responsible for enzyme inhibition, working in conjunction with antioxidant properties and phenolic compounds to produce a synergistic effect in mitigating diabetes, obesity, and associated metabolic disorders.

4.5. Publications and conference presentations

4.5.1. *Paper communicated*

- Das Gupta, B., Kar, A., Singha, S., Gayen, S., Jana, S., Sharma, N., Haldar, P.K., Mukherjee, P.K., 2025b. Metabolite Profiling and Integrated Network Pharmacology Based Mechanism of *Benincasa hispida* (Thunb.) Cogn. Fruit Against Non-insulin-Dependent Diabetes Mellitus. Phytochemical Analysis 36, 884–895. https://doi.org/10.1002/pca.3476.

Chapter 5

Houttuynia cordata Thunb. – Metabolomics integrated network pharmacology and *in vitro* evaluation

5.1. *Houttuynia cordata* **Thunb. – A profile**
5.2. Results
5.3. Discussion
5.4. Comparative estimation
5.5. Conclusion
5.6. Publications and conference presentations

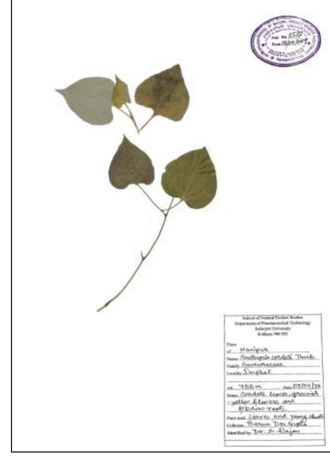
5.1. *Houttuynia cordata* Thunb. – A profile

Scientific classification	**Vernacular names**
Kingdom: Plantae	English: Chameleon plant
Division: Pteridobiotina	Hindi: Simdalu
Class: Angiosperms	Assamese: Musundari, Aich
Order: Piperales	Khasi: Ja mardoh
Family: Saururaceae	Manipuri: Toningkhok, Tokningkhok
Genus: *Houttuynia*	Nepali: Gane
Species: *Houttuynia cordata* Thunb.	Mizo: Uithinthang

5.1.1. *Plant description*

Houttuynia cordata Thunb., a member of the Saururaceae family, is recognised by its distinctive aroma and astringent taste. It is found in Asian and Southeast Asian countries at altitudes ranging from 300 to 2600 meters. In India, it is extensively found in northeast India, encompassing Manipur, Mizoram, Assam, Arunachal Pradesh, and Sikkim. *H. cordata* possesses a thin stem, cordate leaves, greenish-yellow flowers, and fibrous roots. The young shoots and leaves are consumed as raw or cooked vegetables, and the leaves are used as a spice for salads (Pradhan et al., 2023). The whole plant of *H. cordata* has been shown in Figure 5.1.

A



B

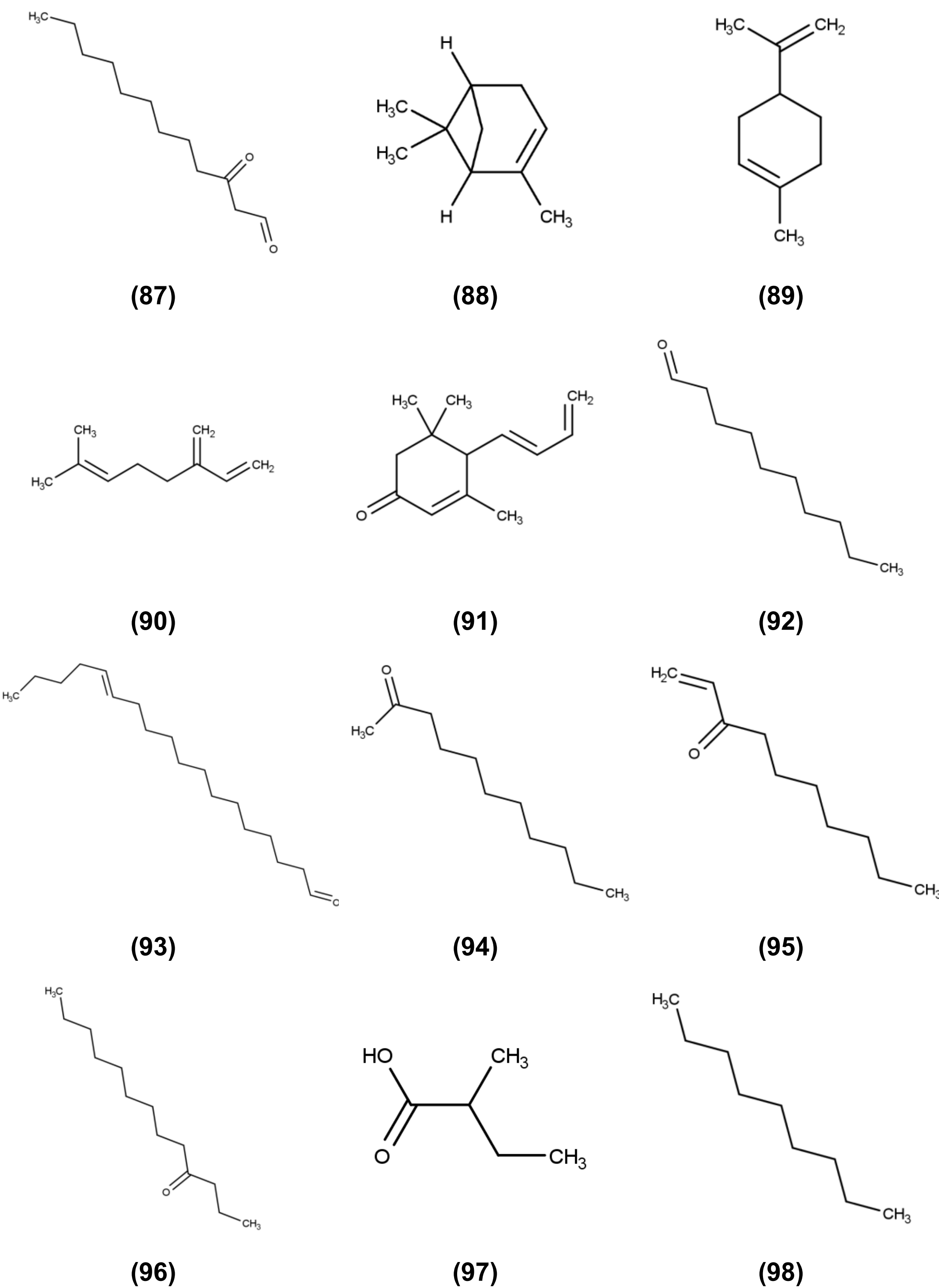
Figure 5.1. A: Leaves of *Houttuynia cordata* Thunb.; B Herbarium

5.1.2. *Traditional uses of H. cordata*

Traditionally, the leaves of *H. cordata* are utilised by northeastern Indian tribes for their anthelmintic properties. The entire plant is utilised to alleviate stomach pain, dysentery, and cholera (Pradhan et al., 2023). According to another report, the whole plant is ingested raw to reduce blood glucose levels (Kumar et al., 2014b). The local tribes of India utilise the shoots to address cardiovascular disorders (Kala, 2005). The traditional folk medicine of Southeast Asian countries employs the entire plant to address hyperglycaemia and hyperlipidaemia. Ancient texts of Japanese traditional medicine document the utilisation of *H. cordata* for combating oxidative stress and inflammation (Wei et al., 2024).

5.1.3. *Phytochemical profile of H. cordata*

H. cordata has been documented to be abundant in alkaloids, followed by flavonoids, volatile oils, and organic acids. The distinctive fishy odour of *H. cordata* resulted from the presence of houttuynin **(87)** (Qing-Song and Sai-Jin, 2015). The ethyl acetate fraction of the aerial parts was found to contain volatile compounds, specifically α-pinene **(88)**, limonene **(89)**, β-myrcene **(90)**, megastigmatrienone **(91)**, decanal **(92)**, (Z)-13-octadecenal **(93)**, 2-undecanone **(94)**, 1-decen-3-one **(95)**, 4-tridecanone **(96)**, methylbutanoic acid **(97)** and methyl n-nononone **(98)** (Kumar et al., 2014a). Ma and fellow researchers identified bioactive alkaloids from the ethanolic extract of *H. cordata* aerial parts, namely piperolactam B **(99)**, C **(100)** and D **(101)**, sauristolactam **(102)**, cepharanone B **(103)**, 7-oxodehydroasimilobine **(104)**, lysicamine **(105)**, atherospermidine **(106)**, liriodenine **(107)**, ouregidione **(108)**, cepharadione A **(109)** and B **(110)**, 1,2-dimethoxy-3-hydroxy-5-oxonoraporphine **(111)** and 1,2,3-trimethoxy-3-hydroxy-5-oxonoraporphine **(112)** (Ma et al., 2017). A separate study discovered three novel alkaloids: houttuynamide B **(113)**, houttuynamide C **(114)**, and houttuycorine **(115)** from the aerial components of *H. cordata*. In addition to the three, the study also identified perlolyrine **(116)** (Ahn et al., 2017).



(87)
(88)
(89)
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(94)
(95)
(96)
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(98)

(99) **(100)** **(101)**

(102) **(103)** **(104)**

(105) **(106)** **(107)**

(108) **(109)** **(110)**

(111) (112) (113)

(114) (115) (116)

5.1.4. *Pharmacological activities of H. cordata*

The diabetic wound-healing efficacy of *H. cordata* decoction-infused hydrogel was examined *in vitro* using reactive oxygen species scavenging and fibroblast proliferation, and *in vivo,* utilising an excision wound model in mice with streptozotocin-induced diabetes. The hydrogel significantly enhanced wound healing by augmenting collagen deposition, neovascularisation, and fibroblast viability (Yang et al., 2025). The pharmacological potential of the *H. cordata* polysaccharide fraction was examined in an *in vivo* MC903-induced atopic dermatitis mouse model, as well as *in vitro* using RAW264.7 macrophages and HaCaT keratinocytes. The polysaccharide-enriched fraction enhanced the skin barrier by elevating Occludin and Claudin-1 levels, while reducing epidermal hyperplasia and mast cell infiltration. The normalisation of collagen and matrix functionality evidenced this. The fraction exhibits antioxidant characteristics and antibacterial activity against *S. aureus*. Reduced nitric oxide, cyclooxygenase-2, and phosphorylated p38 mitogen-activated protein kinase levels were reported in RAW264.7 and HaCaT cells, indicating the anti-inflammatory effect (Huang et al., 2025).

The antiapoptotic efficacy of *H. cordata* root hydroalcoholic extract was examined on human hepatocarcinoma cells, HepG2. The extract induced cytotoxicity in the HepG2 cell line by disrupting cytoskeletal components, including β and γ-tubulin, leading to morphological alterations (Sarkar et al., 2023). The antihyperglycemic effectiveness of the ethanolic extract of the whole plant of *H. cordata* was examined in a streptozotocin-induced diabetic rat model. The extract reduced fasting blood glucose and normalised antioxidant enzyme levels in the liver, pancreas, and adipose tissue (Kumar et al., 2014b). In a separate investigation, *H. cordata* leaves were extracted with green tea in water and subsequently fermented with *L. paracasei* subsp. paracasei NTU 101. The fermented tea suppressed lipogenesis in 3T3-L1 adipocytes by promoting lipolysis, and it additionally diminished body weight and body fat in obese mice (Wang et al., 2018).

5.2. Results

5.2.1. *Metabolite profile of H. cordata*

The percentage yield of HEHC was found to be 15.37% w/w. The UHPLC–QTOF–MS revealed a variety of compounds, which are detailed in Table 5.1. The positive ion chromatogram is depicted in Figure 5.2., displaying the total ion chromatogram. The analysis identified 26 distinct compounds; the details of these compounds, including their chemical formula, theoretical mass (g/mol), observed mass-to-charge ratio (m/z), retention time (RT), mass error (Dif (Tgt, ppm)), and compound class, are presented in Table 5.1. The results confirmed the existence of several compounds known for their therapeutic properties in addressing metabolic disorders, such as diabetes and obesity.

Table 5.1. Compounds identified in the hydroalcoholic extract of *H. cordata* leaves by UHPLC-QTOF-MS.

Sl. No.	RT	Name	Formula	Mass (g/mol)	m/z	Error (ppm)	Class of compounds
1.	2.531	Cinnamic acid	$C_9H_8O_2$	148.0534	166.0872	6.69	Cinnamic acids and

							derivatives
2.	2.629	Dehydrolinalool	$C_{14}H_{20}O_9$	332.1129	355.1027	-2	Organooxygen compounds
3.	3.684	Ethyl p-anisate	$C_{10}H_{12}O_3$	180.0787	203.068	2.63	Benzene and substituted derivatives
4.	3.684	Thymusin	$C_{17}H_{14}O_7$	330.0723	331.081	2	Flavonoids
5.	7.896	Ethyl abietate	$C_{22}H_{34}O_2$	330.2546	331.2621	-3.98	Prenol lipids
6.	8.108	Phloretic acid	$C_9H_{10}O_3$	166.0618	167.0692	-6.53	Phenylpropanoic acids
7.	8.535	Quinic acid	$C_7H_{12}O_6$	192.063	193.0703	-2	Organooxygen compounds
8.	9.470	Scopolin	$C_{16}H_{18}O_9$	355.1036	355.1023	-3.5	Coumarins and derivatives
9.	10.831	Pyrogallol	$C_6H_6O_3$	126.0315	127.0388	5.87	Phenols
10.	11.964	Chlorogenic acid	$C_{16}H_{18}O_9$	354.095	355.1026	-0.5	Organooxygen compounds
11.	12.13	3-Hydroxyphenylpropionic acid	$C_9H_{10}O_3$	166.0631	167.0704	0.84	Phenylpropanoic acids
12.	12.429	Kaempferol	$C_{15}H_{10}O_6$	286.049	287.0564	0.81	Flavonoids
13.	13.029	Luteolin	$C_{15}H_{10}O_6$	286.049	287.0564	0.81	Flavonoids

14.	13.938	Salutaridine	$C_{19}H_{21}NO_4$	328.1552	328.154347	-2.6	Phenanthrenes and derivatives
15.	14.080	Reticuline	$C_{19}H_{23}NO_4$	330.1699	330.1713	0.3	Isoquinolines and derivatives
16.	14.626	Houttuynoid A	$C_{33}H_{38}O_{13}$	642.6031	642.603935	1.3	Flavonoids
17.	14.72	Oxypeucedanin hydrate	$C_{16}H_{16}O_6$	304.0946	327.0839	-0.23	Coumarins and derivatives
18.	15.572	Tricin	$C_{17}H_{14}O_7$	330.0746	353.0642	2	Flavonoids
19.	16.37	Taraxasterone	$C_{30}H_{48}O$	424.3693	425.3768	-2.95	Prenol lipids
20.	16.537	Miliacin	$C_{31}H_{52}O$	440.4029	463.3919	2.35	Prenol lipids
21.	18.295	Phytyl acetate	$C_{22}H_{42}O_2$	338.3184	361.3073	-1.12	Prenol lipids
22.	20.374	Sphinganine	$C_{20}H_{41}NO_2$	327.314	350.3027	0.77	Organic nitrogen compounds
23.	22.155	Trimethyltridecanoic acid	$C_{16}H_{32}O_2$	256.2412	279.2303	2.49	Prenol lipids
24.	26.226	Heptyl butanoate	$C_{11}H_{22}O_2$	186.1619	204.1967	-0.18	Fatty acyls
25.	31.083	3'-O-Methylcatechin	$C_{16}H_{16}O_6$	304.0944	305.1023	-0.98	Flavonoids
26.	32.1	Oleic acid	$C_{18}H_{34}O_2$	282.25	283.	-6.39	Fatty acyls

	74			63	2638		

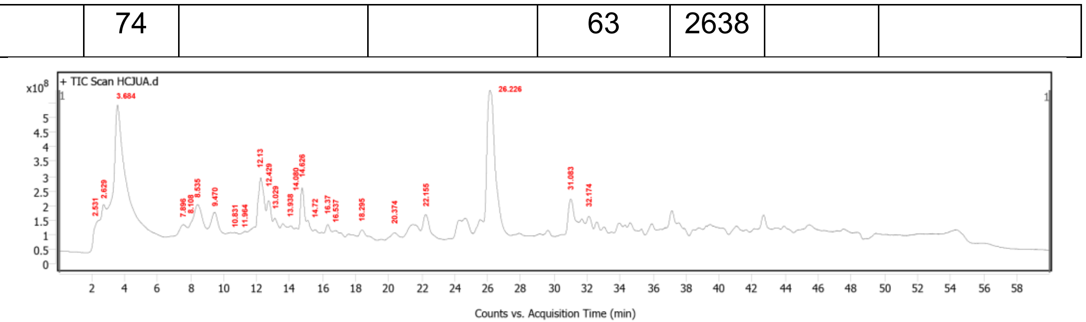


Figure 5.2. Total ion chromatogram of HEHC in the positive ionisation mode

The class of compounds identified includes Cinnamic acids and derivatives – cinnamic acid **(123)**; Organooxygen compounds – dehydrolinalool **(142)**, quinic acid **(143)**, chlorogenic acid **(49)**; Benzene and substituted derivatives – ethyl p-anisate **(144)**; Flavonoids – thymusin **(145)**, kaempferol **(3)**, luteolin **(5)**, houttuynoid A **(146)**, tricin **(147)**, 3'-O-methylcatechin **(148)**; Prenol lipids – ethyl abietate **(149)**, taraxasterone **(150)**, miliacin **(151)**, phytyl acetate **(152)**, trimethyltridecanoic acid **(153)**; Phenylpropanoic acids – phloretic acid **(154)**, 3-hydroxyphenylpropionic acid **(130)**; Coumarins and derivatives – scopolin **(155)**, oxypeucedanin hydrate **(156)**; Phenols – pyrogallol **(157)**; Phenanthrenes and derivatives – salutaridine **(158)**; Isoquinolines and derivatives – reticuline **(159)**; Organic nitrogen compounds – sphinganine **(160)**; Fatty acyls – heptyl butanoate **(161)**, oleic acid **(124)**.

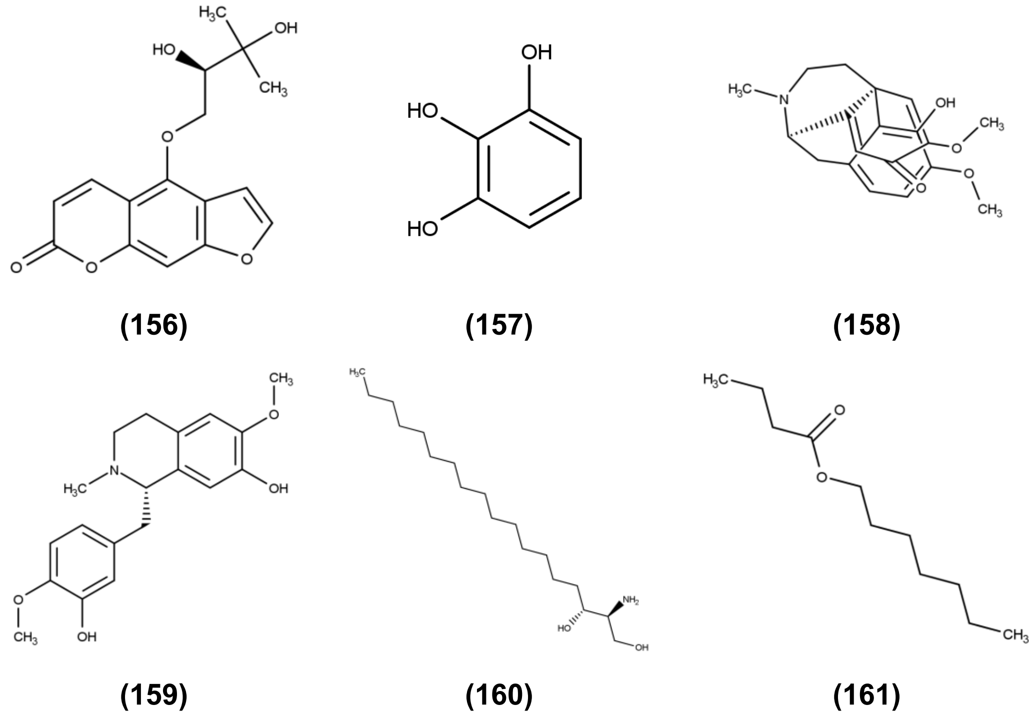
(142) **(143)** **(144)**

(145) **(146)**

(147) **(148)** **(149)**

(150) **(151)** **(152)**

(153) **(154)** **(155)**



5.2.2. *Network analysis*

5.2.2.1. *Identified targets and disease association network*

The compounds (BA) were screened in BindingDB, and the associated genes (TAR) were identified from the UniProt database. The 17 of the 26 compounds exhibiting targets were used to create a bioactive–target (BA–TAR) network, as shown in Figure 5.3.

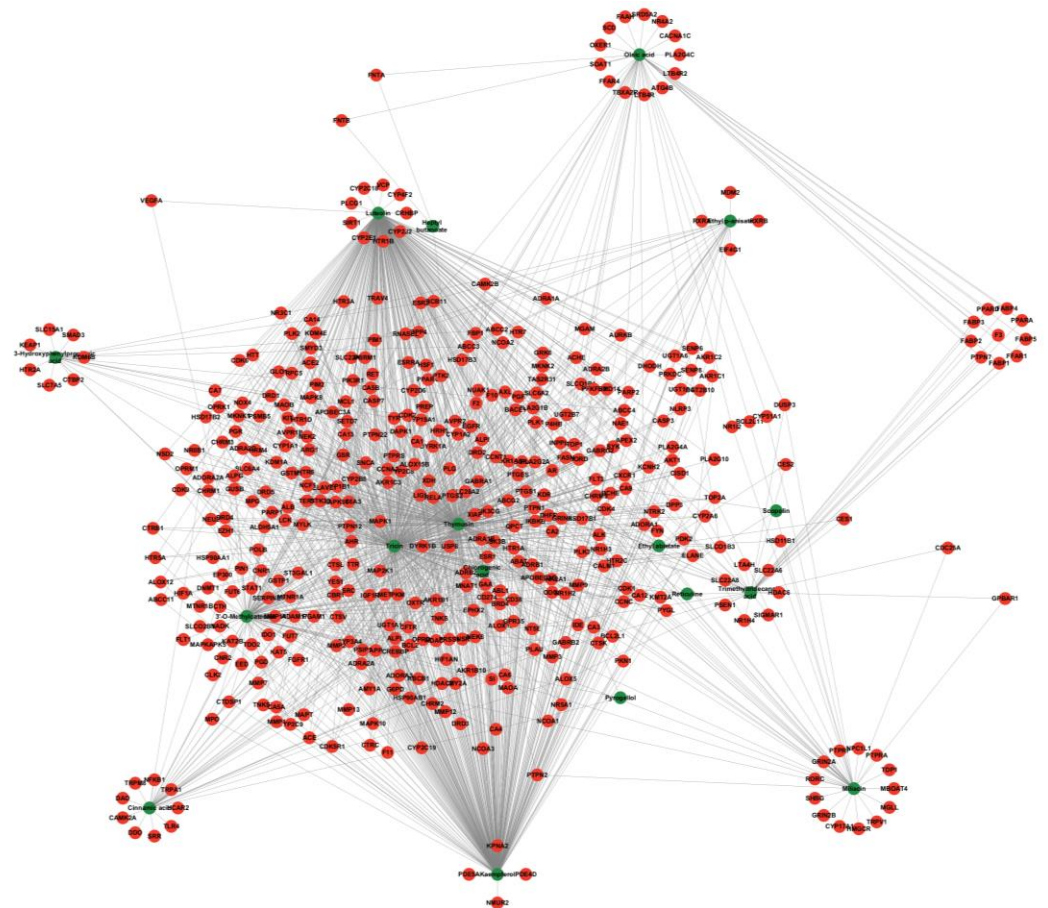
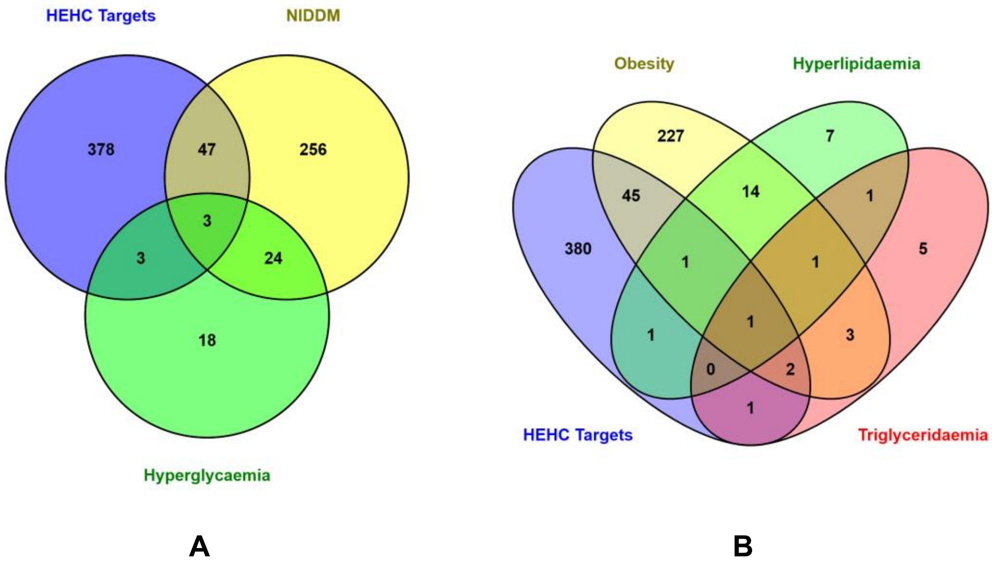


Figure 5.3. The bioactive–target (BA–TAR) network

[Identified compounds are green circles and protein targets are red circles]

The network topology statistics revealed 448 nodes interconnected through 1535 edges, indicating a dense connectivity between the phytocompounds and their targets. The phytocompound Luteolin exhibited the highest connectivity by targeting 300 proteins, and carbonic anhydrase 2 (CA2) was found to be the most targeted protein, targeted by 10 compounds. The disease association was performed in the DisGeNET database, and the common targets for Non-Insulin-Dependent Diabetes Mellitus (NIDDM), Obesity, Hyperlipidaemia, Hypertriglyceridaemia, and Hyperglycaemia are shown in the Venn diagram (Figure 5.4.).



A B

Figure 5.4. Venn diagrams showing the common targets for (A) NIDDM and Hyperglycaemia; (B) Obesity, Hyperlipidaemia and Hypertriglyceridaemia between identified protein targets of HEHC and disease targets in the DisGeNET database.

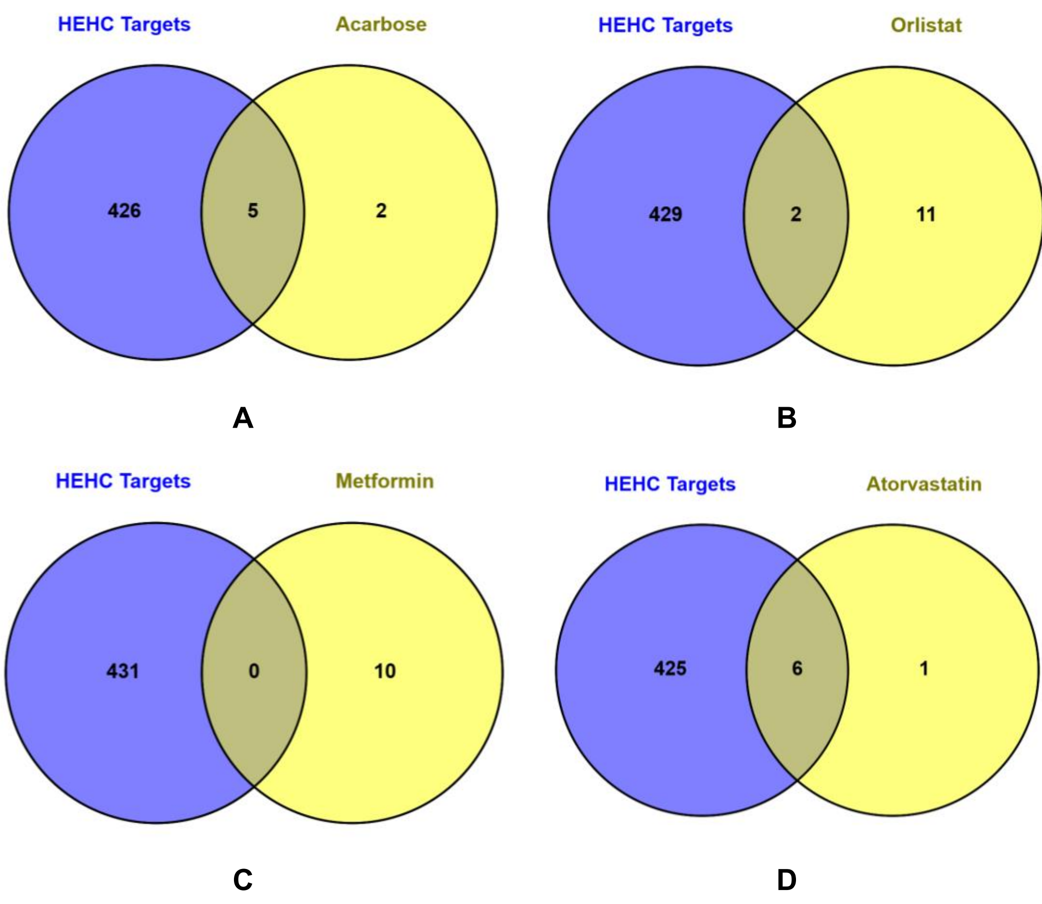
There were 50 common targets for NIDDM, followed by Obesity (49), Hyperglycaemia (6), Triglyceridaemia (4), and Hyperlipidaemia (3) between targets of HEHC and disease targets in the DisGeNET database. The list of common targets is listed in Table 5.2.

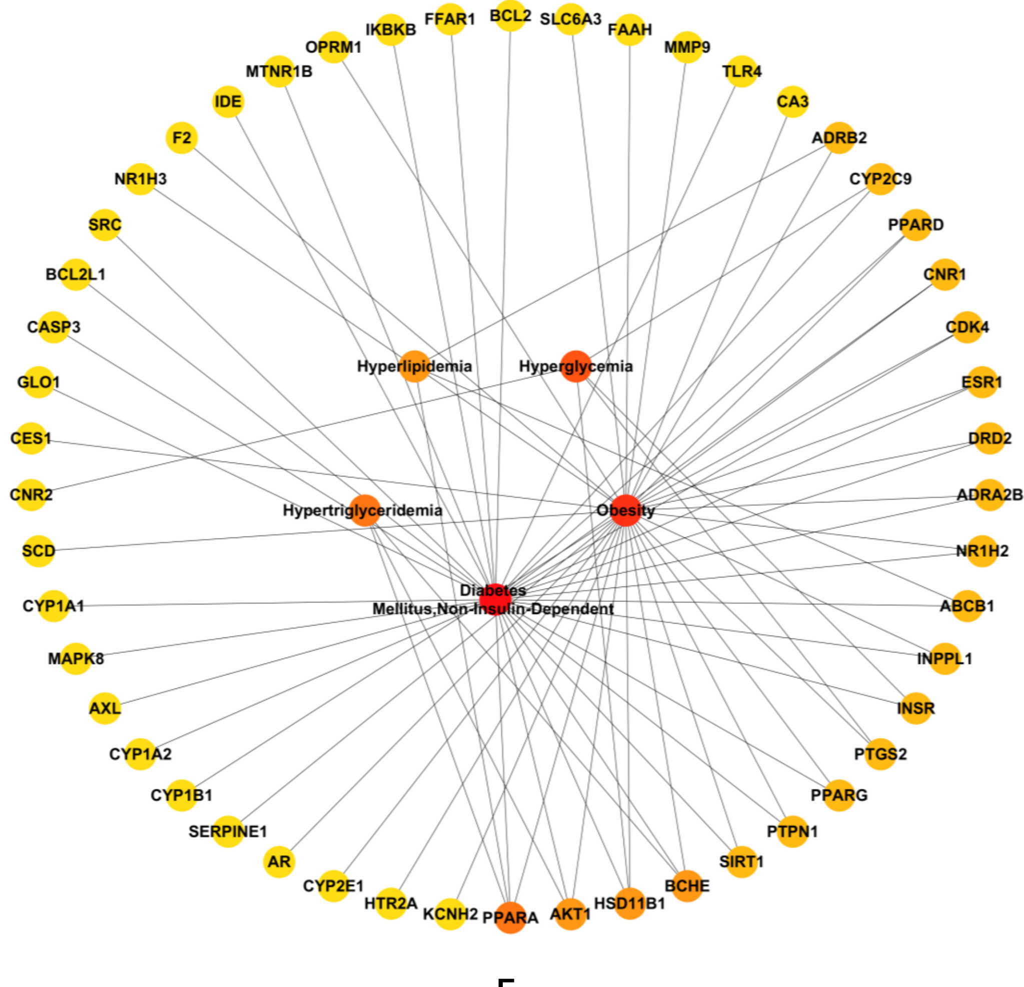
Table 5.2. Shared targets between the identified phytocompounds of *H. cordata* and selected metabolic disorders and reference standards.

Common elements in "Targets" and "selected disorders":		
Disorder	**Number of Common Genes from the obtained targets**	**Common targets**
Hyperlipidaemia	3	ADRB2, PPARA, ABCB1
Triglyceridaemia	4	AKT1, BCHE, PPARA, MET
NIDDM	50	ABCB1, ACE, ADAM17, ADRA2A,

		ADRA2B, AKR1B1, AKT1, AXL, BCHE, BCL2, BCL2L1, BCL2L11, CASP3, CD38, CDK4, CNR1, CYP1A1, CYP1A2, CYP2C9, CYP3A4, DPP4, DRD2, EGFR, ESR1, FABP3, FFAR1, GLO1, GSTM1, GSTP1, HIF1A, HMGCR, HSD11B1, IDE, IKBKB, INPPL1, INSR, MAPK8, MTNR1B, NFKB1, NR1H2, PIK3CG, PIK3R1, PPARA, PPARD, PPARG, PTPN1, SHBG, SIRT1, SRC, TLR4
Hyperglycaemia	6	CNR2, CYP2C9, HSD11B1, INSR, PTGS2, TERT
Obesity	49	ACHE, ADRA2B, ADRB1, ADRB2, AHR, AKR1C3, AKT1, AR, BCHE, CA3, CDK4, CES1, CNR1, CYP1B1, CYP2E1, DRD2, EP300, ESR1, F2, FAAH, FABP2, FASN, HSD11B1, HTR2A, HTR2C, IDO1, INPPL1, KCNH2, MMP9, NCOA3, NR1H2, NR1H3, NR1I2, NR3C1, NTRK2, OPRM1, PARP1, PFKFB3, PGR, PPARA, PPARD, PPARG, PTGS2, PTPN1, SCD, SERPINE1, SIRT1, SLC6A3, TRPV1
Common elements in “Targets” and “selected reference standards”:		
Atorvastatin	6	HDAC1, HDAC2, HDAC6, HMGCR, SLCO1B1, SLCO1B3
Metformin	0	
Orlistat	2	FAAH, FASN
Acarbose	5	AMY1A, AMY2A, GAA, MGAM, SI

The common targets for acarbose, orlistat, metformin, and atorvastatin with HEHC were also screened and are presented in Figure 5.5. While atorvastatin shared HDAC1, HDAC2, HDAC6, HMGCR, SLCO1B1 and SLCO1B3 as common targets with HEHC, no common targets were found between metformin and HEHC. Similarly, acarbose shared AMY1A, AMY2A, GAA, MGAM and SI, and Orlistat shared FAAH and FASN as common targets with HEHC. These common targets suggest a mechanistic overlap and potential of HEHC as an alternative therapeutic. The gene-disease association (TAR–DIS) networks were created using Cytoscape. The top 50 hub proteins acting on NIDDM, Obesity, Hyperlipidaemia, Hypertriglyceridaemia, and Hyperglycaemia, calculated by degree and shortest path length using cytoHubba, are shown in Figure 3.5.





E

Figure 5.5. The common targets for (A) acarbose, (B) orlistat, (C) metformin and (D) atorvastatin with HEHC. (E) The top 50 hub proteins acting on NIDDM, Obesity, Hyperlipidaemia, Hypertriglyceridaemia and Hyperglycaemia

5.2.2.2. *Analysis of protein-protein interactions and associated pathways*

The target genes were visualised in STRING 12.0, and the PPI enrichment analysis showed that the target proteins have significantly more interactions, indicating that they are at least partially biologically connected as a group. The PPI enrichment analysis exhibited 860 edges, which is significantly more than the

expected number of 279. The average node degree was found to be 19.8, suggesting each protein interacts with 19 others (Figure 5.6.).

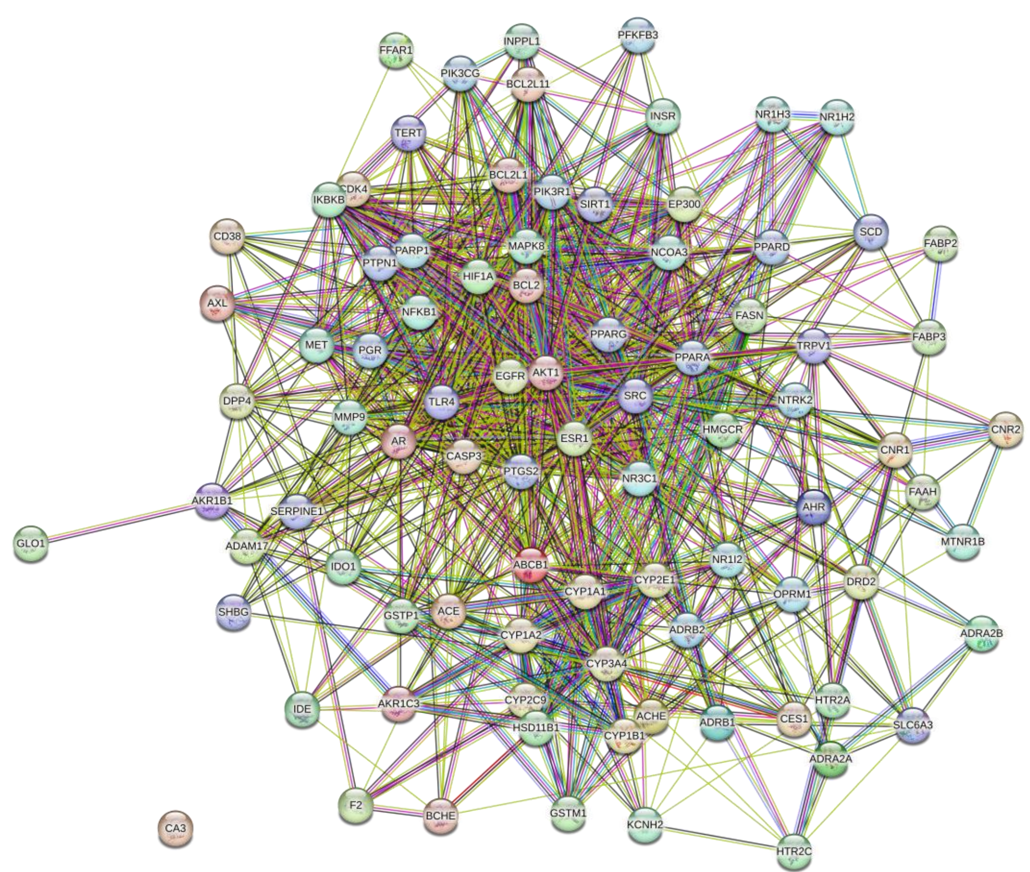


Figure 5.6. Protein-protein interaction network

The PPI enrichment network was filtered by degree and shortest path length using the cytoHubba tool in Cytoscape to calculate the top 10 hub proteins (Figure 5.7.).

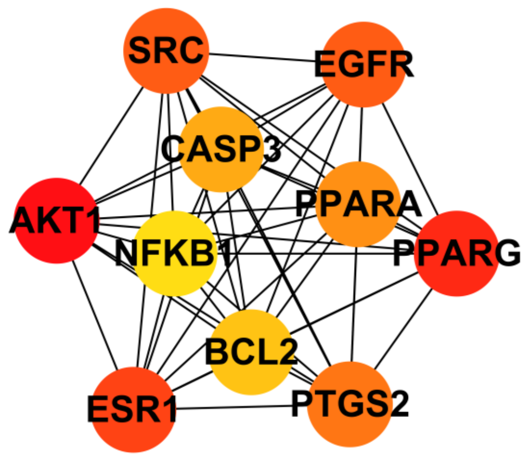


Figure 5.7. PPI enrichment network with the top 10 hub proteins

The pathway enrichment analysis by KEGG demonstrated hsa04910: Insulin signalling pathway, hsa04930: Type II diabetes mellitus, hsa04931: Insulin resistance, hsa04933: AGE-RAGE signalling pathway in diabetic complications, hsa04973: Carbohydrate digestion and absorption, and hsa00040: Pentose and glucuronate interconversions to be involved in NIDDM and Hyperglycaemia. In case of Obesity, Hyperlipidaemia, and Hypertriglyceridaemia, the pathways involved were hsa03320: PPAR signalling pathway, hsa05417: Lipid and atherosclerosis, hsa04152: AMPK signalling pathway, hsa04920: Adipocytokine signalling pathway, and hsa01100: Metabolic pathways. Other important pathways included hsa04151: PI3K-Akt signalling pathway, hsa04310: Wnt signalling pathway, and hsa04970: Salivary secretion (Table 5.3.).

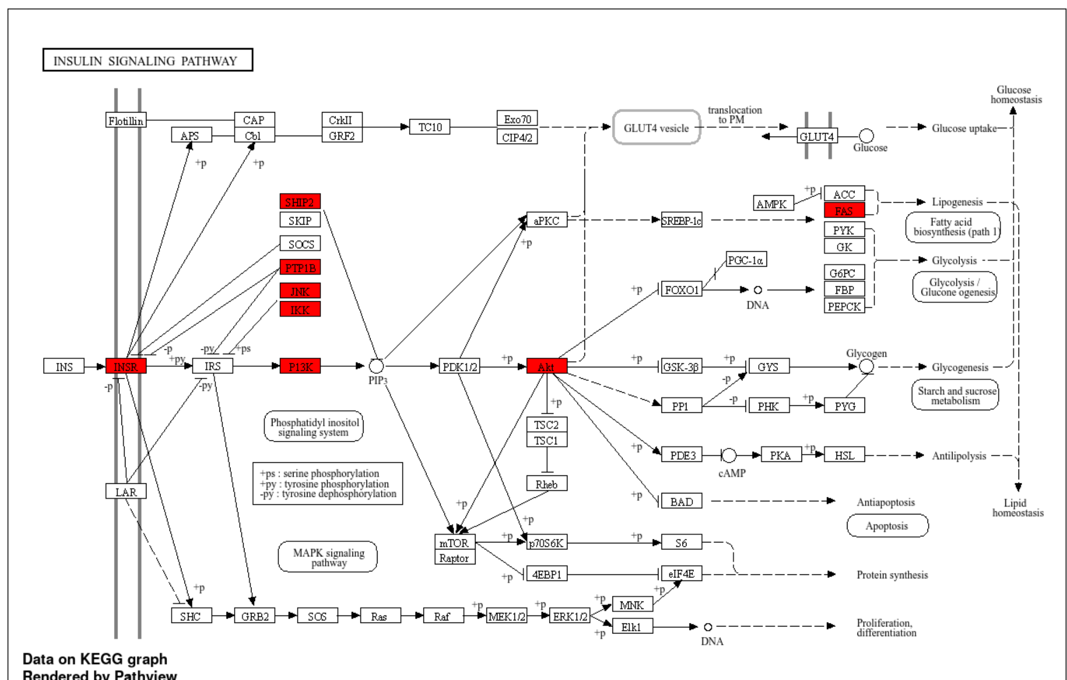
Table 5.3. KEGG enrichment pathways involved in NIDDM, Obesity, Hyperlipidaemia, Hypertriglyceridaemia and Hyperglycaemia.

Enrichment FDR	Pathway	Genes
3.27E-12	Path:hsa05417 Lipid and atherosclerosis	CYP1A1 CYP2C9 AKT1 IKBKB MMP9 NFKB1 PIK3R1 PPARG MAPK8 BCL2 BCL2L1 SRC TLR4 CASP3
1.73E-10	Path:hsa04931 Insulin resistance	NR1H3 AKT1 IKBKB INSR NFKB1 PIK3R1 PPARA MAPK8 PTPN1 NR1H2

8.72E-10	Path:hsa04151 PI3K-Akt signalling pathway	BCL2L11 CDK4 EGFR AKT1 IKBKB INSR MET NFKB1 NTRK2 PIK3CG PIK3R1 BCL2 BCL2L1 TLR4
7.01E-09	Path:hsa04152 AMPK signalling pathway	AKT1 FASN SIRT1 HMGCR INSR PFKFB3 PIK3R1 PPARG SCD
3.20E-08	Path:hsa04933 AGE-RAGE signalling pathway in diabetic complications	CDK4 AKT1 NFKB1 SERPINE1 PIK3R1 MAPK8 BCL2 CASP3
8.76E-08	Path:hsa03320 PPAR signalling pathway	NR1H3 FABP2 FABP3 PPARA PPARD PPARG SCD
2.41E-07	Path:hsa01100 Metabolic pathways	CYP1A1 CYP1A2 CYP2C9 CYP2E1 CYP3A4 FASN AKR1B1 SIRT1 GLO1 GSTM1 GSTP1 HMGCR HSD11B1 IDO1 INPPL1 PFKFB3 PIK3CG PTGS2 SCD CA3 AKR1C3 CD38
2.68E-07	Path:hsa04910 Insulin signalling pathway	AKT1 FASN IKBKB INPPL1 INSR PIK3R1 MAPK8 PTPN1
1.70E-05	Path:hsa04920 Adipocytokine signalling pathway	AKT1 IKBKB NFKB1 PPARA MAPK8
6.33E-05	Path:hsa04930 Type II diabetes mellitus	IKBKB INSR PIK3R1 MAPK8
0.020506249	Path:hsa04973 Carbohydrate digestion and absorption	AKT1 PIK3R1
0.036261499	Path:hsa04310 Wnt signalling pathway	EP300 PPARD MAPK8
0.0610705	Path:hsa04970	ADRB1 ADRB2

54	Salivary secretion	
0.14799566	Path:hsa00040 Pentose and glucuronate interconversions	AKR1B1

The hub gene enriched pathways for NIDDM, Hyperglycaemia, Obesity, Hyperlipidaemia, and Hypertriglyceridaemia are shown in Figure 5.8.



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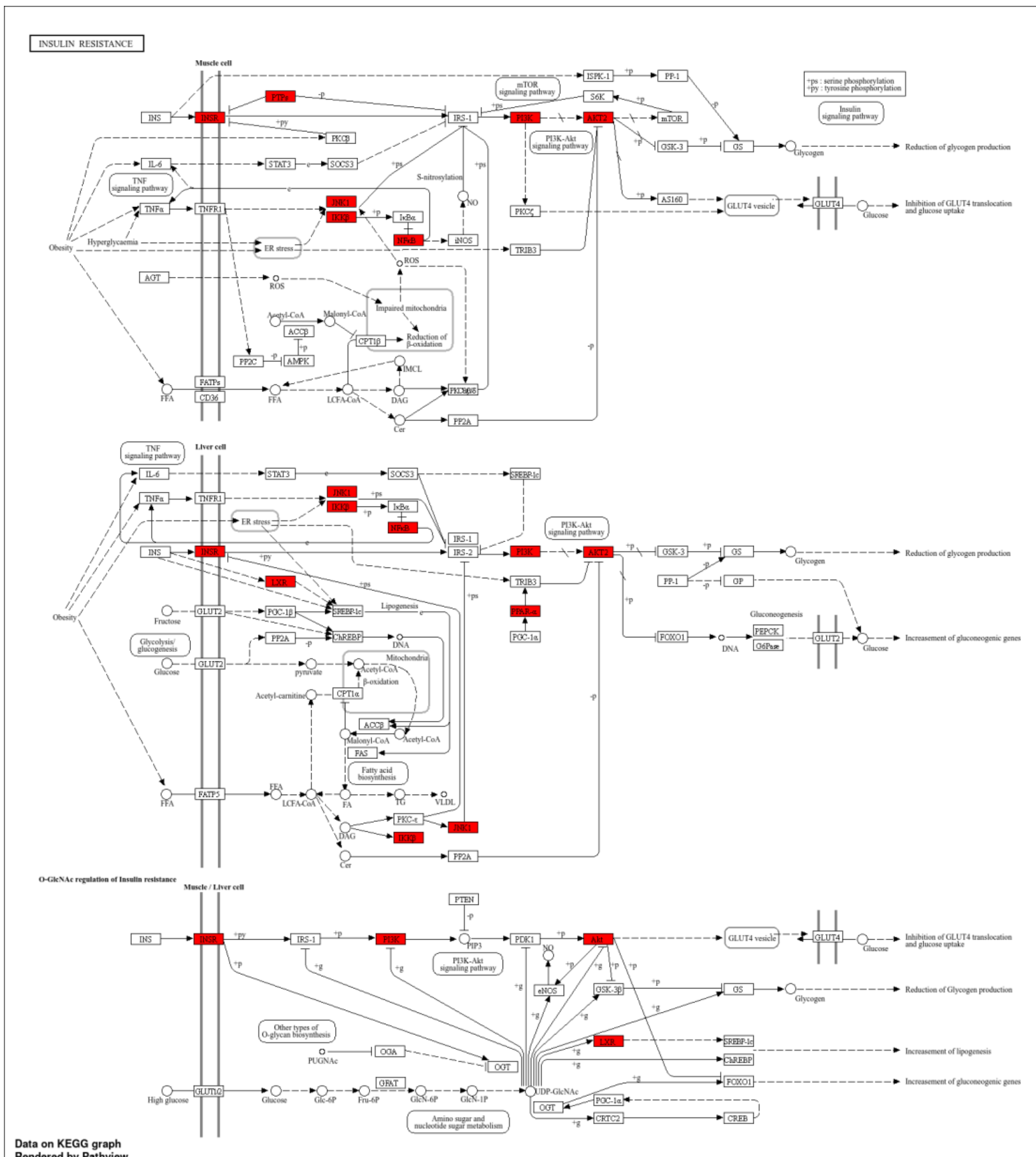

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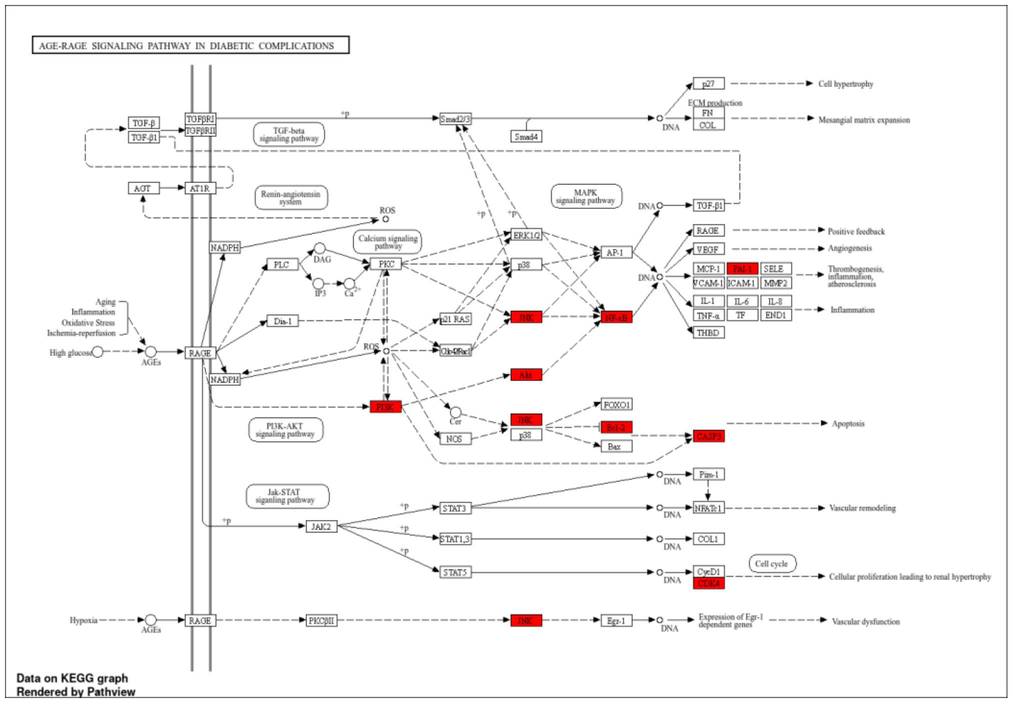

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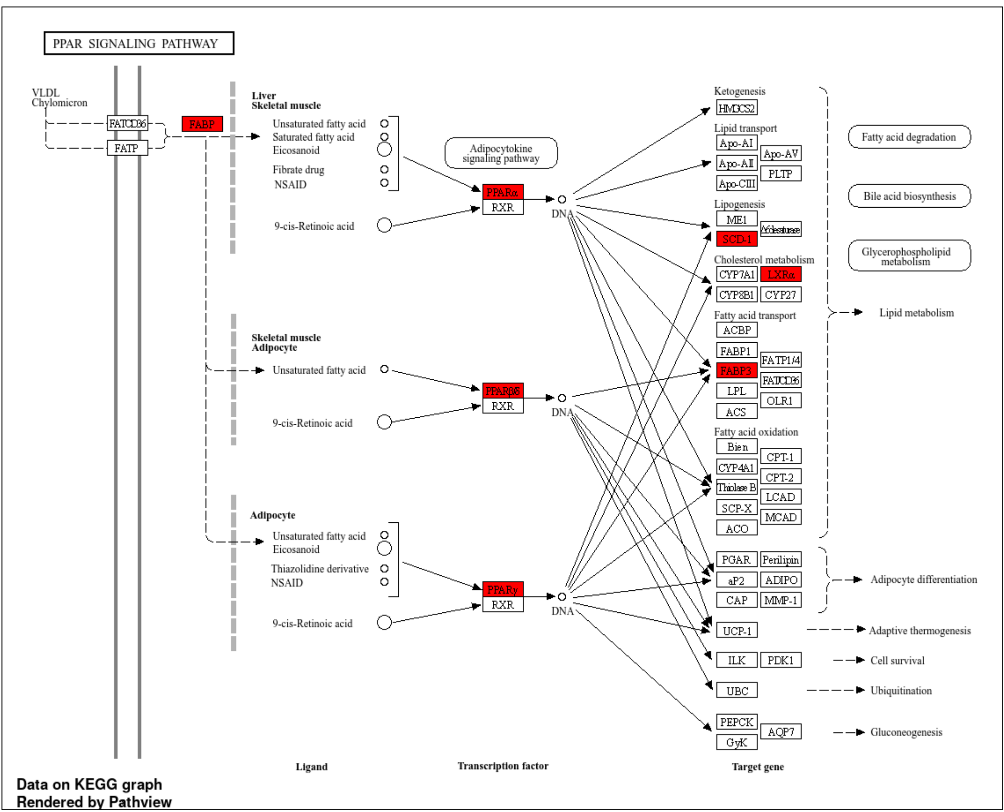

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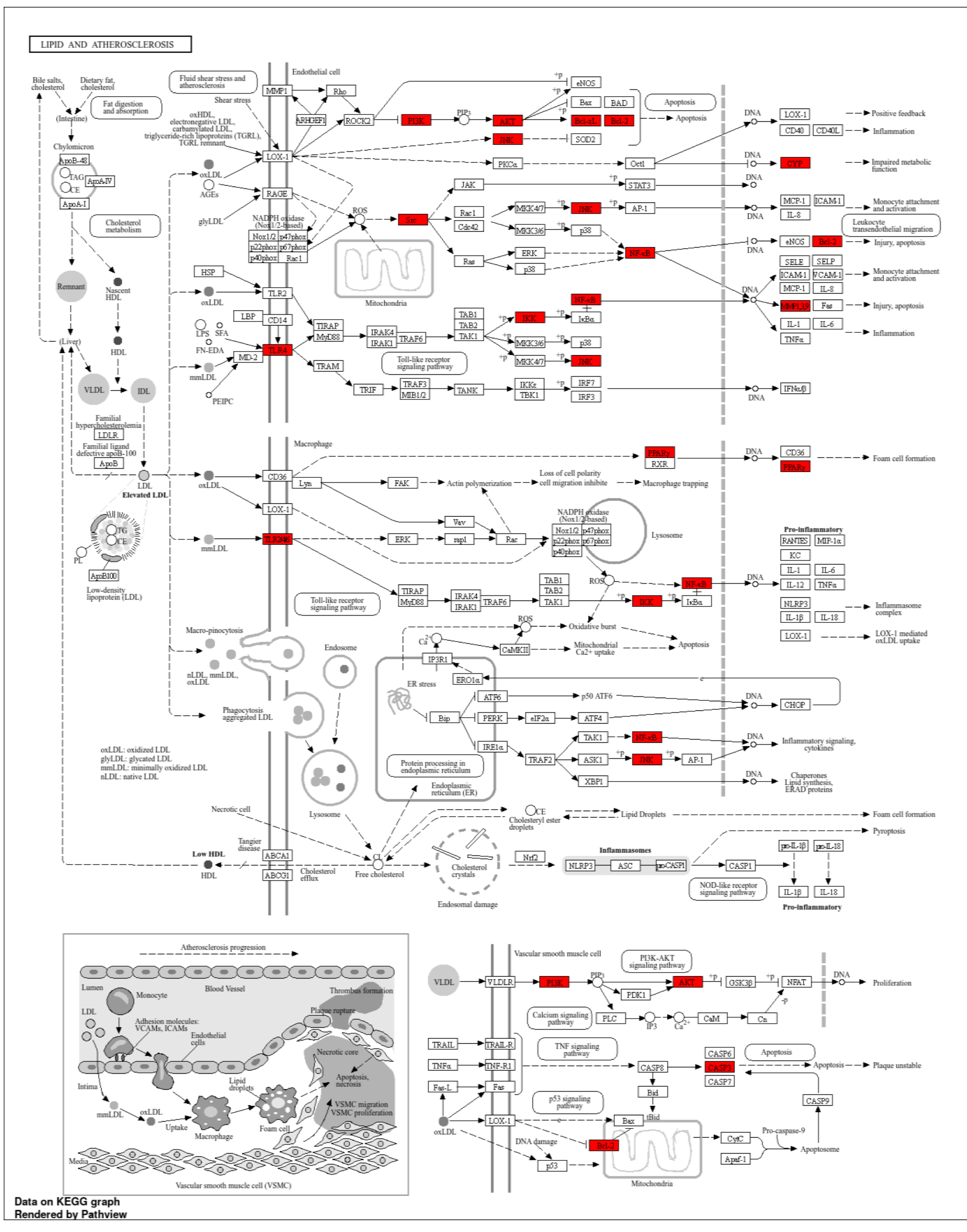

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Figure 5.8. The hub gene enriched pathways A: Insulin signalling pathway; B: Insulin resistance pathway; C: AGE-RAGE signalling pathway in diabetic complications; D: PPAR signalling pathway; E: Lipid and atherosclerosis pathway

The Gene Ontology (GO) analysis, performed using ShinyGO 0.82, explored the biological processes in which the target genes were involved. At FDR cutoff of 0.05 the enriched biological processes included lipid metabolic process (GO:0006629), cellular lipid metabolic process (GO:0044255), lipid biosynthetic process (GO:0008610), fatty acid metabolic process (GO:0006631), lipid transport (GO:0006869), response to insulin (GO:0032868), cellular response to insulin stimulus (GO:0032869), glucose homeostasis (GO:0042593), glucose metabolic process (GO:0006006), regulation of lipid transport (GO:0032368) and regulation of glucose transmembrane transport (GO:0010827) (Table 5.4.). The target gene-enriched biological processes are shown in Figure 5.9.

Table 5.4. GO Biological process pathways involved in NIDDM, Obesity, Hyperlipidaemia, Hypertriglyceridaemia and Hyperglycaemia.

Enrichment FDR	Pathway	Genes
4.67E-23	GO:0006629 lipid metabolic process	PTGS2 SCD PIK3CG PPARD HMGCR FAAH HSD11B1 CYP2E1 PPARG CYP2C9 PIK3R1 FASN PPARA AKR1C3 CES1 NFKB1 FABP3 CYP1A1 CYP3A4 GSTP1 HTR2A GSTM1 CYP1B1 CYP1A2 AKT1 HTR2C F2 NR1H3 AKR1B1 ESR1 SIRT1 NR3C1 CNR1 NR1H2 CDK4 FABP2 INPPL1 TRPV1 SRC ADRA2A NR1I2
4.26E-21	GO:0006631 fatty acid metabolic process	PTGS2 SCD PPARD FAAH CYP2E1 PPARG CYP2C9 FASN PPARA AKR1C3 GSTP1 GSTM1 CYP1B1 CYP1A1 CES1 AKR1B1 SIRT1 CNR1 FABP3 CYP1A2 FABP2 CYP3A4 NR1H3 NR1H2 AKT1
2.90E-19	GO:0008610 lipid biosynthetic process	PTGS2 SCD PIK3CG HMGCR PIK3R1 FASN NFKB1 FABP3 GSTP1 HTR2A GSTM1 AKT1 HTR2C AKR1C3 CES1

		SIRT1 PPARD NR3C1 CDK4 CYP1A1 CYP3A4 INPPL1 NR1H3 NR1H2 PPARA AKR1B1 CYP2E1 CYP2C9 CYP1A2
2.11E-17	GO:0044255 cellular lipid metabolic process	PTGS2 SCD PIK3CG PPARD HMGCR FAAH CYP2E1 PPARG CYP2C9 PIK3R1 FASN PPARA AKR1C3 FABP3 GSTP1 HTR2A GSTM1 CYP1B1 CYP1A1 CYP1A2 HTR2C CYP3A4 CES1 NR1H3 AKR1B1 SIRT1 CNR1 NR1H2 FABP2 INPPL1 AKT1
3.12E-09	GO:0042593 glucose homeostasis	INSR HIF1A SIRT1 PPARD CNR1 FFAR1 AKT1 PIK3R1 ADRA2A ACE PPARG MTNR1B
4.16E-09	GO:0032868 response to insulin	PIK3R1 PPARG SIRT1 PARP1 INSR CDK4 AKT1 INPPL1 PPARA PTPN1 SRC IDE
6.10E-09	GO:0019725 cellular homeostasis	HTR2A HTR2C DRD2 TRPV1 HIF1A OPRM1 IDE FFAR1 BCL2 F2 CD38 SIRT1 PIK3CG PPARD CNR1 MTNR1B PIK3R1 ADRA2A ACE ESR1
7.90E-09	GO:0006869 lipid transport	ABCB1 FABP3 FABP2 NFKB1 PPARG NR1H3 NR1H2 DRD2 ACE PTGS2 PPARD AKT1 CES1 PPARA
6.85E-08	GO:0032869 cellular response to insulin stimulus	PIK3R1 PPARG SIRT1 PARP1 INSR CDK4 AKT1 PTPN1 SRC IDE
8.46E-07	GO:0006006 glucose metabolic process	SIRT1 EP300 INSR NR3C1 AKT1 INPPL1 PPARA SRC PPARD
2.32E-06	GO:0032368	NFKB1 PPARG NR1H3 NR1H2 FABP3

	regulation of lipid transport	AKT1 PPARA
4.91E-05	GO:0010827 regulation of glucose transmembrane transport	INSR ACE TERT AKT1 PIK3R1

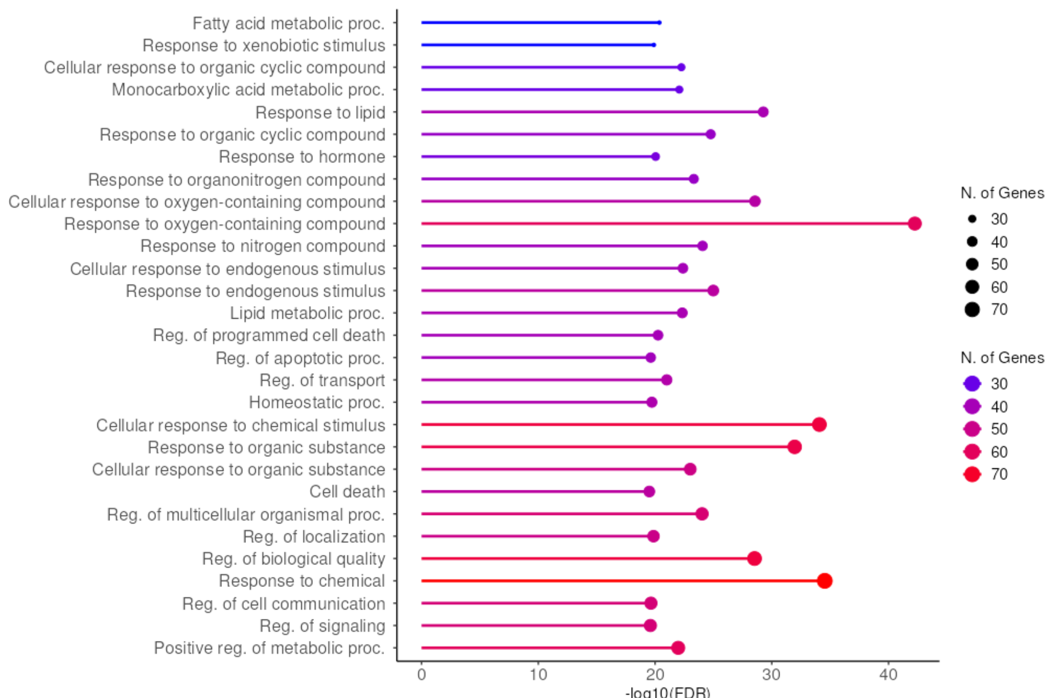
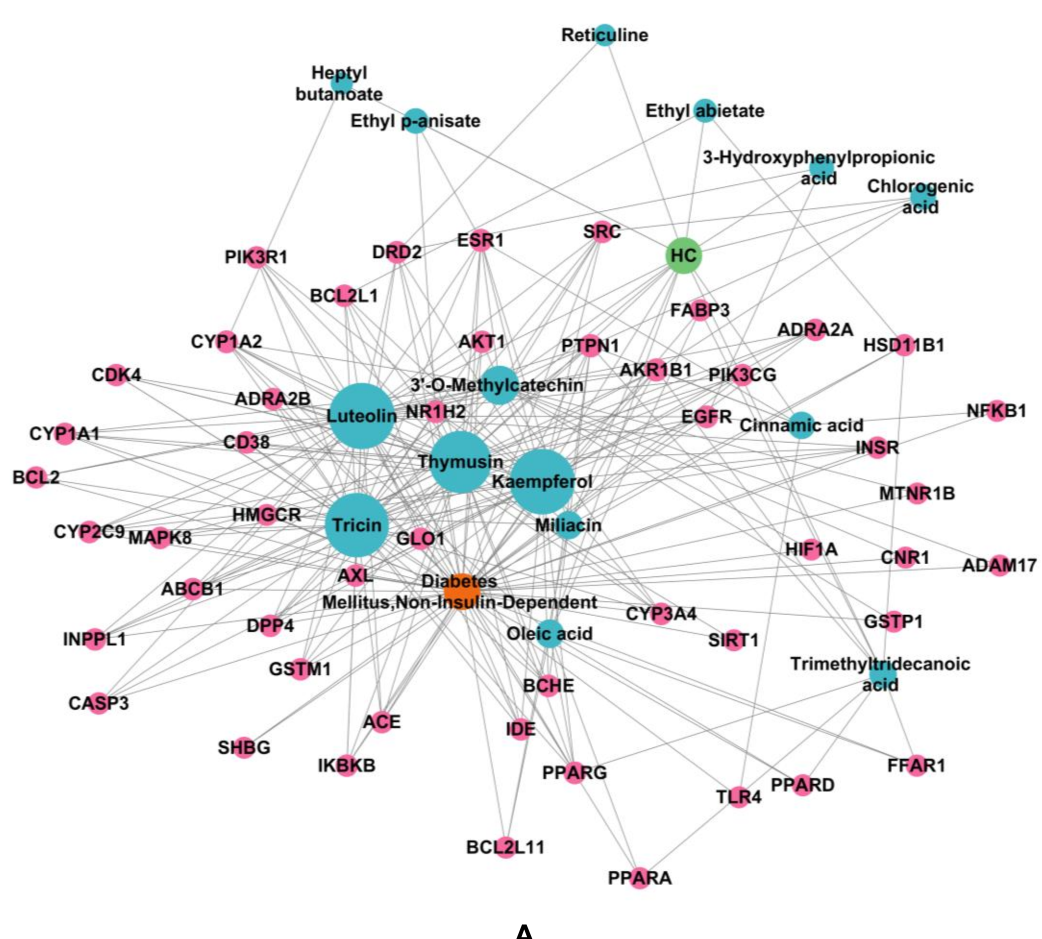


Figure 5.9. Top 30 enriched biological processes involved in the regulation of NIDDM, Obesity, Hyperlipidaemia, Hypertriglyceridaemia and Hyperglycaemia.

5.2.2.3. *Combination network analysis*

The union network of BOT–BA–TAR–DIS for NIDDM, Obesity, Hyperlipidaemia, Hypertriglyceridaemia, and Hyperglycaemia was created in Cytoscape and summarised in yfiles organic layout model. Each network was analysed using the “Analyze Network” tool. Luteolin had the highest level of connectivity in NIDDM and obesity. In hyperlipidaemia and hypertriglyceridaemia, kaempferol had the

highest level of connectivity. In hyperglycaemia, 3'-O-methylcatechin had the highest degree of connection. PTPN1 was recognised as the primary targeted protein in NIDDM, PTGS2 in hyperglycaemia and obesity, ABCB1 in hyperlipidaemia, and MET in hypertriglyceridaemia. Figure 5.10. illustrates all the combination synergy networks.



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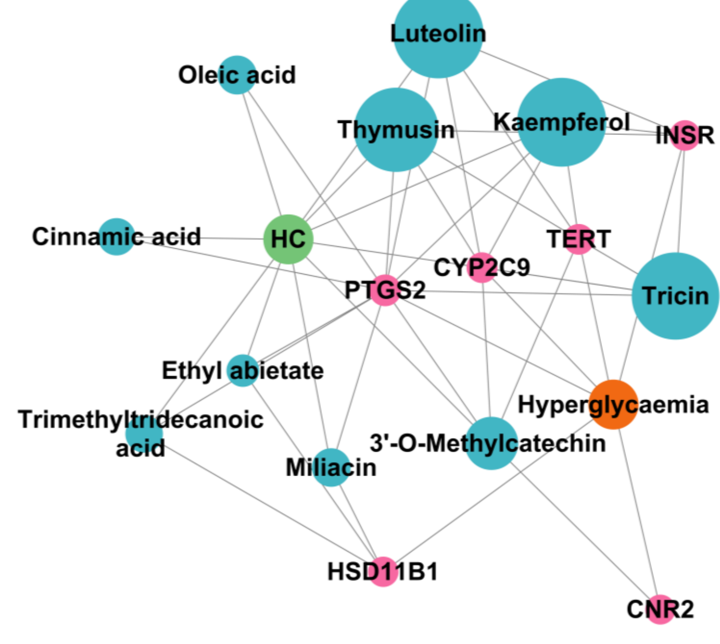

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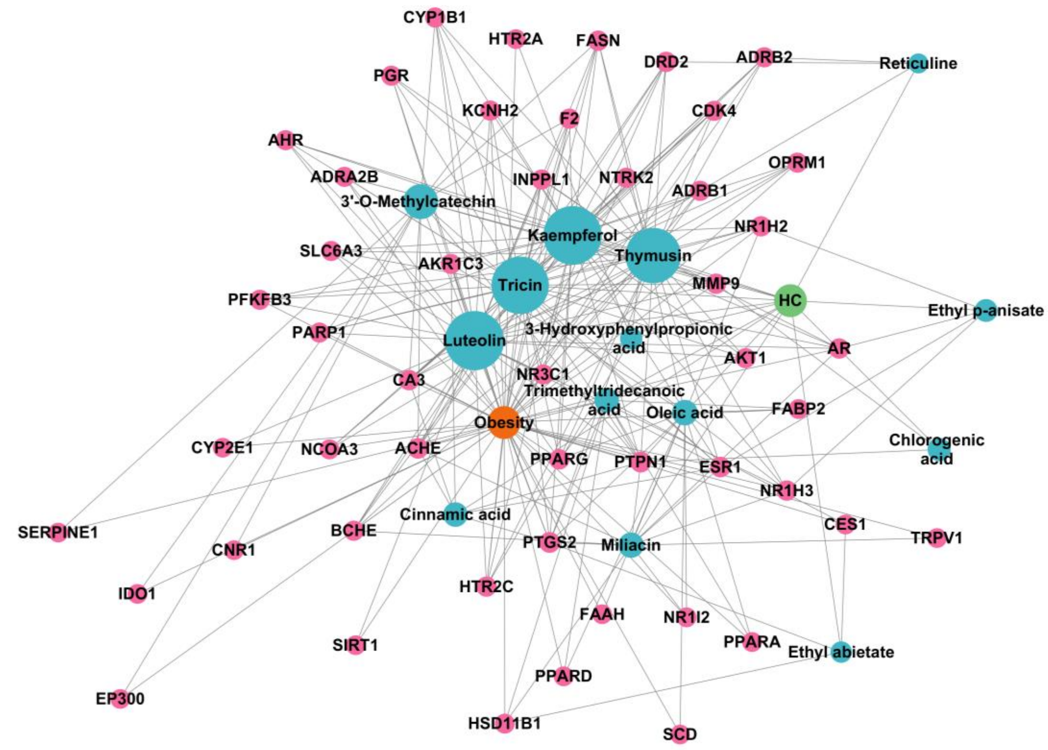

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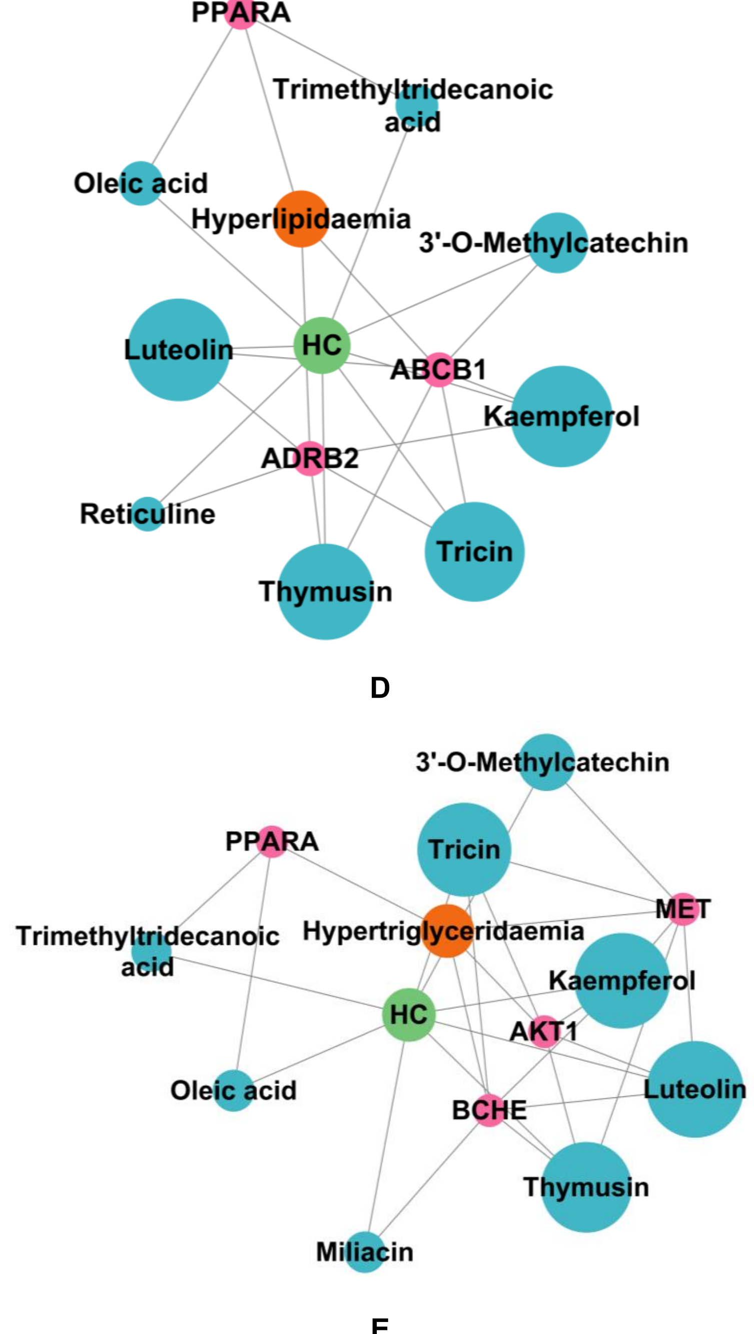


Figure 5.10. Combination synergy network analysis of *Houttuynia cordata* Thunb. (green circle denoted with HC) showing hub nodes interacting to exert the pharmacological effect against A: NIDDM; B: Hyperglycaemia; C: Obesity; D: Hyperlipidaemia and E: Hypertriglyceridaemia. The larger the size of each node, the greater its interaction potential.

[Blue circles are the identified phytoconstituents; Pink circles represent human target proteins; Orange circles represent the disorder]

5.2.3. *Total flavonoid and phenolic content of H. cordata*

The phenolic and flavonoid content was determined from the equations of gallic acid ($y = 0.0012x + 0.1132$, $R^2 = 0.9836$) and Rutin ($y = 0.001x + 0.0362$, $R^2 = 0.9919$), respectively. The standard curve is presented in Figure 5.11. HEHC exhibited a TPC of 12.873 ± 0.952 mg gallic acid equivalent/g and TFC of 7.49 ± 1.1 mg Rutin equivalent/g.

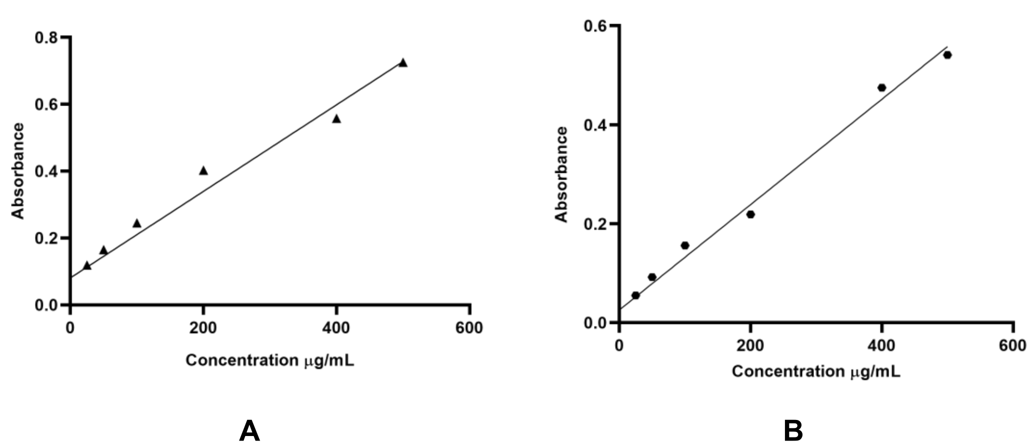


Figure 5.11. Standard curve of (A) Gallic acid and (B) Rutin

5.2.4. *Radical scavenging potential of H. cordata*

HEHC exhibited dose-dependent antioxidant potential (Figure 5.12.). In the DPPH radical scavenging potential assay, HEHC showed an IC_{50} value of 137.8 ± 0.29 µg/mL compared to 53.58 ± 0.064 µg/mL by ascorbic acid. The hydroxyl scavenging capacity of HEHC exhibited an IC_{50} value of 213.1 ± 0.56 µg/mL compared to 59.94 ± 0.49 µg/mL by ascorbic acid. HEHC demonstrated significant antioxidant potential, with an IC_{50} of 262.3 ± 0.54 µg/mL, compared to 120 ± 2.3 µg/mL for ascorbic acid in the nitric oxide scavenging assay.

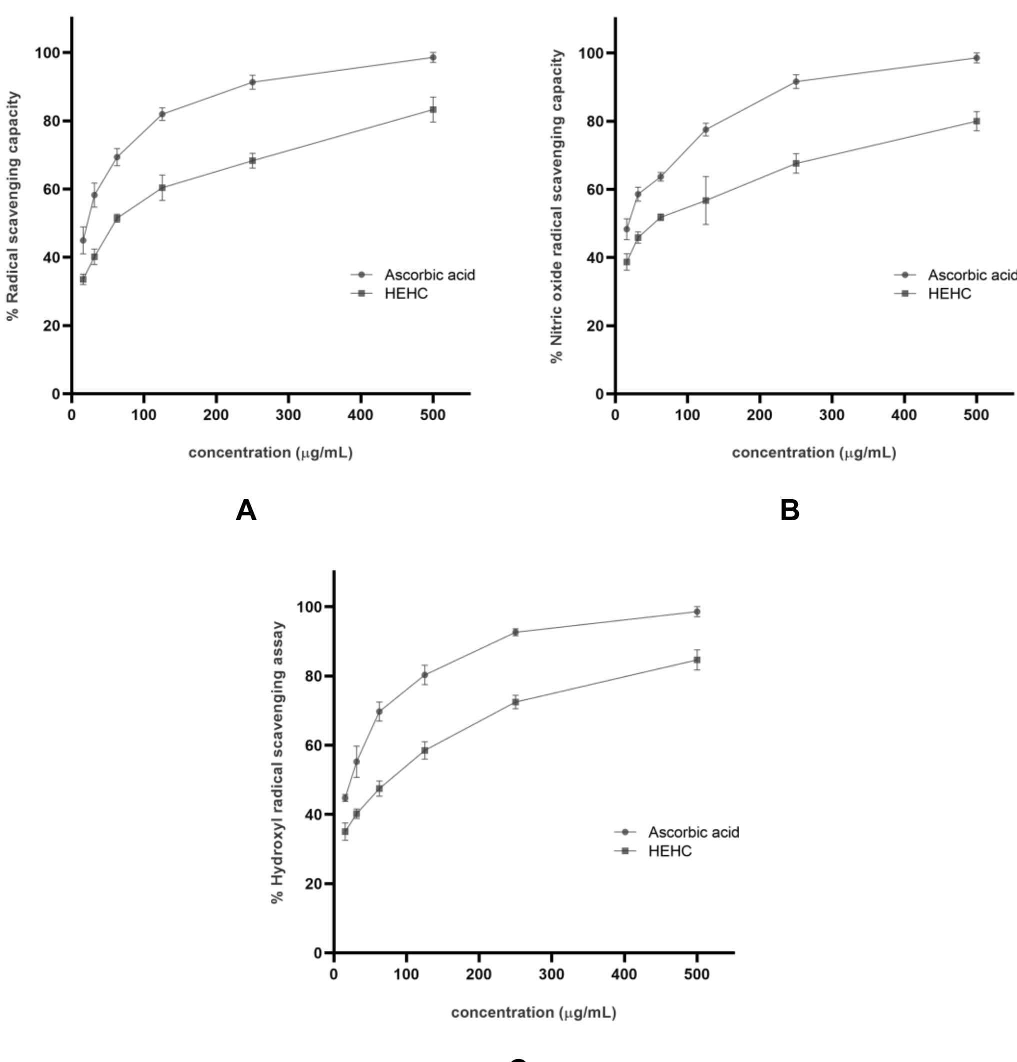


Figure 5.12. Antioxidant potential of *H. cordata* (A) DPPH free radical scavenging potential (B) Nitric oxide (NO) scavenging potential, and (C) Hydroxyl (OH) radical scavenging potential

5.2.5. *Enzyme inhibitory potential of H. cordata*

In the α-glucosidase inhibition assay, the half-maximal inhibitory concentration (IC_{50}) of HEHC was found to be 0.645 ± 0.27 mg/mL compared to 0.294 ± 0.08 mg/mL for acarbose. In the α-amylase inhibition assay, the IC_{50} of HEHC was found to be 1.145 ± 0.08 mg/mL, which is comparable to an IC_{50} of 0.532 ± 0.04 mg/mL for acarbose. In the case of pancreatic lipase, HEHC exhibited an

inhibition potential of 1.02 ± 0.42 mg/mL compared to 0.739 ± 0.09 for Orlistat. The dose–response curve of HEHC and acarbose against α-glucosidase and α-amylase inhibition, and Orlistat against pancreatic lipase inhibition is shown in Figure 5.13. Multiple comparisons of the IC_{50} values with two-way ANOVA between HEHC and acarbose for both enzymes, α-glucosidase and α-amylase, and Orlistat for pancreatic lipase, followed by Dunnett's test, showed significant results ($p < 0.0001$), suggesting that HEHC has inhibited all three enzymes in a strong manner comparable to the standard inhibitors.

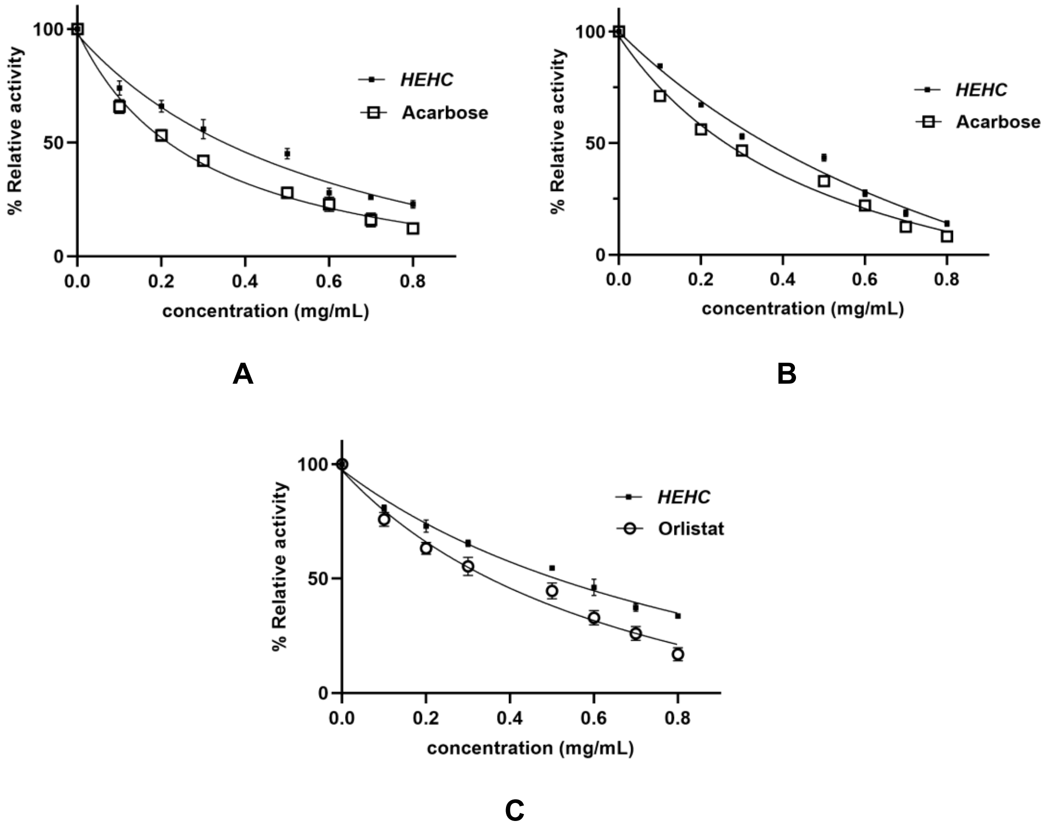
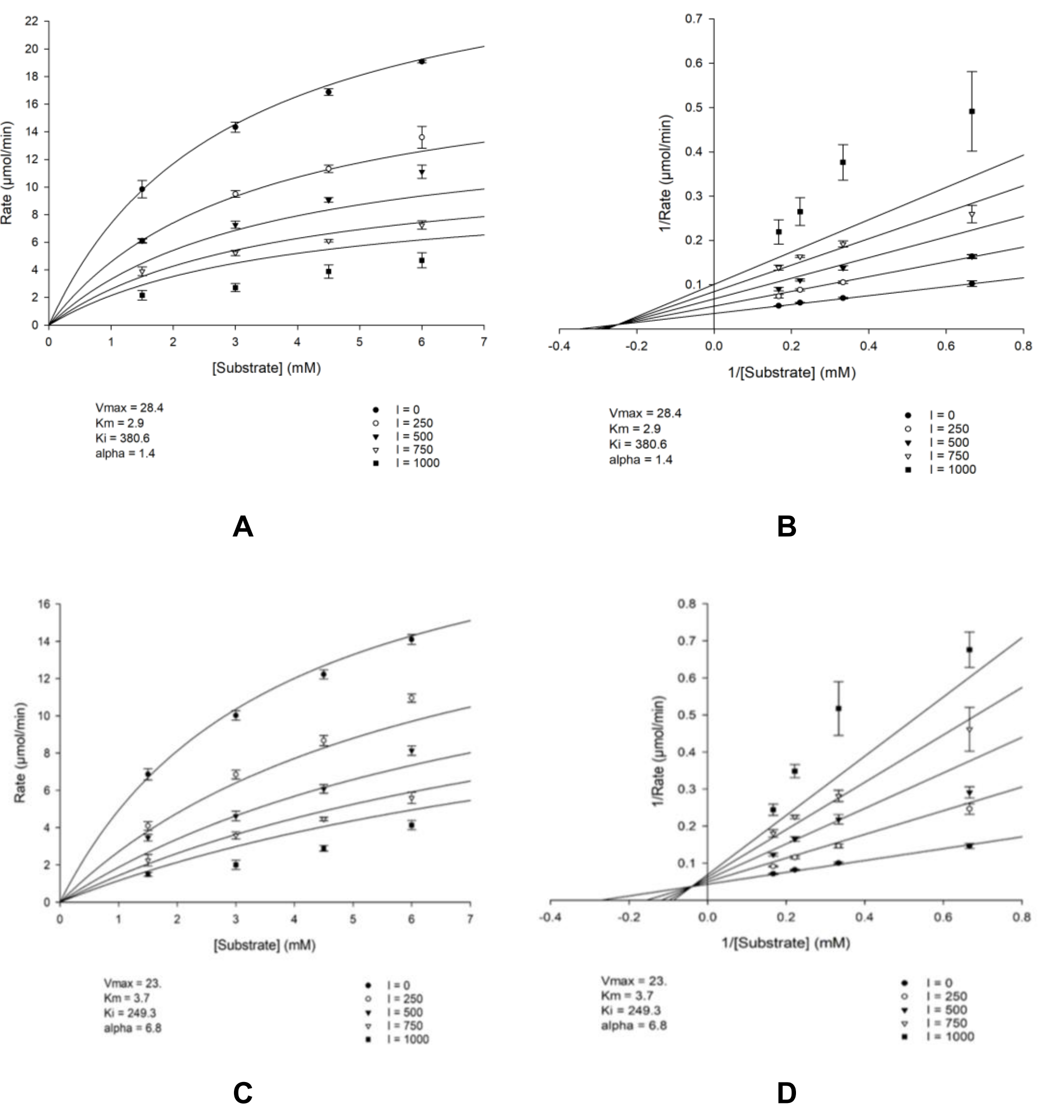


Figure 5.13. Dose–response curve of HEHC and acarbose against (A) α-glucosidase and (B) α-amylase inhibition, and orlistat against (C) Pancreatic lipase inhibition.

(-□-) acarbose (-○-) orlistat and (—■—) HEHC; data are presented as mean ± SD (n = 3).

5.2.6. *Kinetic parameters and inhibition mechanism of H. cordata*

Reaction velocity (v) vs enzyme concentration (I = 0–1000 μg/mL) showed reversible inhibition of α-glucosidase, α-amylase, and pancreatic lipase by HEHC, with an inversely decreasing slope at higher inhibitor concentrations (I). The α-glucosidase inhibition kinetics yielded a V_{max} of 28.4 ± 0.75 mM/min, a K_m of 2.9 ± 0.38 mM, a K_i of 380.6, and an α of 1.4. The α-amylase inhibition kinetics yielded a V_{max} of 23 ± 0.33 mM/min, a K_m of 3.7 ± 0.55 mM, a K_i of 249.3, and an α of 6.8. The pancreatic lipase inhibition kinetics yielded a V_{max} of 152.7 ± 1.54 mM/min and a K_m of 2.6 ± 0.43 mM, a K_i of 188.4, and an α of 5.4 (Figure 5.14).



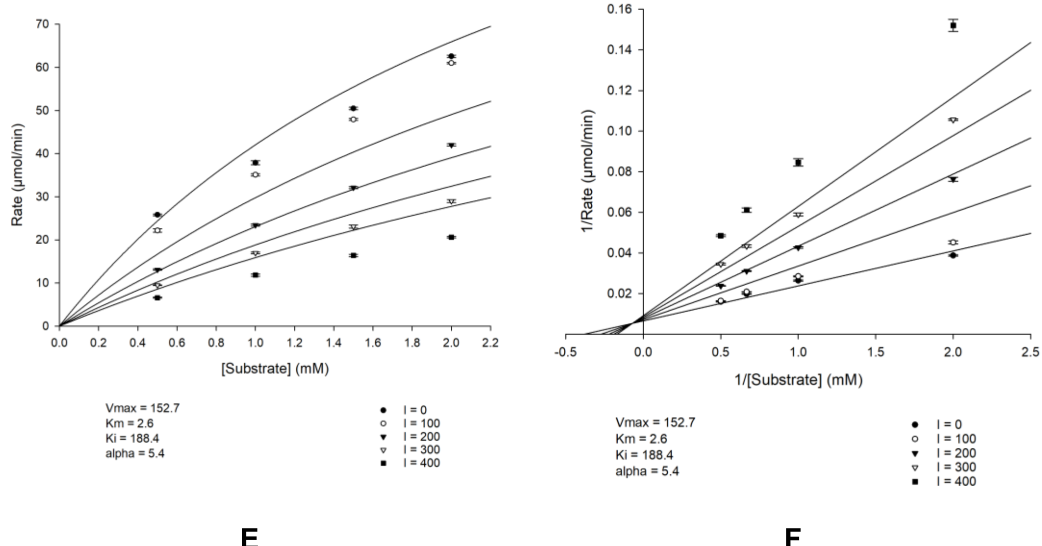


Figure 5.14. Enzyme inhibition kinetics of HEHC (● I=0 µg/ml; ○ I=250 µg/ml; ▼ I=500 µg/ml; ▽ I=750 µg/ml; ■ I=1000 µg/ml).

Michaelis–Menten plot against (A) α-glucosidase, (C) α-amylase and (E) pancreatic lipase inhibitory activity; Lineweaver–Burk plot against (B) α-glucosidase, (D) α-amylase and (F) pancreatic lipase inhibitory activity.

5.2.7. *Correlation Analysis of TPC, TFC, and antioxidant potential with enzyme inhibitory potential*

Pearson correlation analysis between the α-glucosidase and α-amylase inhibitory potential and the TPC & TFC values was evaluated and is shown in Table 5.5. The IC_{50} value of enzymes in mg/mL was correlated with the IC_{50} value (mg/mL) of the antioxidant potential (DPPH, NO, OH) and mg/g of TPC and TFC. It was observed that TPC and TFC of HEHC showed a positive linear relationship with α-amylase, α-glucosidase, and pancreatic lipase, which was reflected in the Pearson r value at $p<0.05$. The enzyme inhibitory activity of HEHC was found to be related to TPC and TFC, indicating that phenolic and flavonoid compounds present in *H. cordata* leaves play a significant role in inhibitory activity, although not as significant as those in *A. hookeri and B. hispida*.

Similarly, Pearson correlation analysis exhibited a strong correlation between the antioxidant potential and enzyme inhibitory activity of HEHC. The Pearson

correlation coefficient, along with the level of significance, has been summarised in Table 5.5. The strong correlation suggests that the antioxidant potential of HEHC was beneficial in inhibiting all three enzymes.

Table 5.5. Pearson correlation analysis of enzyme inhibitor potential of HEHC with total phenolic and flavonoid content and antioxidant potential [p < 0.05].

Pearson correlation coefficient (r) for α-amylase inhibition potential				
DPPH	**NO**	**OH**	**GAE**	**RE**
0.97^{ns}	0.94^{ns}	0.87^{ns}	0.90^{ns}	0.83^{ns}
Pearson correlation coefficient (r) for α-glucosidase inhibition potential				
DPPH	**NO**	**OH**	**GAE**	**RE**
0.97^{ns}	0.94^{ns}	0.87^{ns}	0.90^{ns}	0.83^{ns}
Pearson correlation coefficient (r) for pancreatic lipase inhibition potential				
DPPH	**NO**	**OH**	**GAE**	**RE**
0.97^{ns}	0.94^{ns}	0.87^{ns}	0.90^{ns}	0.83^{ns}

5.3. Discussion

The hydroalcoholic extract of *H. cordata* was found to be rich in flavonoids, prenol lipids and organooxygen compounds. Flavonoids, such as kaempferol (Yao et al., 2024), luteolin (Sojan and Xu, 2025), and tricin (Lee and Imm, 2018) have been previously reported to exhibit therapeutic potential against metabolic disorders. The metabolomics integrated network pharmacology analysis explored thymusin, a flavonoid, as a novel compound showing a high degree of association with the bioactive targets involved in the regulation of non-insulin dependent diabetic mellitus, hyperglycaemia, obesity, hyperlipidaemia, and hypertriglyceridaemia.

The phytocompound target interaction network exhibited a total of 1535 predicted interactions. The Venn diagram analysis suggested that *H. cordata* shared a mechanistic overlap with the reference standards atorvastatin, orlistat and acarbose, suggesting a multitargeted approach in the management of non-insulin dependent diabetic mellitus, hyperglycaemia, obesity, hyperlipidaemia, and

hypertriglyceridaemia. The PPI enrichment analysis demonstrated that the proteins exhibit a high degree of interconnectivity, with interactions occurring 3 times more than expected and a very low p-value, indicating their involvement in shared biological pathways.

The phytocompounds were found to be linked to human gene targets AKT1, PPARA, and PPARG (Bao et al., 2020), as well as EGFR, which have therapeutic implications in hyperglycaemia and hyperlipidaemia (Banerjee et al., 2019). The pathway enrichment analysis explored INSR, PI3K, AKT, PTP1B, and FAS to be modulated by HEHC in the insulin signalling pathway. The upregulation of INSR phosphorylates insulin receptor substrates, which in turn further activate PI3K-AKT in the INSR-PI3K/AKT signalling pathway. The activation of PI3K-AKT leads to the regulation of glucose and lipid metabolism (Lee et al., 2023). Previous studies have shown that PTP1B negatively regulates both insulin and leptin signalling. Inhibition of PTP1B may be able to modulate type 2 diabetes mellitus (Paul et al., 2023). The compounds were also found to modulate PPARα, PPARβ and PPARγ in the PPAR signalling pathway. PPAR was found to metabolise cholesterol and improve lipid profile (Moller and Berger, 2003). The biological processes identified through enrichment analysis normalise lipid and glucose metabolism, restore insulin sensitivity, reduce fat accumulation in tissues, and balance blood sugar and lipid levels.

The combination network analysis revealed that kaempferol and catechin were the most biologically active phytocompounds. Previous studies have reported that kaempferol acts through the PPAR signalling pathway via fatty acid-binding protein (FABP), regulating fatty acid metabolism, oxidation, and lipid storage via PPARα, PPARβ, and PPARγ (Ebrahimi et al., 2015). The potential of kaempferol to improve insulin sensitivity and protect against β-cell dysfunction by upregulating glucose transport-4 (GLUT4) and AMP-dependent protein kinase (AMPK) expression in a high-fat diet animal model has also been previously reported (Banerjee et al., 2023).

The enzyme inhibition analysis results indicate that *H. cordata* successfully inhibited α-glucosidase, α-amylase, and lipase, exhibiting efficacy comparable to

the reference standards. *H. cordata* demonstrated a hybrid inhibition mechanism, exhibiting the most effective inhibitory effect on α-amylase which highlights the beneficial effect of *H. cordata* on postprandial hyperglycaemia. *H. cordata* exhibited a certain level of pancreatic lipase inhibition. The correlation analysis indicated a favourable association between enzyme inhibition potential and both antioxidant and phenolic content; however, these correlations were largely considered non-significant at $p < 0.05$. Thus, the cluster of phytoconstituents collectively contributed to enzyme inhibition, rather than relying solely on polyphenolic concentration.

5.4. Comparative estimation

The metabolite profiling of the hydroalcoholic extracts of *A. hookeri*, *B. hispida* and *H. cordata* revealed the rich cluster phytoconstituents present mainly from flavonoids, fatty acyls, prenol lipids, cinnamic acid derivatives and others. *A. hookeri* (27 compounds) exhibited richer and a more diverse array of bioactive phytocompounds compared to *B. hispida* (17 compounds) and *H. cordata* (26 compounds). The comparative analysis has been illustrated in Figure 5.15.

An integrative approach combining metabolomics and network pharmacology analysis was employed to evaluate the bioactive potential of the uncovered phytocompounds. *A. hookeri* exhibited all 27 compounds identified to be linked to human targets, while *B. hispida* presented 16 compounds, and *H. cordata* revealed 17 compounds related to human targets, indicating a significant assortment of bioactive phytoconstituents in the hydroalcoholic extract of *A. hookeri*. The compound-target relationship, assessed by the BA-TAR network, examined connections via network topology data. *A. hookeri* exhibited distinct system-level interactions with 867 nodes and 11485 edges, resulting in a highly interconnected network, in contrast to the other two plants. This dense connectivity demonstrated a notable polypharmacological and synergistic mechanism-based strategy in the management of metabolic dysregulations such as NIDDM and obesity.

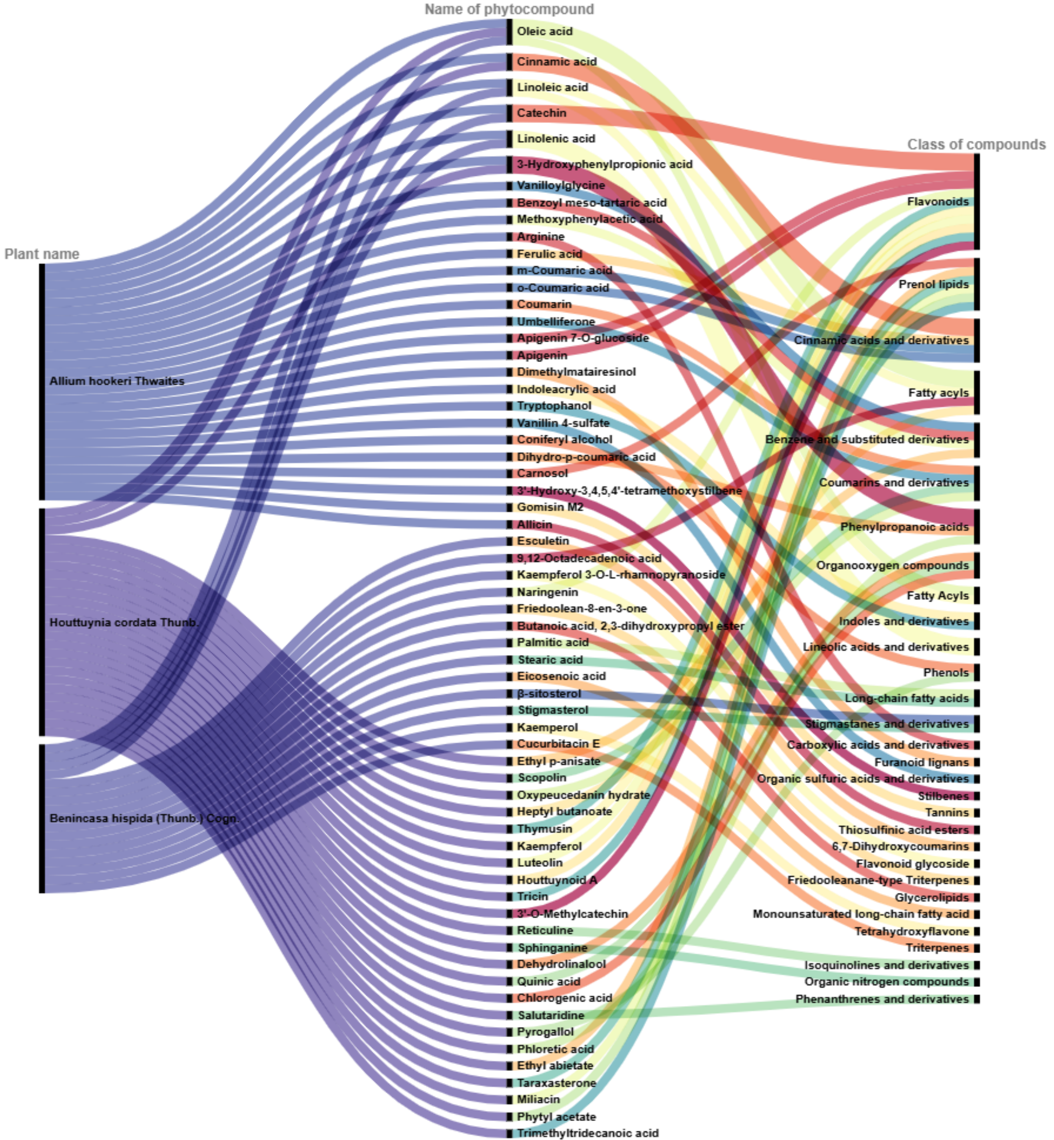


Figure 5.15. Alluvial plot comparative analysis of the cluster of phytocompounds and their classes present in the hydroalcoholic extracts of *A. hookeri*, *B. hispida* and *H. cordata*.

A. hookeri demonstrated a greater number of common genes linked to non-insulin dependent diabetic mellitus, hyperglycaemia, obesity, hyperlipidaemia, and hypertriglyceridaemia. *H. cordata* demonstrated analogous gene correlations similar to those of *A. hookeri*. In instances of hyperlipidaemia and hypertriglyceridaemia, both *A. hookeri* and *H. cordata* exhibited an equivalent

quantity of shared genes. In all instances, *B. hispida* exhibited the fewest shared genes with the DisGeNET database.

The analysis of protein-protein interaction enrichment for targets associated with NIDDM, hyperglycaemia, obesity, hyperlipidaemia, and hypertriglyceridaemia indicated that *A. hookeri* exhibited the highest PPI enrichment. *A. hookeri* demonstrated approximately double the anticipated enrichment and an average node degree of 43, suggesting it operates as a multi-target modulator. In contrast, *B. hispida* and *H. cordata* exhibited significantly fewer interconnections and a lower average node degree. As a result, *A. hookeri* appears to exert a broader regulatory impact, which is relevant to the amelioration of metabolic dysregulations.

The top 10 hub proteins of *A. hookeri* demonstrated extensive mechanistic regulation through proteins linked to glucose homeostasis, inflammation, lipid metabolism, and glycolytic regulation. *B. hispida* and *H. cordata* have common targets with *A. hookeri,* like AKT1 and PPARG, while the other targets primarily focus on inflammation and apoptosis in *B. hispida* and lipid metabolism in *H. cordata*. The pathway enrichment analysis examined pathways associated with the selected metabolic disorders. While all three plants were found to follow similar pathways in the management of selected metabolic dysregulations, the pathways were more enriched with bioactive targets in *A. hookeri*, followed by *H. cordata* and *B. hispida*. These results suggest that *A. hookeri* exhibits a higher level of targeted regulation of metabolic dysregulation. This multi-target network enhances the translational potential and the likelihood of *in vivo* efficacy of *A. hookeri*.

In vitro analysis of the enzyme inhibition potential of *A. hookeri* demonstrated the lowest IC_{50} value among the three enzymes: α-glucosidase, α-amylase, and pancreatic lipase. *A. hookeri* demonstrated significant inhibitory activity, presenting the highest probability of seeing antihyperlipidaemic and antihyperglycaemic effects *in vivo*.

The enzyme kinetics analysis of *A. hookeri* against α-glucosidase, α-amylase, and lipase indicated a strong inhibitory potential against α-glucosidase and α-amylase, which are crucial in the management of NIDDM and hyperglycaemia. Furthermore, the moderate inhibition potential against lipase suggests an inhibitory potential against obesity, hyperlipidaemia, and hypertriglyceridaemia. In α-glucosidase and α-amylase inhibition, *A. hookeri* exhibited the lowest K_m values, moderate to low K_i values, and the highest α values, suggesting a higher affinity towards the free enzyme and potent inhibitory activity. In lipase inhibition kinetics, all three plants exhibited similar V_{max} and K_m values. *A. hookeri* showed moderate K_i and α values, indicating moderate lipase inhibition. *B. hispida* showed better lipase inhibition than the other two.

Thus, the strong inhibition potential of *A. hookeri*, combined with its moderate lipase inhibitory potential, provides a mechanistic rationale for managing hyperglycemia and hyperlipidaemia. Ultimately, concentrating resources on the most promising candidate reduces animal usage, laboratory time, and expenses, while enhancing the likelihood of identifying significant antihyperglycemic and antihyperlipidemic effects. Hence, *A. hookeri* hydroalcoholic extract was considered for further *in vivo* evaluation.

5.5. Conclusion

The comparative analysis of *A. hookeri*, *B. hispida*, and *H. cordata*, utilising metabolite profiling, network pharmacology to elucidate mechanisms of action, and *in vitro* enzyme inhibition assays, demonstrated substantial pharmacological potential against lifestyle-related metabolic disorders, particularly NIDDM and obesity, corroborating the traditional claims. *A. hookeri* emerged as the most potent contender due to its abundant array of bioactive compounds and its exceptional antioxidant and enzyme inhibitory capabilities. These findings demonstrate a promising lead to the therapeutic potential of *A. hookeri* hydroalcoholic extract and can be associated with appropriate *in vivo* investigations into metabolic dysregulations generated by modern lifestyle-oriented diets containing high-fat and high-sugar contents. The following chapter explored the *in vivo* pharmacological potential of *A. hookeri* hydroalcoholic extract

in an experimental mouse model mimicking current lifestyle-oriented dietary patterns.

5.6. Publications and conference presentations

5.6.1. *Paper communicated*

- Das Gupta, B., Gayen, S., Kar, A., Haldar, P. K., & Mukherjee, P. K. Metabolite profiling and network pharmacology analysis of *Houttuynia cordata* Thunb. against hyperlipidaemia and hyperglycaemia. Phytochemical Analysis. Wiley. [Communicated].

5.6.2. *Paper presented*

- Das Gupta, B., Kar, A., Singha, S., Jana, S., Gayen, S., Chowdhury, S., Haldar, P. K., & Mukherjee, P. K. LC-QTOFMS-based metabolite profiling and evaluation of α-glucosidase and α-amylase inhibitory potential of combined plant extract-based nutraceutical formulation from NER. In International Bioresource Conclave & Ethnopharmacology Congress: 22nd International Congress of International Society for Ethnopharmacology & 10th International Congress of Society for Ethnopharmacology: at Institute of Bioresources and Sustainable Development, Imphal, Manipur, India, during February 24–26, 2023.

Chapter 6

In vivo therapeutic evaluation against hyperglycaemia and hyperlipidaemia

6.1. *In vivo* evaluation of antihyperglycaemic and antihyperlipidaemic effects
6.2. Materials and methods
6.3. Results
6.4. Discussion
6.5. Conclusion
6.6. Publications and conference presentations

6.1. *In vivo* evaluation of antihyperglycaemic and antihyperlipidaemic effects

While recent advancements in *in vitro* and *in silico* models have proven crucial in the drug development process, *in vivo* investigations validate the biological efficacy of a drug's pharmacological action. *In vivo* research emulates the intricate biological system, providing toxicity profiles and safety data (Tang and Prueksaritanont, 2010). Regardless of its significance, ethical considerations must be upheld in the utilisation of animals. The utilisation of experimental animals must adhere to ethical norms encapsulated by the 3Rs: reduction, refinement, and replacement (Mukherjee et al., 2022). Various types of animal models are utilised to investigate metabolic disorders, including genetic models, diet-induced models, and chemically induced models. Among these, the diet-induced C57BL/6J model provides the most accurate representation of lifestyle-related aetiology. This procedure is replicable and encapsulates the intricacy of the condition in humans (Janapati and Junapudi, 2024).

Traditional medicine is used in the prevention, immediate treatment prior to diagnosis, acute treatment and recovery phase (Upton, 2022). The adage "Prevention is better than cure" underscores that traditional herbs and formulations offer a holistic strategy for managing chronic conditions before they progress. This method not only targets the underlying cause of an illness but also minimises side effects and is economically viable. Traditional herbs enhance immunity and help maintain the balance of bodily systems, thereby preventing the progression of chronic diseases (Mukherjee et al., 2022). The rapid pace of contemporary living has led to an increase in metabolic dysregulation due to current lifestyle choices. Although therapies exist, they often incur high costs and fail to address the underlying cause. As previously noted, the emphasis has transitioned to developing preventive strategies to alleviate the progression of metabolic dysregulations.

This chapter examines the preventive effect of *A. hookeri* leaf hydroalcoholic extract on the advancement of hyperlipidaemia and hyperglycaemia induced by a high-fat and high-sugar diet in C57BL/6J mice. The current study aims to provide

scientific validation of the traditional claim that this plant serves as "food as medicine" and offer insights for the development of value-added alternative medicines for managing metabolic dysregulations, including diabetes and obesity.

6.2. Materials and methods

6.2.1. *Chemicals and reagents*

Atorvastatin calcium was sourced from Sigma-Aldrich Chemicals (Karnataka, India), and Metformin hydrochloride was obtained from HiMedia Laboratories (Maharashtra, India). Commercially available kits, specifically the Q-Line S+ and Selectra system pack (Q-Line Biotech, New Delhi, India), were obtained for the assessment of serum and haematological parameters. All other analytical grade chemicals were obtained from HiMedia Laboratories (Maharashtra, India).

6.2.2. *Instrumentation*

The 5-part automatic blood cell counter (Medonic M51) was acquired from Boule Medical (Aktiebolag, Sweden), while a fully automated biochemical analyser (Selectra S-Lite) was sourced from ELITech Group (Puteaux, France). Animal tissue sections were prepared with an LEICA CM1520 cryostat (Leica Biosystems, Germany) and examined using an Olympus CX21i light microscope (Olympus Corporation, Tokyo, Japan), in conjunction with a Magcam DC 14 and MagVision image analysis software (Magnus Opto Systems, New Delhi, India).

6.2.3. *Animal Maintenance*

Male C57BL/6J mice, aged 7-8 weeks and weighing 18-20g, were obtained from the State Centre for Laboratory Animal Breeding, Kalyani, West Bengal. The animals were acclimatised for 10 days in the University animal house, provided with commercial rodent pellets (Kalyani Feed Milling Plant, Kalyani, India) and allowed free access to water. The animals were housed in groups of five per cage under controlled conditions of 22 ± 2°C temperature, 50 ± 10% relative humidity, and a 12-hour light/dark cycle. The Institutional Animal Ethical Committee (IAEC) [JU/IAEC-25/82 dated 16/01/2025] authorised the experimental study prior to the commencement of the initial animal experiment (Das Gupta et al., 2025).

6.2.4. *Acute toxicity and oral glucose tolerance test (OGTT)*

The acute toxicity study utilised the up-and-down procedure in accordance with OECD Test Guideline 425. The limit test involved experimental mice of both sexes (n = 5), which were gavaged with 2000 mg/kg body weight of HEAH after an overnight fast. The study examined the animals for indicators of toxicity, including alterations in weight, behaviour, physiology, morphology, and mortality rates (Das Gupta et al., 2025).

The experimental mice, exhibiting normal glycaemic levels (85–90 mg/dL), underwent an overnight fast followed by an OGTT. The experimental animals were divided into four groups (n = 5). Group I (normal control) received distilled water at a dosage of 5 mL/kg body weight, administered orally. Groups II and III received HEAH at doses of 200 and 400 mg/kg body weight, orally, based on the acute toxicity study. Group IV was administered an oral dose of 150 mg/kg body weight of Metformin. Subsequent to these treatments, each group received an oral administration of glucose at a dosage of 2 g/kg body weight. Blood samples for the OGTT were obtained from the tail vein at baseline (0 minutes) and at 30, 60, and 120 minutes post-glucose administration, with measurements taken using a single-touch glucometer (Gayen et al., 2024).

6.2.5. *Experimental design*

The experimental animals were divided into six groups, each consisting of five animals. The experimental dose was selected based on the results of an acute toxicity study. A dose of 200 mg/kg b.w. and 400 mg/kg b.w. ($1/10^{th}$ and $1/5^{th}$ of 2000 mg/kg b.w. respectively) was selected. The experimental animals were fed with high-fat and high-sugar diet (HFSD) consisting of 50% fat, 25.6% carbohydrate, 14.4% protein and 10% sugar for a period of 8 weeks (60 days) along with the test drug (HEAH) and reference control drug (RC) containing Metformin and Atorvastatin (Thakur et al., 2024; Banerjee et al., 2023). The experimental animals, after a 10-day acclimatisation period, were fasted overnight, and fasting blood glucose (FBG) levels were noted. The animal experimentation setup is illustrated in Figure 6.1., and the treatment schedule is presented in Table 6.1.

Table 6.1. Treatment regimen of HEAH, administered once daily per oral for 60 days (n=5)

Group code	Group name	Details
NC	Normal control	Normal pellet diet + Normal saline (5 ml/kg, p.o.)
DC	Disease control	HFSD only
LD	Test drug – Low dose	HFSD + HEAH (200 mg/kg b.w., p.o.)
HD	Test drug – High dose	HFSD + HEAH (400 mg/kg b.w., p.o.)
RC	Reference control	HFSD + Metformin (150 mg/kg b.w., p.o.) + Atorvastatin (10 mg/kg b.w., p.o.)
COMB	A combination of the test drug and the reference control drug	HFSD + HEAH (400 mg/kg b.w., p.o.) + Metformin (75 mg/kg b.w., p.o.) + Atorvastatin (05 mg/kg b.w., p.o.)

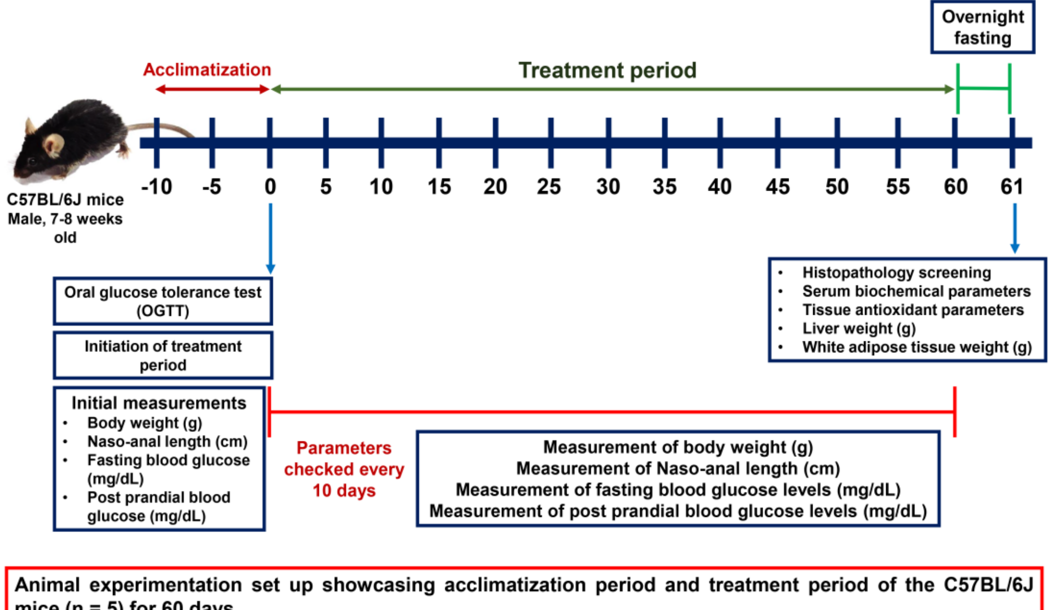


Figure 6.1. Experimental setup with C57BL/6J mice (7-8 weeks old, Male) fed with a high-fat and high-sugar diet (HFSD) for 60 days and treated with HEAH and reference control drugs

6.2.6. *Estimation of fasting blood glucose level*

The fasting blood glucose levels were measured every 10 days using a glucometer. The blood glucose levels were recorded in milligrams per deciliter (mg/dL) and then compared with disease control and normal control levels after the treatment period (Gayen et al., 2024).

6.2.7. *Measurement of body weight and body mass index parameters*

The body weight of the experimental animals was measured using a digital weighing scale every 10 days to assess changes in body weight (g) and the percentage change in body weight. The body mass index (BMI) was determined by measuring body weight (g)/Naso-anal length (cm^2) every 10 days, with the percentage change in BMI subsequently calculated. Similarly, Lees index (LI) was determined by the cubic root of body weight (g)/Naso-anal length (cm). After the treatment period, the mice were euthanised (overdose of ketamine, i.p.) and the heart, liver, and adipose tissues were excised, washed in PBS, and measured to quantify the accumulated subcutaneous fat and visceral fat (Thakur et al., 2024). The data from the test groups (LD and HD) were compared with those from the normal control (NC), disease control (DC), and reference control (RC) groups.

6.2.8. *Estimation of serum biochemical parameters*

Blood samples were collected to assess the blood and serum biochemical parameters from ketamine + xylazine (80 + 10mg/kg b.w., i.p.) anaesthetised animals by cardiac puncture, following overnight fasting and maintaining a 24-hour gap after the last dose (post-treatment period). The glycosylated haemoglobin (HbA1c) was calculated using an automatic blood cell counter (5-part) (Gayen et al., 2024). The serum was separated by centrifugation for 10 minutes at 3000 rpm, and quantification was performed using a biochemical auto-analyser with commercially available kits (Q-Line S+, Selectra system pack, Q-Line Biotech, New Delhi, India). The separated serum was utilised to analyse lipid parameters, including total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL), Apolipoprotein A-I (Apo A-I), and

Apolipoprotein B (Apo B) (Banerjee et al., 2023). To measure kidney and liver damage, serum levels of aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine transaminase (ALT), total protein, blood urea nitrogen (BUN), and serum creatinine were quantified (Gayen et al., 2024). The urine samples were collected using a metabolic cage (post-treatment period) and used to calculate urinary output, urine microalbumin, and urine creatinine clearance (Kumari et al., 2021).

6.2.9. *Estimation of tissue antioxidant parameters*

After the treatment period, the experimental animals were euthanised (overdose of ketamine, i.p.) and the pancreas, liver and kidney were collected and homogenised individually in 10mL of phosphate buffer (20mM, pH: 7.4). The homogenised mixture underwent centrifugation at 12,000 rpm for 30 minutes at 4°C utilising a Remi cooling centrifuge (RM-12C). After centrifugation, the supernatants were collected and analysed to assess the levels of lipid peroxidation (LPO), glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) (Gayen et al., 2024). Absorbance was measured using the Spectra Max iD3 Multi-Mode Microplate Reader (Molecular Device, USA).

6.2.10. *Tissue histopathological study*

The harvested organs were rinsed in normal saline and preserved in a 10% formalin solution for 24 hours. The tissues were embedded in paraffin wax and sectioned (5-10μm) in a rotary microtome (Leica Biosystems, Germany). The tissues were affixed to slides and stained with haematoxylin and eosin. The tissues were visualised for any structural damage in a light microscope, and pictomicrographs were captured through MagVision software (Das Gupta et al., 2025).

6.2.11. *Statistical analysis*

The results were visualised graphically and statistically analysed using GraphPad Prism 8.0.2 software (GraphPad Software, Inc., Boston, MA, USA). All results (n=3) were statistically analysed and presented as mean value ± standard

deviation (SD). A two-way ANOVA, followed by Dunnett's multiple comparison test at p<0.0001, was conducted to analyse the significant differences between the control, untreated, and treated groups.

6.3. Results

6.3.1. *Acute oral toxicity and OGTT analysis*

The acute oral toxicity study with HEAH showed no significant changes in the dietary habits, body weight, or behavioural pattern. The extract did not produce any signs of drowsiness or diarrhoea. All the animals survived, suggesting that the LD_{50} is greater than the test dose (2000 mg/kg). The OGTT results indicated an elevated blood glucose level during the initial 30 minutes of glucose administration, which gradually decreased to near-normal levels at 60 minutes and returned to normal by 120 minutes, as presented in Table 6.2.

Table 6.2. Results of Oral Glucose Tolerance Test (OGTT) at mg/dL. Mean ± SD

Groups	0 min	30 min	60 min	120 min
NC	83.6 ± 2.42	187 ± 8.67	145.6 ± 4.45	120.2 ± 2.56
LD	90.6 ± 2.42	179 ± 5.22	140.8 ± 5.84	118 ± 4.56
HD	88.4 ± 4.96	169 ± 5.59	130.2 ± 4.40	109.2 ± 2.71
RC	87 ± 7.69	171.6 ± 6.28	137 ± 9.34	108.8 ± 4.12

The 400 mg/kg b.w dose of HEAH and the 150 mg/kg b.w dose of Metformin showed comparable results in downregulating elevated blood glucose levels around 120 minutes after the induction of oral glucose (Figure 6.2.).

6.3.2. *Effect of HEAH on blood glucose and glycosylated haemoglobin levels*

The fasting blood glucose levels of HFSD-induced C57BL/6J mice (DC, LD, HD, RC, and COMB) were significantly elevated compared to those of NC during the experimental study. Daily administration of HEAH extract at doses of 200 and 400 mg/kg b.w., p.o. significantly ($p < 0.05$) maintained blood glucose levels near

normal compared to the disease control group. Metformin (150 mg/kg b.w. p.o.) and Atorvastatin (10 mg/kg b.w. p.o.) treated mice in RC also exhibited maintained blood glucose levels compared to DC (Table 6.3.).

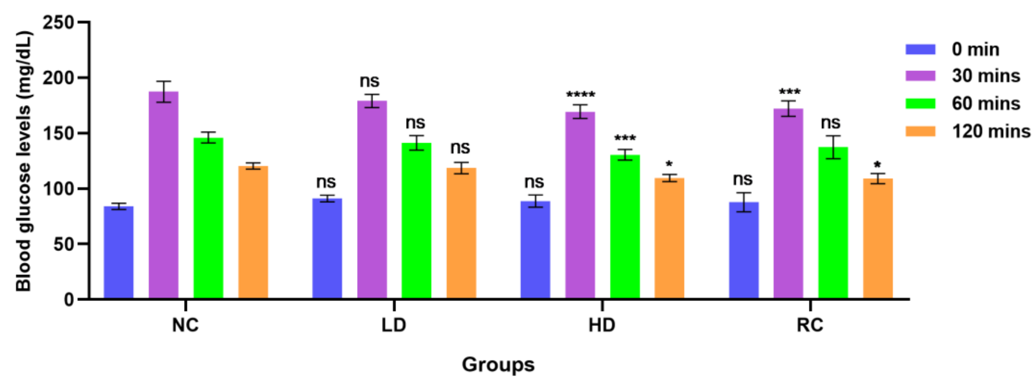


Figure 6.2. Effect of HEAH on OGTT. Each value is expressed as Mean ± SD, where n=5.

[NC: Normal Control, DC: Diabetic Control, LD: Test drug – Low dose (HEAH 200 mg/kg b.w.), HD: Test drug – High dose (HEAH 400 mg/kg b.w.), RC: Metformin treated group (150 mg/kg b.w.)]

Table 6.3. Comparison of fasting blood glucose levels and post-prandial blood glucose levels upon treatment with HEAH and the reference standard. Mean ± SD

Days	Group name					
	NC	DC	LD	HD	RC	COMB
Fasting Blood Glucose Levels						
Day 0	93.6 ± 5.08	98.2 ± 5.56	100.8 ± 7.68	97.8 ± 2.48	108.2 ± 2.04	97.8 ± 2.64
Day10	103.6 ± 4.22	100.2 ± 3.76	104.8 ± 3.54	97.8 ± 2.48	132.2 ± 7.39	97.8 ± 2.64
Day20	113.6 ± 5.95	155.4 ± 18.95	180.6 ± 34.75	128.2 ± 16.63	155 ± 17.99	153 ± 13.58

Day30	114.2 ± 8.23	184.6 ± 22.76	136.4 ± 18.21	121.2 ± 5.34	156.8 ± 16.62	150.8 ± 19.22
Day40	114.2 ± 8.63	218.4 ± 41.17	146.4 ± 21.43	124 ± 14.64	147 ± 7.18	120.6 ± 16.06
Day50	115.6 ± 4.32	236.2 ± 46.21	140.6 ± 17.23	112.6 ± 13.75	111 ± 9.78	102 ± 2.28
Day60	121 ± 18.97	259 ± 35.37	128.8 ± 23.30	105.4 ± 11.02	91.8 ± 15.37	90.4 ± 9.37
Post-prandial Blood Glucose Levels						
Day0	138 ± 8.32	135.2 ± 4.66	136.6 ± 7.61	133.2 ± 4.31	138.8 ± 7.30	128.6 ± 8.71
Day10	176 ± 15.01	159.2 ± 8.73	155.2 ± 11.30	201.2 ± 90.24	190.8 ± 29.97	154.6 ± 7.26
Day20	181.8 ± 16.28	195.8 ± 30.29	206 ± 22.99	179.6 ± 12.72	185.4 ± 7.36	170.6 ± 18.02
Day30	168 ± 20.95	246 ± 37.27	140.6 ± 7.91	142 ± 9.40	157.6 ± 19.68	161.8 ± 10.13
Day40	154.6 ± 7.14	279.8 ± 35.98	139.6 ± 16.67	139.4 ± 8.59	157.2 ± 12.98	145.4 ± 10.98
Day50	154.6 ± 7.14	347 ± 56.14	145.6 ± 9.93	130.4 ± 8.11	123.2 ± 7.08	127.6 ± 9.00
Day60	154.6 ± 7.14	458.2 ± 99.69	134 ± 8.27	117.8 ± 5.19	94 ± 13.16	108.6 ± 7.09

HD showed better maintenance of blood glucose levels compared to LD and was then combined with half doses of Metformin (75 mg/kg b.w., p.o.) and Atorvastatin (5 mg/kg b.w., p.o.). COMB exhibited near-normal blood glucose levels compared to RC (Figure 6.3.).

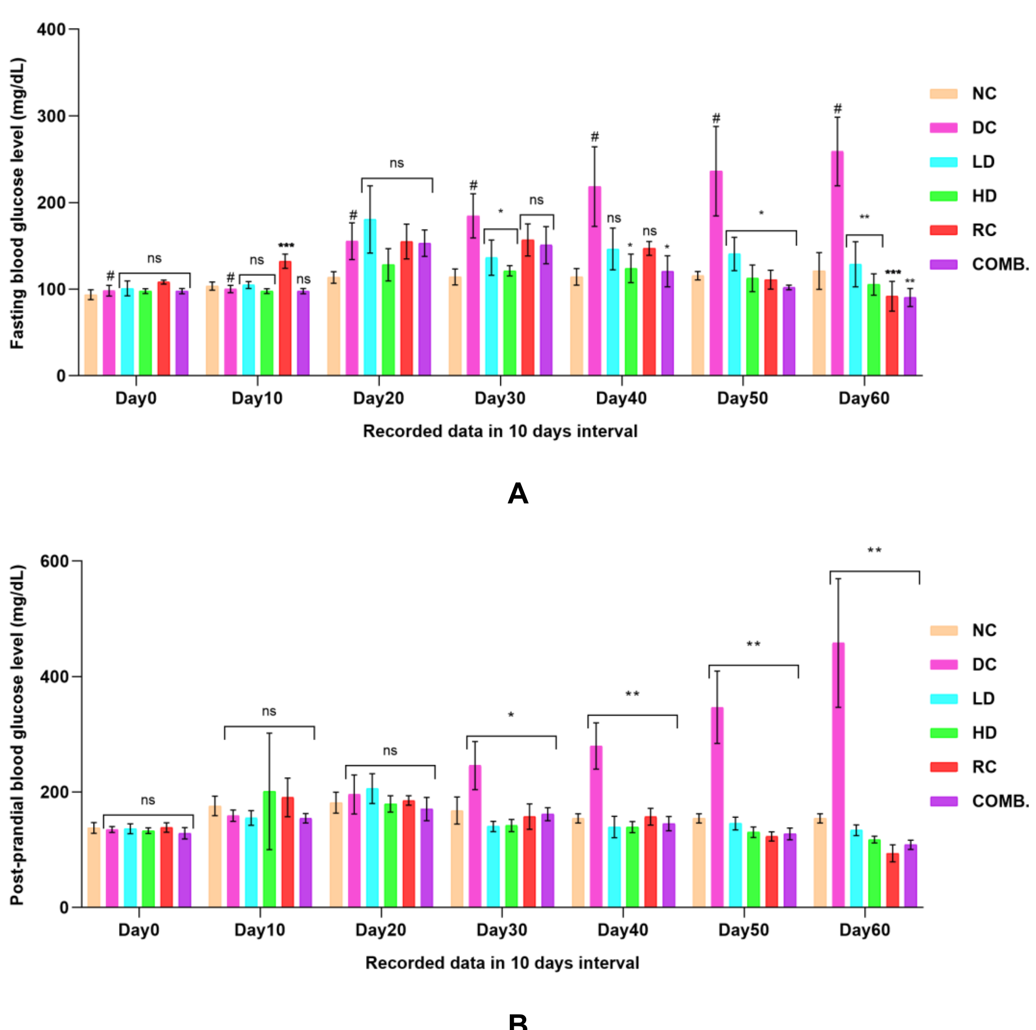


Figure 6.3. Effect of HEAH on (A) Fasting blood glucose levels; (B) Post-prandial blood glucose levels. Each value is expressed as Mean ± SD, where n=5.

[NC: Normal control, DC: Disease control, LD: Test drug – Low dose, HD: Test drug – High dose, RC: Reference control; COMB: Combination of test drug and reference control]

After a 60-day treatment period, the FBG and post-prandial blood glucose levels were observed to reach near-normal levels in the treatment groups, with the HD and COMB groups showing comparable results to the RC group. The HbA1c

levels were recorded on the 61st day, and the treatment groups were found to have significantly (> 0.05) lower HbA1c levels than DC (Figure 6.4.).

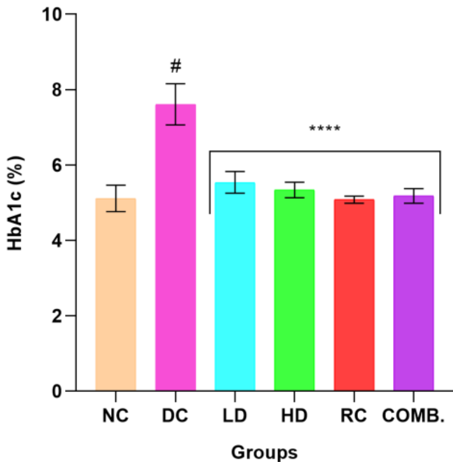


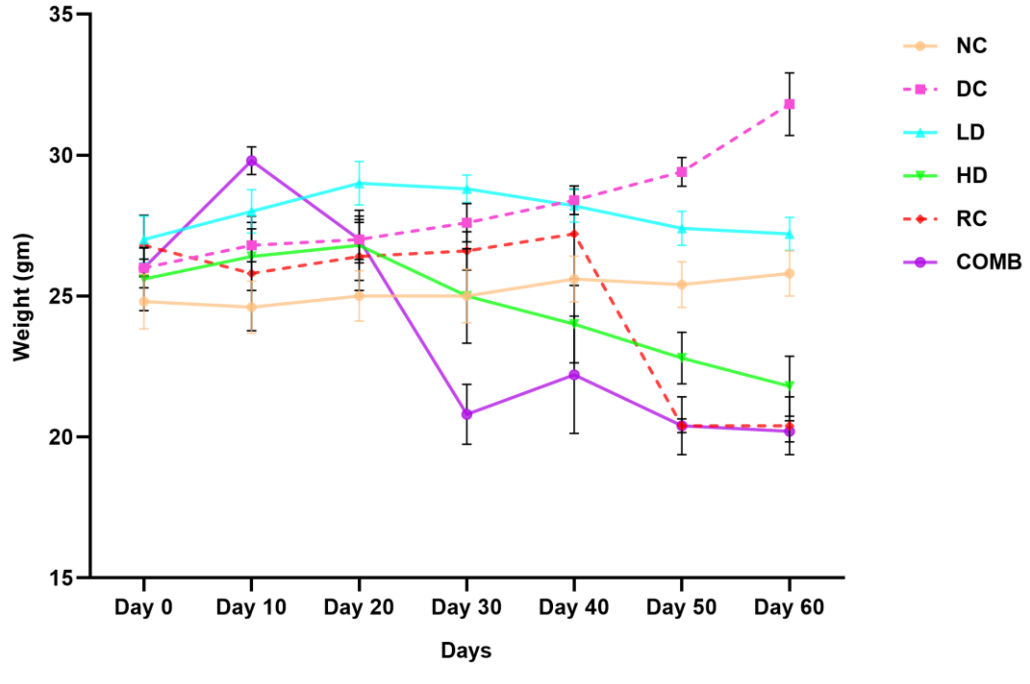
Figure 6.4. Effect of HEAH on glycated haemoglobin (HbA1c) levels. Each value is expressed as Mean ± SD, where n=5.

[NC: Normal control, DC: Disease control, LD: Test drug – Low dose, HD: Test drug – High dose, RC: Reference control; COMB: Combination of test drug and reference control]

6.3.3. *Effect of HEAH on anthropometric parameters*

The body weight changes were recorded and shown in Figure 6.5. The body weight of DC was seen to have a significant weight gain compared to the treatment groups. Groups LD, HD, RC, and COMB were observed to control weight gain. LD exhibited weight control at near-normal levels, whereas HD, RC, and COMB reduced body weight to less than day 1.

DC exhibited a high LI, which was positively correlated with increased body mass index (BMI). The HEAH and reference control treated groups demonstrated a significant reduction in LI and BMI. Figure 6.5. illustrates the comprehensive timeline of animal experimentation. The white adipose tissue (WAT) was collected from each group and measured. The DC showed an accumulation of significantly more WAT than the HEAH-treated groups (Figure 6.5.).



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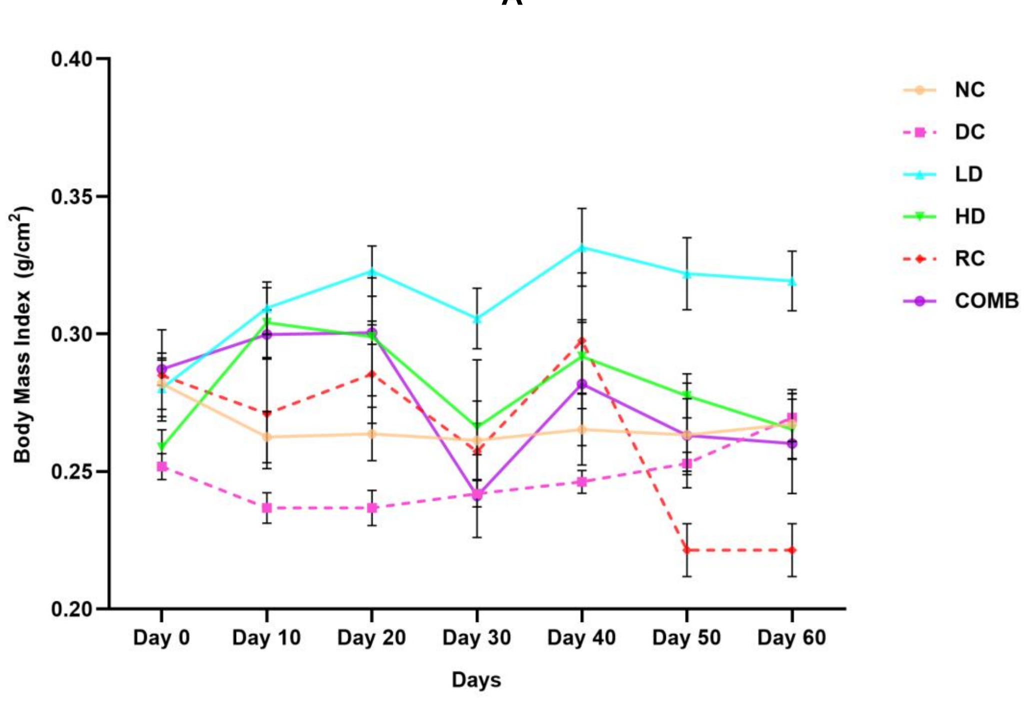

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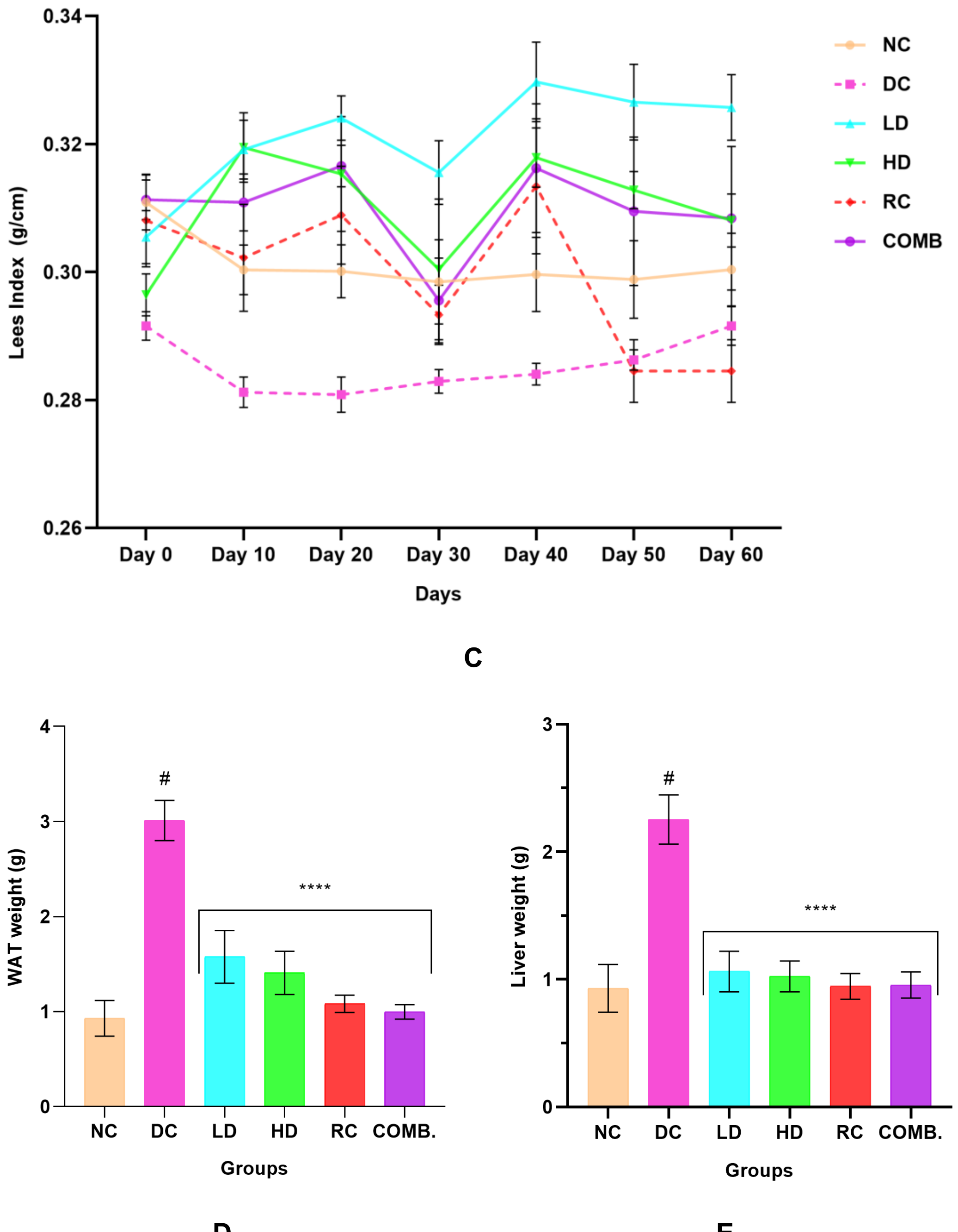


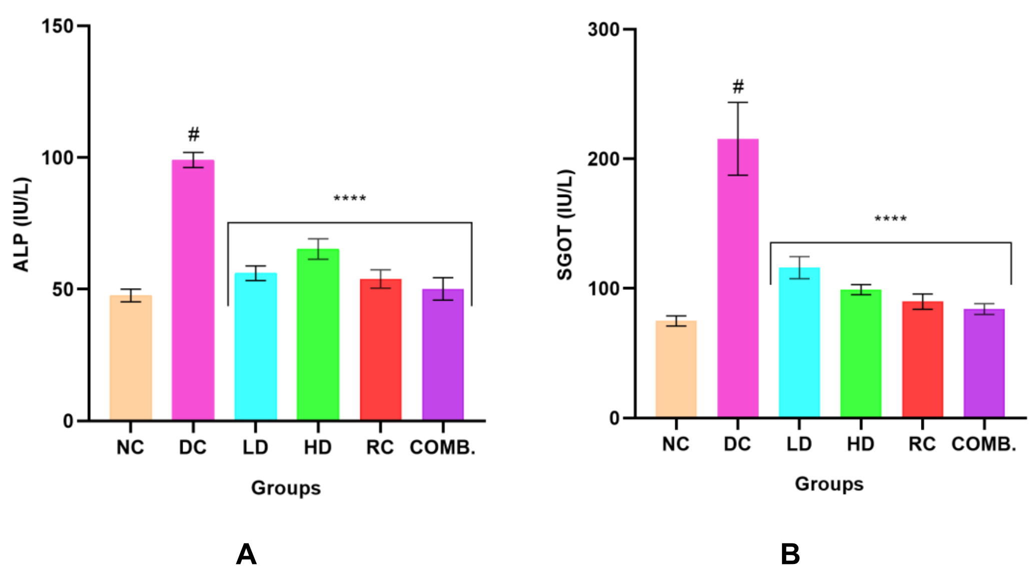
Figure 6.5. Effect of HEAH on (A) Body weight changes; (B) Body Mass Index (BMI) changes; (C) Lees Index (LI) changes; (D) Weight of accumulated white adipose tissue (WAT) and (E) Weight of liver. Each value is expressed as Mean ± SD, where n=5.

[NC: Normal control, DC: Disease control, LD: Test drug – Low dose, HD:

Test drug – High dose, RC: Reference control; COMB: Combination of test drug and reference control]

6.3.4. *Blood and serum biochemical parameters*

The serum biochemical parameters of LD, HD, RC and COMB were compared with DC and analysed for ALP, AST, ALT, Total protein, total bilirubin, triglyceride levels, total cholesterol, HDL cholesterol, serum creatinine, blood urea nitrogen and uric acid. The test groups showed dose-dependent activity, maintaining levels of AST, ALT, total bilirubin, ALP, and total protein. In contrast, DC showed elevated levels of AST, ALT, total bilirubin, and ALP, and decreased levels of total protein. Similarly, LD, HD, RC, and COMB exhibited dose-dependent maintenance of triglyceride levels, total cholesterol, and HDL cholesterol levels. The hepatic function markers of the HEAH-treated groups compared to the reference control are illustrated in Figure 6.6.



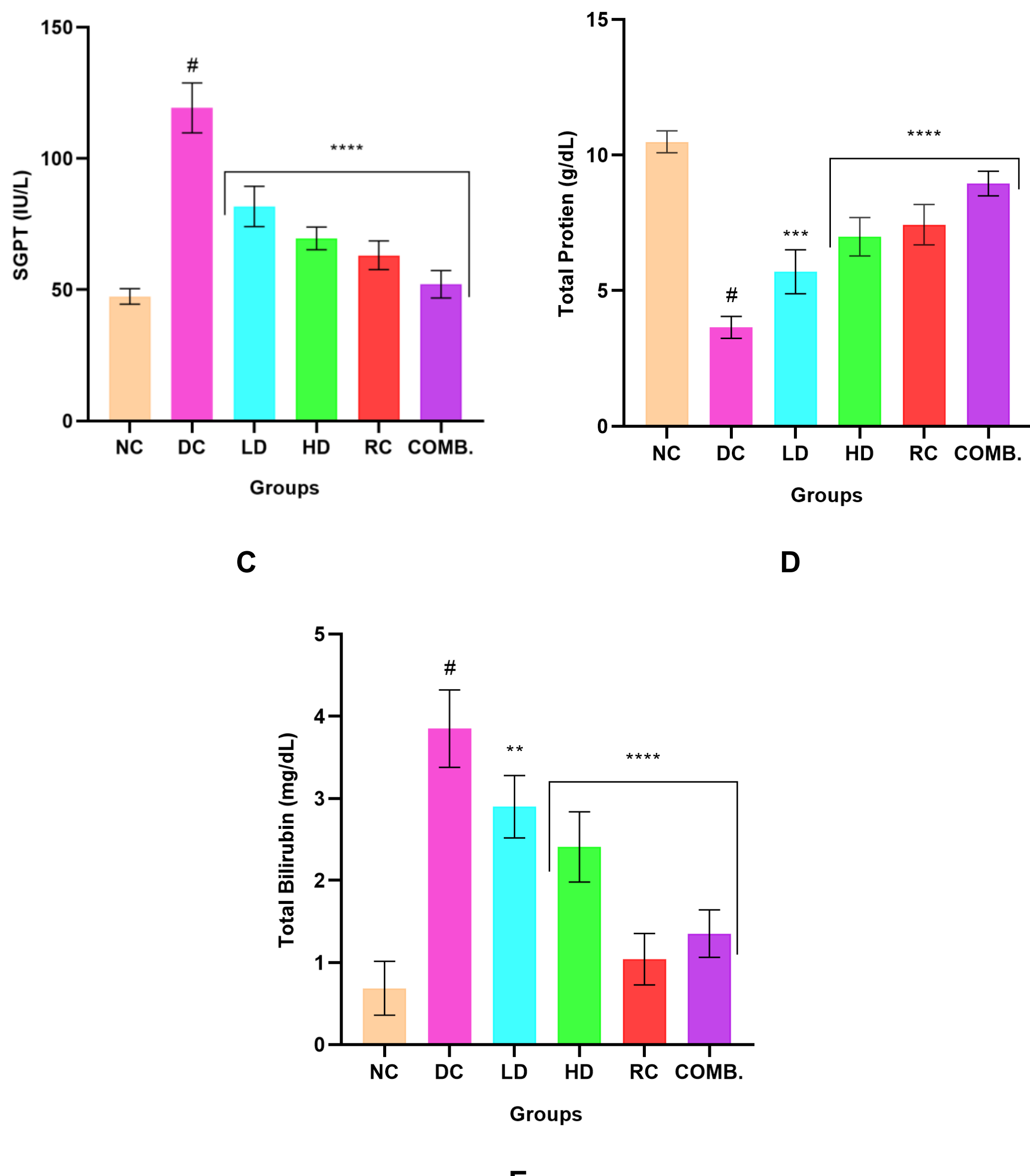
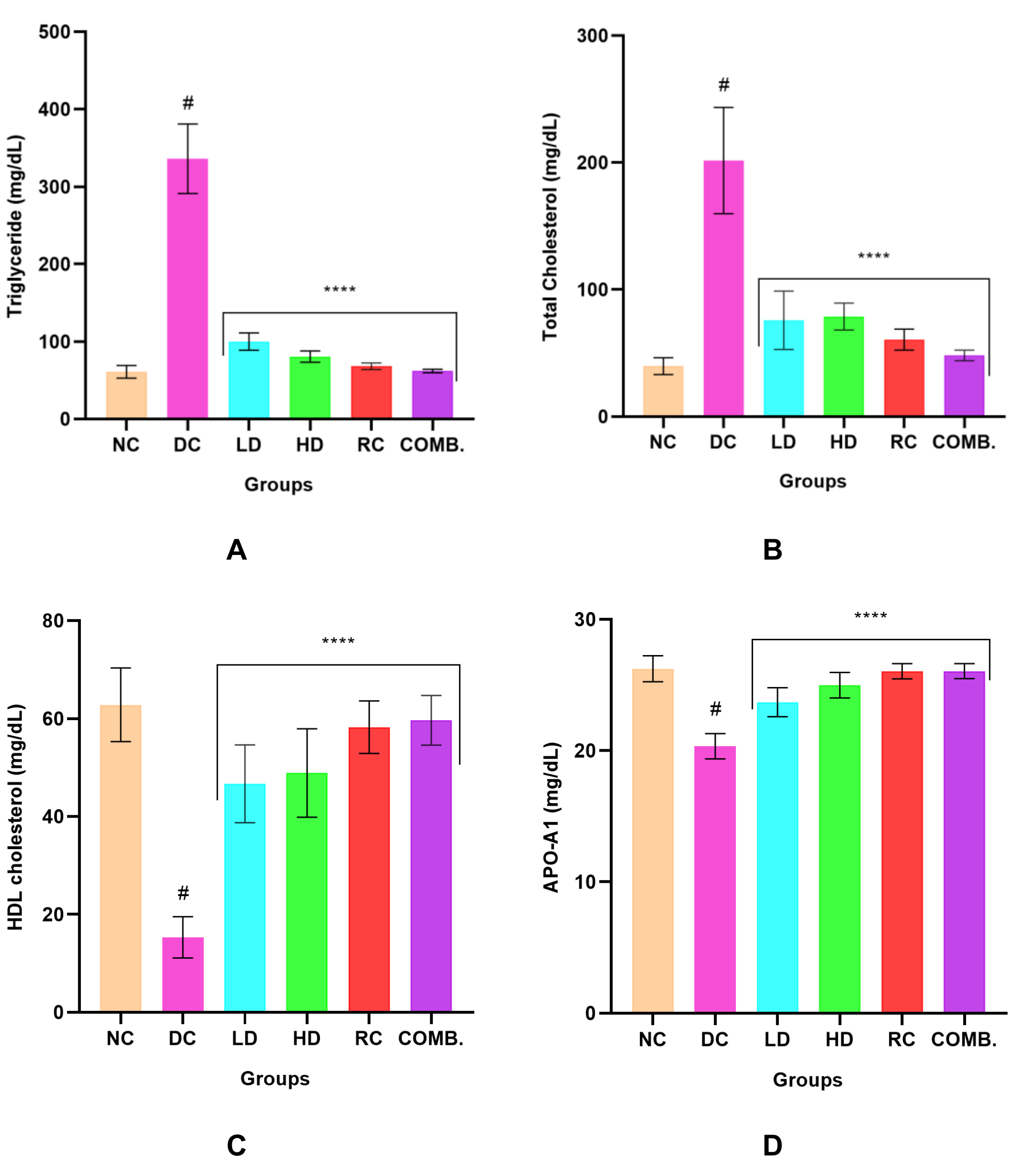
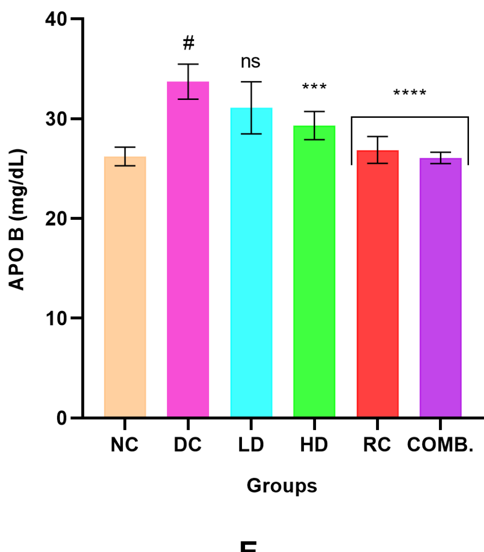


Figure 6.6. Effect of HEAH on hepatic function markers (A) Alkaline Phosphatase (ALP); (B) Aspartate Aminotransferase (AST); (C) Alanine Aminotransferase (ALT); (D) Total protein; (E) Total bilirubin. Each value is expressed as Mean ± SD, where n=5.

[NC: Normal control, DC: Disease control, LD: Test drug – Low dose, HD: Test drug – High dose, RC: Reference control; COMB: Combination of test drug and reference control]

The HFSD groups exhibited a significant reduction in plasma apo A-I ($p < 0.001$), whereas plasma apo B levels were significantly increased ($p < 0.001$) compared to the other groups. The administration of HEAH and reference drugs in the HFSD fed groups was seen to maintain the Apo A-1 and Apo B levels compared to DC (Figure 6.7.).





E

Figure 6.7. Effect of HEAH on lipid profile (A) Triglycerides; (B) Total cholesterol; (C) HDL cholesterol; (D) Apolipoprotein A1 (APO-A1); (E) Apolipoprotein B (APO B). Each value is expressed as Mean ± SD, where n=5.

[NC: Normal control, DC: Disease control, LD: Test drug – Low dose, HD: Test drug – High dose, RC: Reference control; COMB: Combination of test drug and reference control]

To determine the efficiency of the kidney, a kidney function test was conducted, and the HEAH-treated groups significantly maintained the levels of serum creatinine, blood urea nitrogen, and uric acid, whereas the DC group showed elevated levels of these markers. All the results of LD, HD, and COMB were compared to those of RC, and COMB was found to be the most effective, followed by HD and LD, in maintaining normal levels of serum and blood parameters in the experimental mice with a high-fat and high-sugar diet (Figure 6.8).

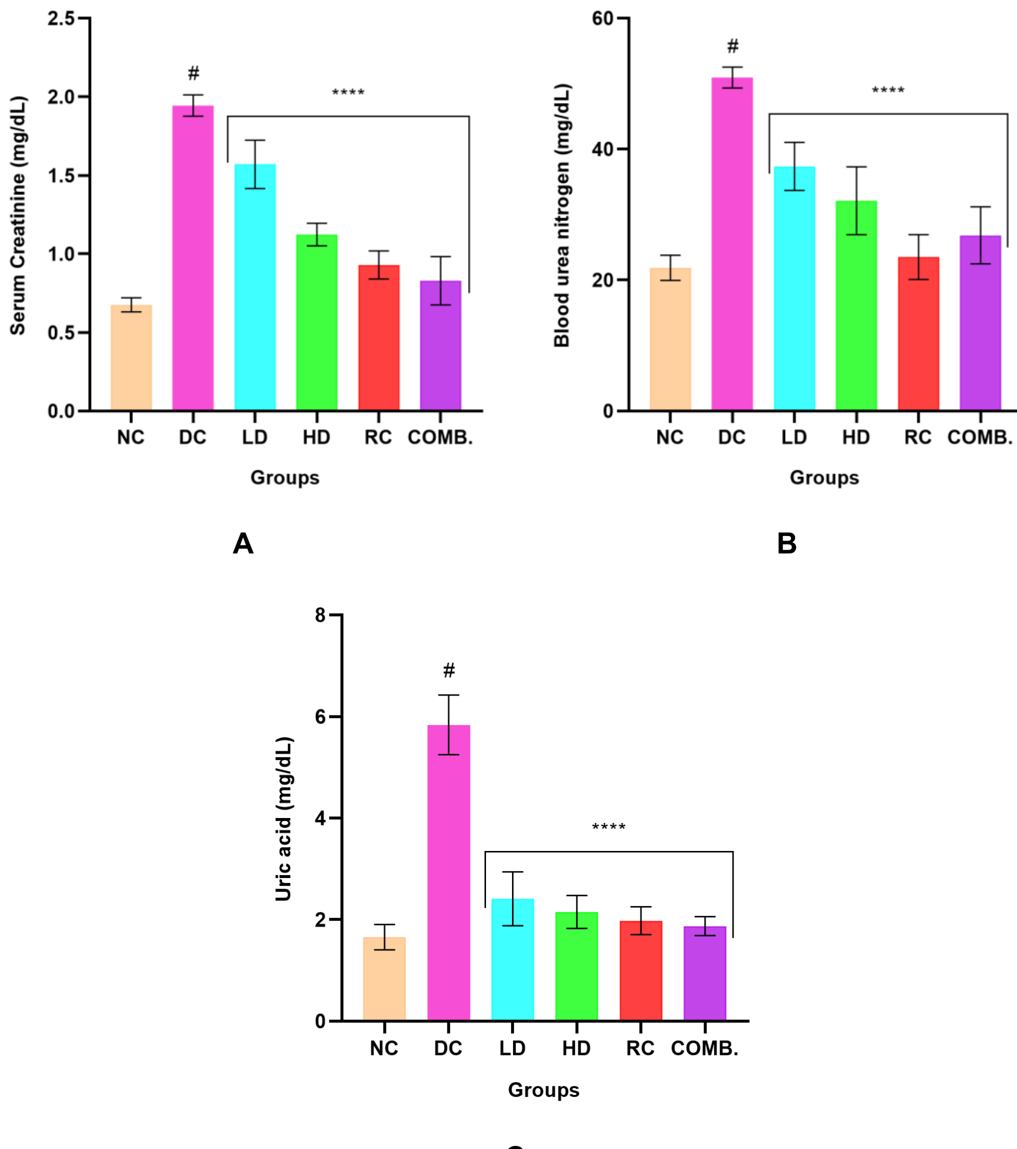


Figure 6.8. Effect of HEAH on renal function markers (A) Serum creatinine; (B) Blood Urea Nitrogen (BUN); (C) Uric acid. Each value is expressed as Mean ± SD, where n=5.

[NC: Normal control, DC: Disease control, LD: Test drug – Low dose, HD: Test drug – High dose, RC: Reference control; COMB: Combination of test drug and reference control]

The urine microalbumin levels, urinary creatinine clearance and urine volume were calculated for individual experimental animals in each group. The urine

microalbumin levels, urine volume, and urinary creatinine clearance were significantly maintained upon treatment with HEAH compared to DC (Figure 6.9.). The LD, HD, and COMB groups exhibited close similarity in regulating urine microalbumin, urinary creatinine clearance, and urine volume with RC.

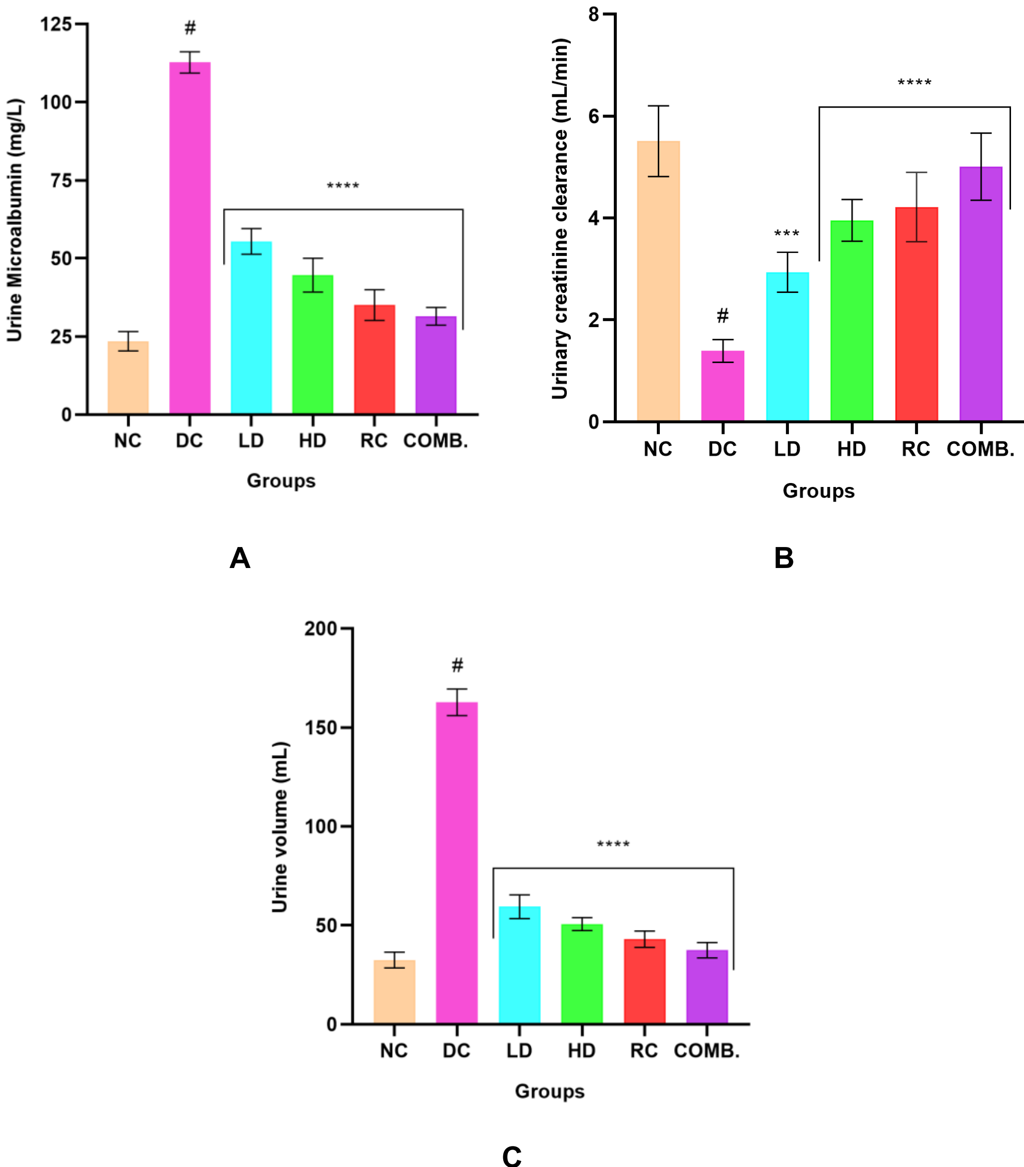
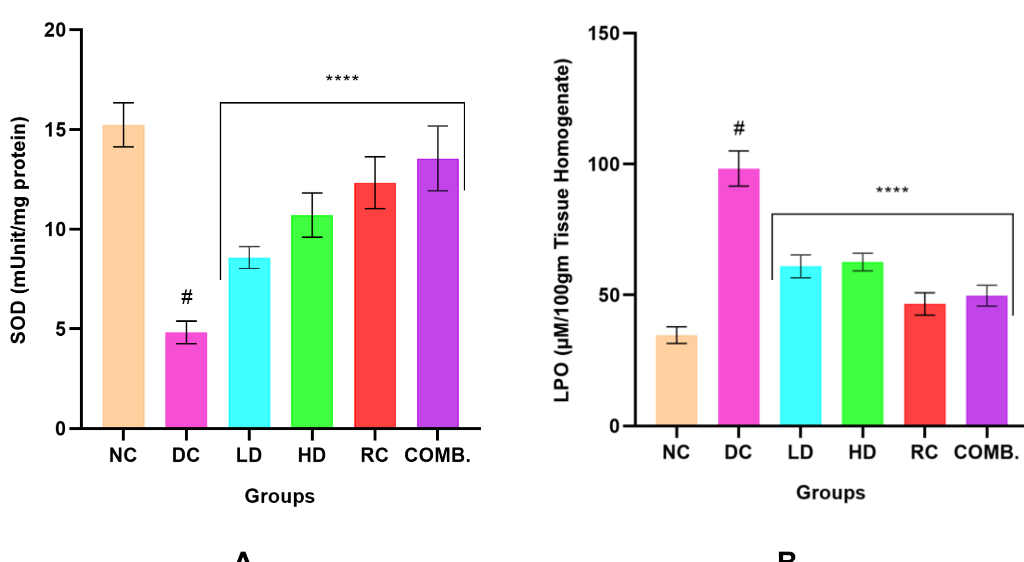


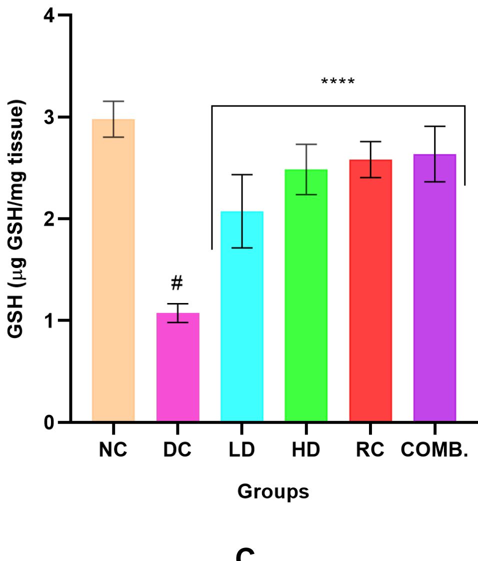
Figure 6.9. Effect of HEAH on renal function markers (A) Urine microalbumin; (B) Urinary creatinine clearance test; (C) Urinary output/urine volume. Each value is expressed as Mean ± SD, where n=5.

[NC: Normal control, DC: Disease control, LD: Test drug – Low dose, HD: Test drug – High dose, RC: Reference control; COMB: Combination of test drug and reference control]

6.3.5. *Tissue antioxidant parameters*

The kidney and liver tissue antioxidant parameters SOD, LPO and GSH levels were monitored at the end of the study, and the levels were compared between LD, HD and COMB with DC, NC and RC. The HEAH-treated groups significantly ($p < 0.05$) maintained malondialdehyde levels, superoxide radicals, and reduced glutathione levels close to those of the NC and comparable to those of the RC (Figure 6.10.).





C

Figure 6.10. Effect of HEAH on oxidative markers (A) Superoxide dismutase (SOD); (B) Lipid Peroxidation (LPO); (C) Reduced glutathione (GSH). Each value is expressed as Mean ± SD, where n=5.
[NC: Normal control, DC: Disease control, LD: Test drug – Low dose, HD: Test drug – High dose, RC: Reference control; COMB: Combination of test drug and reference control]

6.3.6. *Histopathological parameters*

The histopathological examination of the pancreas, liver, and kidney tissues of HEAH-treated experimental mice and the reference control was compared to that of disease control and normal control mice (Figure 6.11.). The pancreas DC showed hydropic degeneration and necrosis, contributing to β-cell dysfunction and impaired insulin secretion. The HEAH-treated groups (LD, HD, and COMB) exhibited nominal disintegration of the islets of Langerhans and preserved β-cells, comparable with the RC.

In liver tissues, hepatocellular damage in DC was characterised by disseminated vacuolisation in hepatocytes and sinusoidal dilation. These conditions were mild in LD, HD, and COMB, but negligible in COMB, with minimal cellular degeneration and no significant sinusoidal dilation.

The kidney tissues of HEAH-treated mice exhibited significantly lower glomerulosclerosis than those of untreated mice. Additionally, glomerular membrane thickening was observed to be less in HEAH-treated groups, particularly at HD (400 mg/kg b.w., p.o.) and combination group [HEAH (400 mg/kg b.w., p.o.) + Metformin (75 mg/kg b.w., p.o.) + Atorvastatin (05 mg/kg b.w., p.o.)] compared to DC. The renal histology of the COMB was similar to that of the normal control group, with minimal tubular dilation, followed by HD, RC, and LD.

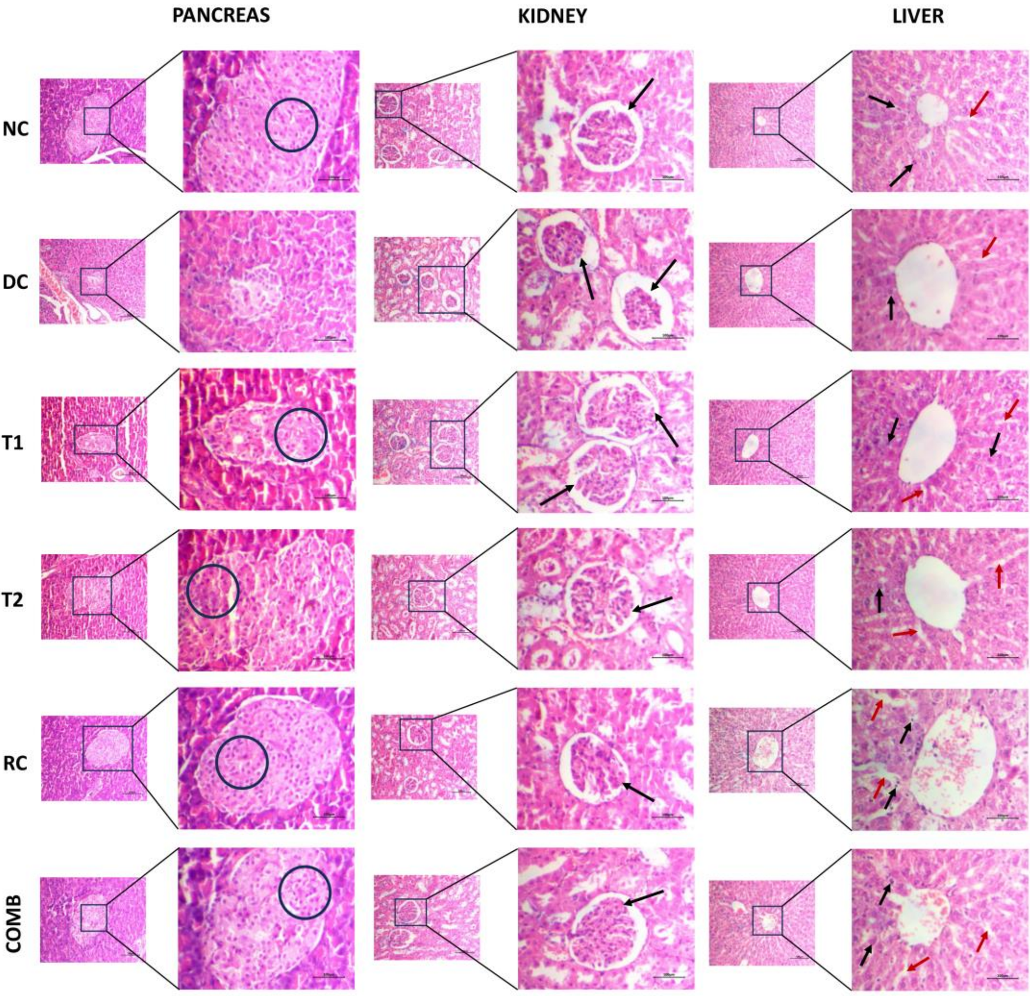


Figure 6.11. Histopathology images with H&E stain of the Pancreas, Kidney, and Liver, showing tissue-level changes in disease control groups and HEAH-treated groups. Scale Bar 100 µm.

[In pancreas, the β-cell density is shown in circles; In kidney, the black arrows indicate glomerular membrane thickening and In Liver tissues, hepatocellular damage was characterised by disseminated vacuolisation in hepatocytes (Black arrow) and sinusoidal dilation (Red arrow)].

6.4. Discussion

The *in-vivo* study with HEAH treatment for 60 days demonstrated the ability to maintain blood glucose levels and prevent weight gain and adipose tissue deposition. Mice treated with HEAH at doses of 200 and 400 mg/kg body weight exhibited regulated fasting and post-prandial blood glucose levels, near-normal glycated haemoglobin levels, and stable anthropometric parameters, including BMI and Lees index, when compared to the disease control group. HEAH at 400 mg/kg body weight improved the therapeutic efficacy of Metformin and Atorvastatin by reducing the required dose to half that of the usual dose. The HEAH-treated groups were also found to be less prone to high-fat and high-sugar diet-induced hair loss and stomach upset. At 400 mg/kg body weight, the white adipose tissue deposition was found to be 1.5 times less than in the disease control group, whereas the combination group of HEAH and reference control drugs exhibited 3 times less fat deposition. Hepatomegaly was observed in the disease control group, with a threefold increase in weight compared to the HEAH-treated groups.

The HD, RC and COMB. groups showed better glycaemic control than LD but experienced extensive weight loss with final weights less than their initial weights. LD was able to maintain its body weight in relation to the initial weight. Histological examinations of the pancreas, kidney, and liver conducted 60 days post-administration of HEAH revealed no degenerative alterations in these organs. All treated groups exhibited a significant reduction in tissue damage when compared to the disease control group. The combination dose of HEAH and reference control drugs, Metformin and Atorvastatin, had retained a greater β-cell density in the pancreas compared to the LD, HD, and RC treated groups. Liver sinusoidal dilation and disseminated vacuolisation were marked in the DC group, which appeared to be negligible in HEAH and COMB. treated groups. The

HD and COMB. groups were able to maintain the renal histology similar to that of the normal control groups. The present study demonstrated that HEAH exhibited prophylactic intervention in C57BL/6J mice in a dose-dependent manner. Chapter 3 examined the mechanism of action indicated by the phytocompounds of HEAH. The modulation of the INSR-PI3K-AKT pathway may influence blood glucose regulation, whereas the upregulation of the transcription factor PPARA may account for the reduced fat formation reported in HEAH-treated mice. The likely mode of action of *A. hookeri* leaves is depicted in Figure 6.12.

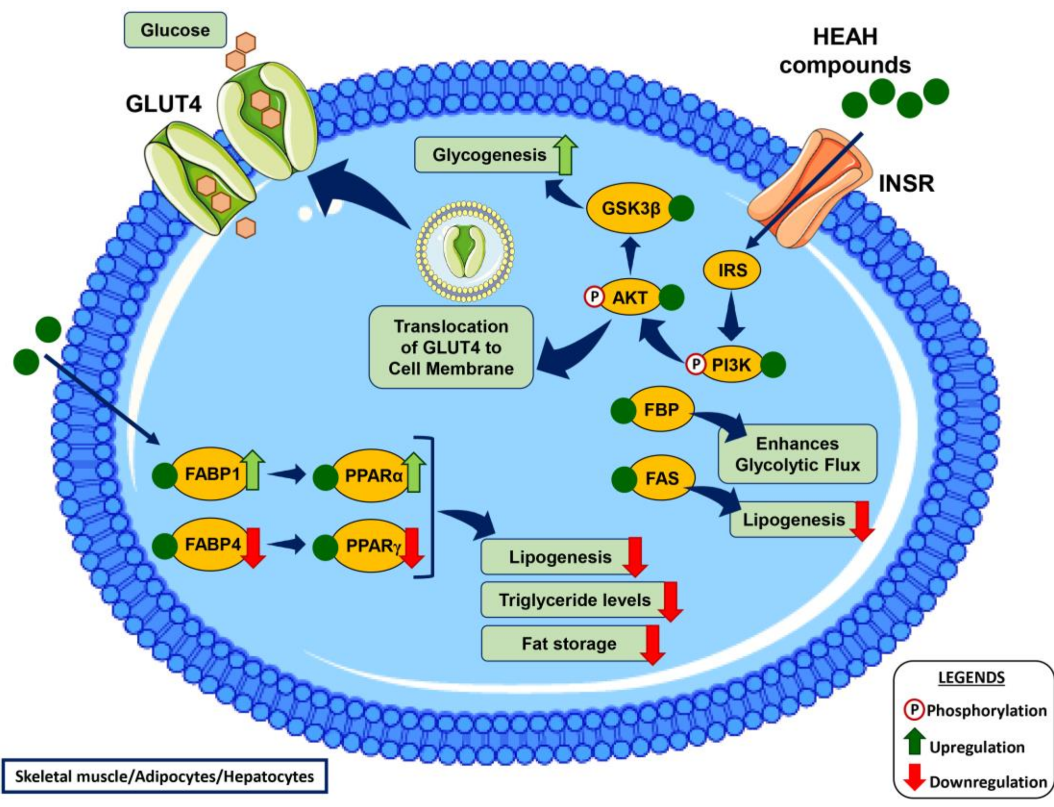


Figure 6.12. Probable mechanism of action of the phytocompounds of HEAH in glucose and lipid homeostasis

6.5. Conclusion

The current study encompasses the traditional evidence of *A. hookeri*, a popular medicinal food plant of the northeastern region of India. It leads to the development of an enriched extract of *A. hookeri*, providing a preventive strategy

for the progression of NIDDM and Obesity resulting from the current dietary lifestyle. The research demonstrated a significant reduction in the dosage of standard medications, Metformin and Atorvastatin, to 50% when administered alongside HEAH hydroalcoholic extract. Furthermore, studies should be conducted at the molecular and transcriptomic levels to validate the probable mechanism of action in greater detail, along with safety and toxicity studies on CYP isoenzymes and normal cell lines. These findings will prove to be quintessential in the development of safe and efficacious nutraceuticals from *A. hookeri* to prevent the risk of progression of metabolic disorders, such as diabetes and Obesity. This study will serve as a gateway to explore medicinal food plants of northeast India for human health and well-being and foster the bioeconomy of the region.

6.6. Publications and conference presentations

6.6.1. *Paper communicated*

- Das Gupta, B., Gayen, S., Chowdhury, S., Chatterjee, T., Kar, A., Duangyod, T., Charoensup, R., Haldar, P. K., & Mukherjee, P. K. Integrative metabolomics and network pharmacology - Exploring the antidiabetic and antiobesity potential of *Allium hookeri* Thwaites in C57BL/6J mice. Phytomedicine. Elsevier. [Communicated].

6.6.2. *Paper presented*

- Das Gupta, B., Gayen, S., Chowdhury, S., Kar, A., Haldar, P. K., & Mukherjee, P. K. Evaluation of therapeutic potential of polyherbal product derived from medicinal food plants of Eastern Himalayan Region against diabetes. In 12th International Congress of Society for Ethnopharmacology & International Conference on Innovations in Drug Technology & Phytopharmaceuticals at KLE College of Pharmacy, KLE Academy of Higher Education and Research, Belagavi, Karnataka, India during March 6–8, 2025.

Chapter 7

Summary and conclusion

7.1. Summary
7.2. Conclusion
7.3. Future prospect
7.4. Publications and conference presentations

7.1. Summary

The metabolomics-integrated network pharmacology approach identifies bioactive phytoconstituents that interact with functional targets and modulate pathways to address a condition. This thesis examined the mechanisms of action of three therapeutic food plants from the northeastern region of India: *A. hookeri*, *B. hispida*, and *H. cordata*, which possess notable ethnopharmacological potential, as evident in the exhaustive literature survey. The identification of bioactive phytomolecules was conducted using UHPLC-QTOF-MS, followed by the targeted identification of potent phytomolecules. This was complemented by functional association network analysis and an investigation into the mechanisms of action through network pharmacology. Furthermore, the medicinal food plants were evaluated *in vitro* by enzyme inhibition assays targeting α-amylase, α-glucosidase, and pancreatic lipase, as well as *in vivo* in a mouse model simulating a modern lifestyle-induced hyperglycemic and hyperlipidemic condition.

Chapter 1 explores the field of metabolomics and its implications for translating traditional knowledge through this scientific lens. The importance of metabolomics in ensuring the quality of herbal pharmaceuticals and in the discovery of medications for metabolic disorders has been highlighted, alongside the development of synergy and integration within systems biology. The integration of metabolomics with network pharmacology analysis has proven to be essential in overcoming the limitations inherent in the reductionist approach to drug discovery, particularly by employing combination synergy to investigate the synergistic effects found in traditional medicinal plants. The chapter further explores a network pharmacology-based approach to elucidate the mechanisms of action of traditional medicinal plants, thereby validating traditional claims. The methodology of mechanistic elucidation was further investigated, beginning with the untargeted or pseudo-targeted identification of bioactive compounds, followed by an examination of protein–target interactions, pathway analysis, network construction, and the analysis of combinatorial synergy.

These instruments may be employed to cultivate personalised healthcare systems and in the repurposing of traditional medicine. This chapter further explores the depth of metabolomics-integrated network pharmacology analysis in relation to metabolic and lifestyle-related disorders, highlighting the merits of this approach in advancing alternative therapeutics utilising herbal medicine. The chapter examines the application of traditional medicine, specifically the use of medicinal plants from the northeastern region (NER) of India, in addressing diabetes, obesity, and their associated complications. Case studies of several prominent medicinal plants and their bioactive compounds from the northeast region, addressing type 2 diabetes mellitus and obesity, were included along with their potential mechanisms of action. The chemical structures of the bioactive phytoconstituents identified through a literature review were also presented throughout.

Chapter 2 outlines the scope and rationale of the thesis, as well as the study objectives and work plan design. The framework of the study was developed based on the objectives and the experimental analysis performed.

Chapter 3 presents an exhaustive literature survey of *Allium hookeri* Thwaites, encompassing its scientific classification, vernacular names, traditional uses, phytochemical profile, and pharmacological activities. The methodology section of the chapter describes the collection, extraction, metabolite profiling, network pharmacology analysis, determination of total phenolic and flavonoid content, antioxidant potential, *in vitro* enzyme inhibition assays against α-amylase, α-glucosidase, and pancreatic lipase, and *in vitro* enzyme kinetic assays of *A. hookeri*, *B. hispida*, and *H. cordata*. The extraction utilised a microwave-assisted technique to produce an enriched hydroalcoholic extract of *A. hookeri* (HEAH), achieving a percentage yield of 19.34% w/w. Metabolite profiling of HEAH in positive ionisation mode utilising UHPLC–QTOF–MS identified 27 compounds. The structures of the phytocompounds have also been illustrated. The network pharmacology analysis identified protein targets for the identified phytocompounds and generated a disease association network for NIDDM, obesity, hyperlipidaemia, hypertriglyceridemia, and hyperglycaemia.

Apigenin demonstrated the highest connectivity, and aldose reductase (AKR1B1) was identified as the most targeted protein. The disease association was subsequently analysed to identify common targets between HEAH and the disease conditions, as well as between HEAH and the reference standards: acarbose, Orlistat, Metformin, and Atorvastatin. Atorvastatin exhibited shared targets with HEAH, including HDAC1, HDAC2, SLCO1B1, and SLCO1B3, whereas no common targets were identified between Metformin and HEAH. Acarbose and HEAH share common targets, including AMY1A, AMY2A, GAA, MGAM, and SI, while Orlistat shares ABHD16A, FAAH, and FASN as common targets with HEAH. The identified common targets indicate a mechanistic overlap and the potential of HEAH as an alternative therapeutic approach. The protein-protein enrichment analysis revealed that target proteins exhibit significantly greater interaction levels than expected, suggesting a partial biological connection among them as a collective entity. The PPI enrichment analysis showed that each protein interacts with at least 43 other proteins.

The KEGG pathway enrichment analysis identified several pathways associated with NIDDM and hyperglycaemia, including hsa04910: Insulin signalling pathway, hsa04930: Type II diabetes mellitus, hsa04931: Insulin resistance, hsa04933: AGE-RAGE signalling pathway in diabetic complications, hsa04973: Carbohydrate digestion and absorption, hsa00010: Glycolysis/Gluconeogenesis, and hsa00040: Pentose and glucuronate interconversions. In the context of obesity, hyperlipidaemia, and hypertriglyceridaemia, the relevant pathways include hsa03320: PPAR signalling pathway, hsa05417: Lipid and atherosclerosis, hsa04152: AMPK signalling pathway, hsa04920: Adipocytokine signalling pathway, and hsa01100: Metabolic pathways. Additional significant pathways comprised hsa04151: PI3K-Akt signalling pathway, hsa04310: Wnt signalling pathway, hsa04972: Pancreatic secretion, and hsa04970: Salivary secretion. The combination network analysis indicated that Apigenin exhibited the highest degree of connectivity, while AKR1B1 emerged as the most targeted protein across all selected disease conditions. The hub nodes involving the bioactive compounds and protein targets were correlated with previous studies.

The total flavonoid and phenolic content of *A. hookeri* demonstrated a total phenolic content (TPC) of 18.503 ± 0.903 mg gallic acid equivalent/g and a total flavonoid content (TFC) of 10.73 ± 0.76 mg Rutin equivalent/g. In the DPPH radical scavenging potential assay, HEAH exhibited an IC_{50} value of 128.5 ± 0.031 μg/mL, whereas ascorbic acid demonstrated an IC_{50} value of 53.58 ± 0.064 μg/mL. The hydroxyl scavenging capacity of HEAH demonstrated an IC_{50} value of 210.5 ± 3.4 μg/mL, in contrast to 59.94 ± 0.49 μg/mL for ascorbic acid. HEAH demonstrated notable antioxidant activity, exhibiting an IC_{50} of 234 ± 3.5 μg/mL, in contrast to 120 ± 2.3 μg/mL for ascorbic acid in the nitric oxide scavenging assay. The inhibition assay of α-glucosidase by *A. hookeri* yielded an IC_{50} of 0.517 ± 0.07 mg/mL, in contrast to 0.294 ± 0.08 mg/mL for acarbose. The α-amylase inhibition assay revealed an IC_{50} of HEAH at 1.138 ± 0.57 mg/mL, in comparison to an IC_{50} of 0.532 ± 0.04 mg/mL for acarbose. HEAH demonstrated an inhibition potential of 0.809 ± 0.06 mg/mL for pancreatic lipase, compared to 0.739 ± 0.09 mg/mL for Orlistat.

The enzyme kinetics assay indicated a potential for mixed inhibition by *A. hookeri*. The changes in V_{max} and K_m values suggest that HEAH does not solely compete with the substrate for the active site. It may interact with both the unbound enzyme and the enzyme–substrate complex. The Pearson correlation analysis indicated a positive correlation between the antioxidant potential and the phenolic and flavonoid content of *A. hookeri*, implying a multi-target pharmacological mechanism that may involve the reduction of reactive oxygen species (ROS) and the promotion of enzyme inhibition.

Chapter 4 presents a comprehensive literature review of *Benincasa hispida* (Thunb.) Cogn., encompassing its scientific classification, common names, traditional applications, phytochemical composition, and pharmacological properties. The methodology section of the chapter outlines the metabolite profiling of *B. hispida* hydroalcoholic extract (HEBH). The UHPLC–QTOF–MS analysis revealed 17 distinct compounds, including polyphenols, flavonoids, phenols, triterpenes, flavanones, stigmastanes, and amino acids. The structures of the identified compounds have been depicted. The network pharmacology

analysis began with the bioactive–target (BA–TAR) network, which encompasses 16 compounds and their associated targets. Apigenin exhibited the highest connectivity, whereas aldose reductase (ESR2) was identified as the most targeted protein. The Venn diagram analysis revealed the shared targets for acarbose, Orlistat, Metformin, and Atorvastatin in relation to HEBH. Atorvastatin and HEBH both target HMGCR, whereas Metformin and HEBH share PRKAB1 as a common target. Acarbose and HEBH both target GAA, while Orlistat and HEBH share FAAH and PNLIP as common targets. The Venn diagram analysis indicated that HEBH exhibited a mechanistic overlap with the reference standards atorvastatin, Metformin, Orlistat, and acarbose.

The PPI enrichment analysis revealed that target proteins exhibit interaction levels that are significantly higher than anticipated, suggesting a partial biological association among them as a collective group. Each protein was found to interact with a minimum of 19 other proteins. The KEGG pathway enrichment analysis indicated that the identified genes are associated with hsa04910 (Insulin signalling pathway), hsa04930 (Type II diabetes mellitus), hsa04931 (Insulin resistance), and hsa04933 (AGE-RAGE signalling pathway) in relation to diabetic complications linked to NIDDM and hyperglycaemia. Regarding obesity, hyperlipidaemia, and hypertriglyceridemia, the genes were associated with several critical pathways: hsa03320 (PPAR signalling pathway), hsa05417 (lipid and atherosclerosis), hsa04152 (AMPK signalling pathway), hsa04920 (adipocytokine signalling pathway), and hsa01100 (metabolic pathways). Other notable pathways included hsa04151 (PI3K-Akt signalling pathway), hsa04310 (Wnt signalling pathway) and hsa04972 (Pancreatic secretion).

In combination network analysis, kaempferol demonstrated the strongest connectivity in NIDDM, hyperglycaemia, obesity, and hyperlipidaemia. FABP1 has been identified as the primary targeted protein in NIDDM, hyperglycaemia, and hyperlipidaemia, whereas ESR1 is recognised as the most targeted protein in obesity. In hypertriglyceridemia, catechin exhibited the strongest association, whereas PPARA was identified as the most targeted protein. The total flavonoid and phenolic content of *B. hispida* demonstrated a total phenolic content (TPC) of

15.353 ± 1.019 mg gallic acid equivalent/g and a total flavonoid content (TFC) of 12.42 ± 0.97 mg Rutin equivalent/g. In the DPPH radical scavenging potential assay, HEBH exhibited an IC_{50} value of 199.6 ± 0.57 µg/mL, whereas ascorbic acid demonstrated an IC_{50} value of 53.58 ± 0.064 µg/mL. The hydroxyl scavenging capacity of HEBH demonstrated an IC_{50} value of 239.2 ± 0.24 µg/mL, in contrast to 59.94 ± 0.49 µg/mL for ascorbic acid. HEBH demonstrated notable antioxidant activity, exhibiting an IC_{50} of 339.5 ± 0.65 µg/mL, in contrast to 120 ± 2.3 µg/mL for ascorbic acid in the nitric oxide scavenging assay. The inhibition assay of α-glucosidase by *B. hispida* yielded an IC_{50} of 1.394 ± 0.16 mg/mL, in contrast to 0.294 ± 0.08 mg/mL for acarbose. The α-amylase inhibition assay revealed an IC_{50} of HEBH at 1.905 ± 0.21 mg/mL, in comparison to an IC_{50} of 0.532 ± 0.04 mg/mL for acarbose. HEBH demonstrated an inhibition potential of 2.564 ± 0.08 mg/mL for pancreatic lipase, in contrast to 0.739 ± 0.09 for Orlistat.

The enzyme kinetics assay demonstrated a potential for mixed inhibition by *B. hispida*. The reaction velocity (v) in relation to enzyme concentration demonstrated reversible inhibition of α-glucosidase, α-amylase, and pancreatic lipase by HEBH, characterised by a decreasing slope at elevated inhibitor concentrations (I). The total phenolic content (TPC) and total flavonoid content (TFC) of HEBH exhibited a positive linear correlation with α-amylase, α-glucosidase, and pancreatic lipase, as indicated by the Pearson r value at $p<0.05$. The enzyme inhibitory activity of HEBH correlates with TPC and TFC, suggesting that the phenolic and flavonoid compounds in *B. hispida* fruits contribute significantly to this activity, although to a lesser extent than *A. hookeri*. Pearson correlation analysis demonstrated a strong correlation between the antioxidant potential and enzyme inhibitory activity of HEBH. The significant correlation indicates that the antioxidant potential of HEBH was effective in inhibiting all three enzymes, albeit to a lesser extent than *A. hookeri*. The correlation study revealed a positive relationship between enzyme inhibition potential and both antioxidant and phenolic content; however, most correlations were non-significant, with the exception of the correlation with flavonoid content, which was significant at $p < 0.05$. Thus, flavonoids were identified as the primary bioactive molecules responsible for enzyme inhibition, functioning in conjunction

with antioxidant properties and phenolic compounds to create a synergistic effect in mitigating diabetes, obesity, and related metabolic disorders.

Chapter 5 presents a comprehensive literature review of *Houttuynia cordata* Thunb., covering its scientific classification, common names, traditional uses, phytochemical composition, and pharmacological properties. The percentage yield of HEHC was determined to be 15.37% w/w. The UHPLC–QTOF–MS analysis identified 26 distinct compounds, comprising flavonoids, prenol lipids, and organooxygen compounds. The structures of the identified compounds are illustrated. Seventeen compounds demonstrating target association were utilised to construct a bioactive target network, identifying luteolin as the most interacting phytocompound. Carbonic anhydrase 2 (CA2) emerged as the most targeted protein. The Venn diagram analysis indicated that Atorvastatin shared HDAC1, HDAC2, HDAC6, HMGCR, SLCO1B1, and SLCO1B3 as common targets with HEHC; however, no common targets were identified between Metformin and HEHC. Acarbose and HEHC share common targets, including AMY1A, AMY2A, GAA, MGAM, and SI, while Orlistat shares FAAH and FASN as common targets with HEHC. The identified common targets indicate a mechanistic overlap and the potential of HEHC as an alternative therapeutic approach.

The PPI enrichment analysis revealed that target proteins exhibit interactions, suggesting a partial biological association among them as a collective group. Moreover, each protein was observed to interact with at least 19 additional proteins. The KEGG pathway enrichment analysis revealed that the identified genes are linked to the following pathways: hsa04910 (Insulin signalling pathway), hsa04930 (Type II diabetes mellitus), hsa04931 (Insulin resistance), hsa04933 (AGE-RAGE signalling pathway in diabetic complications), hsa04973 (Carbohydrate digestion and absorption), and hsa00040 (Pentose and glucuronate interconversions). The genes associated with obesity, hyperlipidaemia, and hypertriglyceridemia were linked to multiple essential pathways, including hsa03320 (PPAR signalling pathway), hsa05417 (Lipid and atherosclerosis), hsa04152 (AMPK signalling pathway), hsa04920 (Adipocytokine signalling pathway) and hsa01100 (Metabolic pathways). Additional significant

pathways comprised hsa04151 (PI3K-Akt signalling pathway), hsa04310 (Wnt signalling pathway), and hsa04970 (Salivary secretion).

In combination network analysis, each network was analysed utilising the "Analyse Network" tool. Luteolin exhibited the highest connectivity in NIDDM and obesity. Kaempferol exhibited the highest connectivity in cases of hyperlipidaemia and hypertriglyceridemia. In hyperglycemia, 3'-O-methylcatechin exhibited the most substantial degree of association. PTPN1 is identified as the principal targeted protein in noninsulin-dependent diabetes mellitus, PTGS2 in hyperglycaemia and obesity, ABCB1 in hyperlipidaemia, and MET in hypertriglyceridaemia. The total flavonoid and phenolic content of H. cordata revealed a total phenolic content (TPC) of 12.873 ± 0.952 mg gallic acid equivalent/g and a total flavonoid content (TFC) of 7.49 ± 1.1 mg Rutin equivalent/g. In the DPPH radical scavenging potential assay, HEHC showed an IC_{50} value of 137.8 ± 0.29 μg/mL, while ascorbic acid presented an IC_{50} value of 53.58 ± 0.064 μg/mL. The hydroxyl scavenging capacity of HEHC exhibited an IC_{50} value of 213.1 ± 0.56 μg/mL, compared to 59.94 ± 0.49 μg/mL for ascorbic acid. HEHC exhibited significant antioxidant activity, with an IC_{50} of 262.3 ± 0.54 μg/mL, compared to 120 ± 2.3 μg/mL for ascorbic acid in the nitric oxide scavenging assay. The inhibition assay of α-glucosidase by *H. cordata* yielded an IC_{50} of 0.645 ± 0.27 mg/mL, in contrast to 0.294 ± 0.08 mg/mL for acarbose. The α-amylase inhibition assay revealed an IC_{50} of HEHC at 1.145 ± 0.08 mg/mL, in comparison to an IC_{50} of 0.532 ± 0.04 mg/mL for acarbose. HEHC demonstrated an inhibition potential of 1.02 ± 0.42 mg/mL for pancreatic lipase, in contrast to 0.739 ± 0.09 for Orlistat. The enzyme inhibition analysis results indicate that *H. cordata* successfully inhibited α-glucosidase, α-amylase, and lipase, exhibiting efficacy comparable to the reference standards. *H. cordata* demonstrated a hybrid inhibition mechanism, exhibiting the most effective inhibitory effect on α-amylase. This highlights the beneficial effect of *H. cordata* on postprandial hyperglycaemia. *H. cordata* exhibited a certain level of pancreatic lipase inhibition. The correlation analysis indicated a favourable association between enzyme inhibition potential and both antioxidant and phenolic content; however, these correlations were largely considered non-significant at $p < 0.05$. Thus, the

cluster of phytoconstituents collectively contributed to enzyme inhibition, rather than relying solely on polyphenolic concentration.

This chapter presents a comparative analysis of the results derived from the hydroalcoholic extracts of *A. hookeri*, *B. hispida*, and *H. cordata*. *A. hookeri* demonstrated a greater richness and diversity of bioactive phytocompounds compared to *B. hispida* and *H. cordata*. An integrative approach utilising metabolomics and network pharmacology analysis was used to assess the bioactive potential of the identified phytocompounds. *A. hookeri* demonstrated 27 compounds associated with human targets, whereas *B. hispida* showed 16 compounds, and *H. cordata* revealed 17 compounds linked to human targets. This suggests a notable variety of bioactive phytoconstituents in the hydroalcoholic extract of *A. hookeri*. *A. hookeri* demonstrated unique system-level interactions characterised by 867 nodes and 11,485 edges, leading to a highly interconnected network, unlike the other two plants. This dense connectivity illustrates a significant polypharmacological and synergistic mechanism-based approach in addressing metabolic dysregulations, including non-insulin-dependent diabetes and obesity. *A. hookeri* exhibited a higher quantity of shared genes associated with noninsulin-dependent diabetes mellitus, hyperglycemia, obesity, hyperlipidaemia, and hypertriglyceridemia. *H. cordata* exhibited gene correlations comparable to those of *A. hookeri*. In cases of hyperlipidaemia and hypertriglyceridaemia, *A. hookeri* and *H. cordata* demonstrated an equal number of shared genes. *B. hispida* consistently demonstrated the lowest number of shared genes with the DisGeNET database.
The analysis of protein-protein interaction enrichment for targets associated with NIDDM, hyperglycaemia, obesity, hyperlipidaemia, and hypertriglyceridaemia suggests that *A. hookeri* may have a wider regulatory influence pertinent to the improvement of metabolic dysregulations. The ten primary hub proteins of *A. hookeri* exhibited significant mechanistic regulation via proteins associated with glucose homeostasis, inflammation, lipid metabolism, and glycolytic regulation. *B. hispida* and *H. cordata* share common targets with *A. hookeri*, such as AKT1 and PPARG, whereas the other targets predominantly address inflammation and apoptosis in *B. hispida* and lipid metabolism in *H. cordata*. All three plants

exhibited similar pathways in managing selected metabolic dysregulations; however, the pathways were more enriched with bioactive targets in *A. hookeri*, followed by *H. cordata* and *B. hispida*. The results suggest that *A. hookeri* exerts a more pronounced regulatory effect on metabolic dysregulation. This multi-target network enhances the translational potential and increases the likelihood of *in vivo* efficacy of *A. hookeri*.

A. hookeri exhibited the lowest IC_{50} value in the *in vitro* analysis of enzyme inhibition potential among the three enzymes: α-glucosidase, α-amylase, and pancreatic lipase. *A. hookeri* exhibited notable inhibitory activity, indicating a high likelihood of anti-hyperlipidaemic and anti-hyperglycaemic effects *in vivo*. The enzyme kinetics analysis of *A. hookeri* against α-glucosidase, α-amylase, and lipase demonstrated a significant inhibitory potential against α-glucosidase and α-amylase, which are important in the management of NIDDM and hyperglycaemia. The moderate inhibition potential against lipase indicates a potential inhibitory effect on obesity, hyperlipidaemia, and hypertriglyceridaemia. *A. hookeri* demonstrated the lowest K_m values, moderate to low K_i values, and the highest α values in the inhibition of α-glucosidase and α-amylase, indicating a higher affinity for the free enzyme and significant inhibitory activity. All three plants demonstrated comparable V_{max} and K_m values in lipase inhibition kinetics. *A. hookeri* demonstrated moderate K_i and α values, indicating moderate lipase inhibition. *B. hispida* exhibited superior lipase inhibition compared to the other two species. Therefore, the significant inhibitory potential of *A. hookeri*, along with its moderate lipase inhibitory effects, provides a mechanistic basis for managing hyperglycaemia and hyperlipidaemia.

Chapter 6 examines the *in vivo* assessment of the antihyperglycaemic and antihyperlipidaemic properties of *A. hookeri* leaf hydroalcoholic extract in C57BL/6J mice subjected to a high-fat and high-sugar diet. The methodology section outlines the specifics of experimental animal maintenance, acute toxicity assessments, and the oral glucose tolerance test (OGTT). It details the experimental design, fasting blood glucose level estimation, body weight and

body mass index measurements, serum biochemical parameter assessments, tissue antioxidant parameter evaluations, and tissue histopathological analyses.

The acute oral toxicity study with HEAH demonstrated no significant alterations in dietary habits, body weight, or behavioural patterns. The extract exhibited no indications of drowsiness or diarrhoea. All animals survived, indicating that the LD_{50} is greater than the administered dose of 2000 mg/kg. The OGTT results demonstrated an increased blood glucose level at 30 minutes post-glucose administration, which progressively declined to near-normal levels at 60 minutes and normalised by 120 minutes. Daily administration of HEAH extract at doses of 200 and 400 mg/kg b.w., p.o. Significantly ($p < 0.05$), the blood glucose levels were maintained near normal levels compared to the disease control group. T2 showed better maintenance of blood glucose levels compared to T1 and was then combined with half doses of Metformin (75 mg/kg b.w. p.o.) and Atorvastatin (5 mg/kg b.w. p.o.) to form COMB, which exhibited near-normal blood glucose levels comparable to those of RC. The HbA1c levels were recorded on the 61st day, and the treatment groups were found to have significantly (> 0.05) lower HbA1c levels than DC.

The body weight of DC exhibited a significant increase compared to the treatment groups. Groups T1, T2, RC, and COMB demonstrated control over weight gain. T1 demonstrated weight regulation to levels approaching normal, while T2, RC, and COMB decreased body weight to below that observed on day 1. DC demonstrated a significant LI, positively correlated with an increase in body mass. The HEAH and reference control treated groups demonstrated a significant decrease in LI and BMI. The DC exhibited a significantly greater accumulation of WAT compared to the HEAH-treated groups. The test groups exhibited dose-dependent activity and stable levels of AST, ALT, total bilirubin, ALP, and total protein, while the DC group displayed elevated levels of AST, ALT, total bilirubin, ALP, and reduced levels of total protein. T1, T2, RC, and COMB demonstrated a dose-dependent effect on maintaining triglyceride levels, total cholesterol, and HDL cholesterol levels. The HEAH-treated groups significantly maintained serum creatinine, blood urea nitrogen, and uric acid levels, whereas the disease control group exhibited elevated levels. The results of T1, T2, and

COMB were compared to those of RC, revealing that COMB was the most effective in maintaining normal serum and blood parameters in experimental mice on a high-fat, high-sugar diet, followed by T2 and T1. The T1, T2, and COMB groups demonstrated a close similarity in their regulation of urine microalbumin, urinary creatinine clearance, and urine volume in relation to RC. The HEAH-treated groups significantly ($p < 0.05$) maintained malondialdehyde levels, superoxide radicals, and reduced glutathione levels, which were similar to those in the NC and comparable to those in the RC.

The DC pancreas exhibited hydropic degeneration and necrosis, leading to β-cell dysfunction and impaired insulin secretion. The HEAH-treated groups (T1, T2, and COMB) demonstrated minimal disintegration of the islets of Langerhans and maintained β-cell integrity similar to the RC. Hepatocellular damage in DC was characterised by widespread vacuolisation in hepatocytes and dilation of sinusoids in liver tissues. The conditions observed in T1, T2, and COMB were mild, while in COMB, they were negligible, characterised by minimal cellular degeneration and an absence of significant sinusoidal dilation. The kidney tissues demonstrated that HEAH-treated mice exhibited significantly reduced glomerulosclerosis compared to untreated mice. Furthermore, glomerular membrane thickening was noted to be reduced in the HEAH-treated groups, especially at T2 (400 mg/kg b.w., p.o.) and in the combination group [HEAH (400 mg/kg b.w., p.o.) + Metformin (75 mg/kg b.w., p.o.) + Atorvastatin (05 mg/kg b.w., p.o.)] in comparison to the DC group. The renal histology of the COMB exhibited similarities to the normal control group, characterised by minimal tubular dilation, which was subsequently observed in T2, RC, and T1.

7.2. Conclusion

Metabolomics-integrated network pharmacology approach elucidates the interactions of phytoconstituents with diverse physiologically active targets, highlighting the multi-molecule, multi-target characteristics of plant extracts in delivering therapeutic effects. This distinctive approach to screening bioactive phytocompounds has expedited the lead identification process in phytomarker development and drug discovery from traditional medicine for the management of

lifestyle-related metabolic disorders. The northeastern region of India is rich in a diverse array of medicinal herbs and food plants that have been traditionally used for generations to manage lifestyle-related metabolic disorders. Despite their ethnopharmacological potential, there is a scarcity in the utilisation of these medicinal and food plants in newer drug development. The discovery and drug development process requires proper scientific validation and therapeutic evaluation of traditional claims, citing the chemical matrix and mechanistic approach in the management of lifestyle-related metabolic disorders.

In relation to traditional uses, three plants from the northeastern region of India were selected for metabolite profiling, network pharmacology to elucidate mechanisms of action, and *in vitro/in vivo* therapeutic evaluation based on their enzyme inhibitory potential and preventive potential against diet-induced lifestyle-related metabolic disorders. The metabolomics analysis of the plant extracts helped identify the bioactive phytocompounds present, which exhibited biological activity. The network pharmacology approach further mapped the bioactive phytocompounds with a probable mechanism of action in the management of lifestyle-related metabolic disorders. *A. hookeri* demonstrated a greater richness and diversity of bioactive phytocompounds along with a strong network of multi-molecule multi-target interactions compared to *B. hispida* and *H. cordata*. The results of the enzyme inhibition study indicated that the compounds of *A. hookeri*, *B. hispida*, and *H. cordata* serve as major contributors to α-glucosidase, α-amylase, and pancreatic lipase enzyme inhibition, which could play a useful role in the management of lifestyle-related metabolic disorders, especially Non-Insulin Dependent Diabetes Mellitus and Obesity. The *in vivo* assessment of the therapeutic potential against diet-induced hyperglycemia and hyperlipidaemia resulted in the exploration of the prophylactic potential of *A. hookeri.* These results will prove to be quintessential in the development of safe and efficacious nutraceuticals from *A. hookeri* to prevent the risk of progression of metabolic disorders, such as Non-Insulin Dependent Diabetes Mellitus and Obesity.

7.3. Future prospect

Thus, the present work addresses several phytochemical and therapeutic aspects of the three selected plants of NER, widely used in the daily diet and traditional system of medicine. The integrative approach of metabolomics and network pharmacology, combined with therapeutic evaluation in in vitro and in vivo settings, may be beneficial in the development of value-added formulations for managing lifestyle-related metabolic disorders, particularly NIDDM and Obesity. Furthermore, studies should be conducted at the molecular and transcriptomic levels to validate the probable mechanism of action in greater detail, along with safety and toxicity studies on CYP isoenzymes and normal cell lines. The exploration of traditional diets and medicinal plants in the management of lifestyle disorders will not only be applicable in the development of novel alternative therapeutics but will also serve as a gateway to fostering the bioeconomy of Northeast India.

7.4. Publications and conference presentations

7.4.1. *Paper communicated*

- Mukherjee, P.K., Banerjee, S., Das Gupta, B., Kar, A., 2022. Chapter 1 - Evidence-based validation of herbal medicine: Translational approach, in: Mukherjee, Pulok K. (Ed.), Evidence-Based Validation of Herbal Medicine (Second Edition). Elsevier, pp. 1–41. https://doi.org/10.1016/B978-0-323-85542- 6.00025-1
- Gayen, S., Das Gupta, B., Mukherjee, P.K., Haldar, P.K., Network Pharmacology in the Scientific Validation of Traditional Medicine for Management of Metabolic Disorders, in: Nissapatron, V., Bodade, R., Bala, A., Pandey, R., Gaurav, A. (Ed.), Network Pharmacology : Exploring New Horizons in Drug Discovery. Elsevier. ISBN: 9780443440892. [Communicated]
- Das Gupta, B., Gayen, S., Chowdhury, S., Chatterjee, T., Kar, A., Duangyod, T., Charoensup, R., Haldar, P. K., & Mukherjee, P. K. Integrative metabolomics and network pharmacology - Exploring the antidiabetic and antiobesity potential of *Allium hookeri* Thwaites in C57BL/6J mice. Phytomedicine. Elsevier. [Communicated].

- Das Gupta, B., Kar, A., Singha, S., Gayen, S., Jana, S., Sharma, N., Haldar, P.K., Mukherjee, P.K., 2025b. Metabolite Profiling and Integrated Network Pharmacology Based Mechanism of *Benincasa hispida* (Thunb.) Cogn. Fruit Against NIDDM. Phytochemical Analysis 36, 884–895. https://doi.org/10.1002/pca.3476.
- Das Gupta, B., Gayen, S., Kar, A., Haldar, P. K., & Mukherjee, P. K. Metabolite profiling and network pharmacology analysis of *Houttuynia cordata* Thunb. against hyperlipidaemia and hyperglycaemia. Phytochemical Analysis. Wiley. [Communicated].

7.4.2. *Paper presented*

- Das Gupta, B., Gayen, S., Haldar, P. K., Sharma, N., Mukherjee, P. K., & Kar, A. Metabolomics integrated network pharmacology analysis for combination synergy-based approach for exploring traditionally used medicinal plants of NER for the management of diabetes and obesity. In 11th Convention of the Society for Ethnopharmacology & International Conference (SECON 2024) at BRIC-Institute of Bioresources and Sustainable Development, Gangtok, Sikkim, India during November 15-16, 2024.
- Das Gupta, B., Kar, A., Haldar, P. K., Sharma, N., & Mukherjee, P. K. UHPLC-QTOF-MS/MS analysis coupled with validated network pharmacology approach to reveal synergy-based mechanism of action of medicinal food plants from NER in the treatment of diabetes and related disorders. In P. K. Haldar (Ed.), Proceedings of the International Conference on Indian Medicinal Plants in Drug Discovery: Tradition, Science & Innovation (ISBN: 978-93-48215-95-6). School of Natural Product Studies, Jadavpur University, Kolkata, India during January 21–22, 2025.
- Das Gupta, B., Kar, A., Singha, S., Jana, S., Gayen, S., Chowdhury, S., Haldar, P. K., & Mukherjee, P. K. LC-QTOFMS-based metabolite profiling and evaluation of α-glucosidase and α-amylase inhibitory potential of combined plant extract-based nutraceutical formulation from NER. In

International Bioresource Conclave & Ethnopharmacology Congress: 22nd International Congress of International Society for Ethnopharmacology & 10th International Congress of Society for Ethnopharmacology: at Institute of Bioresources and Sustainable Development, Imphal, Manipur, India, during February 24–26, 2023.

- Das Gupta, B., Gayen, S., Chowdhury, S., Kar, A., Haldar, P. K., & Mukherjee, P. K. Evaluation of therapeutic potential of polyherbal product derived from medicinal food plants of Eastern Himalayan Region against diabetes. In 12th International Congress of Society for Ethnopharmacology & International Conference on Innovations in Drug Technology & Phytopharmaceuticals at KLE College of Pharmacy, KLE Academy of Higher Education and Research, Belagavi, Karnataka, India during March 6–8, 2025.

Chapter 8

8.1. References

8.1. References

Adeeyo, A.O., Amao, I.O., Ngandjui, Y.A.T., Alabi, M.A., Msagati, T.A.M., 2024. Metabolic profiling of plant and other natural products: Platforms, applications and quality evaluation. Microchemical Journal 207, 111779. https://doi.org/10.1016/j.microc.2024.111779

Ahmad, N., Hasan, N., Ahmad, Z., Zishan, M., Zohrameena, S., 2016. Momordica charantia: for traditional uses and pharmacological actions. Journal of Drug Delivery and Therapeutics 6, 40–44. https://doi.org/10.22270/jddt.v6i2.1202

Ahn, J., Chae, H.-S., Chin, Y.-W., Kim, J., 2017. Alkaloids from aerial parts of *Houttuynia cordata* and their anti-inflammatory activity. Bioorganic & Medicinal Chemistry Letters 27, 2807–2811. https://doi.org/10.1016/j.bmcl.2017.04.072

Akash, M.S.H., Rehman, K., Liaqat, A., 2018. Tumor Necrosis Factor-Alpha: Role in Development of Insulin Resistance and Pathogenesis of Type 2 Diabetes Mellitus. Journal of Cellular Biochemistry 119, 105–110. https://doi.org/10.1002/jcb.26174

Alkhalidy, H., Moore, W., Wang, Y., Luo, J., McMillan, R.P., Zhen, W., Zhou, K., Liu, D., 2018. The Flavonoid Kaempferol Ameliorates Streptozotocin-Induced Diabetes by Suppressing Hepatic Glucose Production. Molecules 23, 2338. https://doi.org/10.3390/molecules23092338

Anjana, R.M., Unnikrishnan, R., Deepa, M., Pradeepa, R., Tandon, N., Das, A.K., Joshi, S., Bajaj, S., Jabbar, P.K., Das, H.K., Kumar, A., Dhandhania, V.K., Bhansali, A., Rao, P.V., Desai, A., Kalra, S., Gupta, A., Lakshmy, R., Madhu, S.V., Elangovan, N., Chowdhury, S., Venkatesan, U., Subashini, R., Kaur, T., Dhaliwal, R.S., Mohan, V., Anjana, R.M., Unnikrishnan, R., Pradeepa, R., Deepa, M., Sudha, V., Nirmal, E., Subashini, R., Venkatesan, U., Nath, L.M., Lakshmy, R., Tandon, N., Mahanta, J., Madhu, S.V., Das, A.K., Pandey, A., Dhaliwal, R.S., Kaur, T., Rao, P.V., Rao, M.N., Jampa, L., Kaki, T., Das, H.K., Borah, P.K., Kumar, A., Sharma, S., Bhansali, A., Dash, K., Shrivas, V.K., Krishnan, A., Desai, A., Dias, A., Saboo, B., Padhiyar,

J.M., Kalra, S., Kalra, B., Mokta, J.K., Gulepa, R., Dhandhania, V.K., Adhikari, P., Rao, S., Jabbar, P.K., Jayakumari, C., Jain, S.M., Gupta, G., Joshi, S., Yajnik, C.S., Joshi, P.P., Ningombam, S., Singh, T.B., Budnah, R.O., Basaiawmoit, M.R., Rosangluaia, Lalramenga, P.C., Suokhrie, V., Tunyi, S., Tripathy, S.K., Sahu, N.C., Purty, A.J., John, M., Gupta, A., Gupta, B.L., Shrivastava, S.K., Tobgay, K.J., Kaleon, T.T., Reang, T., Das, S.K., Bajaj, S., Mathur, M.K., Modi, S., Kakkar, R., Chowdhury, S., Ghosh, S., 2023. Metabolic non-communicable disease health report of India: the ICMR-INDIAB national cross-sectional study (ICMR-INDIAB-17). The Lancet Diabetes & Endocrinology 11, 474–489. https://doi.org/10.1016/S2213-8587(23)00119-5

Ayam, V., 2011. Allium hookeri, Thw. Enum. A lesser known terrestrial perennial herb used as food and its ethnobotanical relevance in Manipur. African Journal of Food, Agriculture, Nutrition and Development 11, 5389–5412. https://doi.org/10.18697/ajfand.47.9330

Azmi, A.S., 2012. Network Pharmacology for Cancer Drug Discovery: Are We There Yet? Future Medicinal Chemistry 4, 939–941. https://doi.org/10.4155/fmc.12.44

Banerjee, S., Bhattacharjee, P., Kar, A., Mukherjee, P.K., 2019. LC–MS/MS analysis and network pharmacology of *Trigonella foenum-graecum* – A plant from Ayurveda against hyperlipidemia and hyperglycemia with combination synergy. Phytomedicine, 90th birthday of Professor Hildebert Wagner 60, 152944. https://doi.org/10.1016/j.phymed.2019.152944

Banerjee, S., Tiwari, A., Kar, A., Chanda, J., Biswas, S., Ulrich-Merzenich, G., Mukherjee, P.K., 2023. Combining LC-MS/MS profiles with network pharmacology to predict molecular mechanisms of the hyperlipidemic activity of *Lagenaria siceraria* stand. Journal of Ethnopharmacology 300, 115633. https://doi.org/10.1016/j.jep.2022.115633

Bao, S., Wu, Y.-L., Wang, X., Han, S., Cho, S., Ao, W., Nan, J.-X., 2020. Agriophyllum oligosaccharides ameliorate hepatic injury in type 2 diabetic

db/db mice targeting INS-R/IRS-2/PI3K/AKT/PPAR-γ/Glut4 signal pathway. Journal of Ethnopharmacology 257, 112863. https://doi.org/10.1016/j.jep.2020.112863

Basile, G., Qadir, M.M.F., Mauvais-Jarvis, F., Vetere, A., Shoba, V., Modell, A.E., Pastori, R.L., Russ, H.A., Wagner, B.K., Dominguez-Bendala, J., 2022. Emerging diabetes therapies: Bringing back the β-cells. Molecular Metabolism 60, 101477. https://doi.org/10.1016/j.molmet.2022.101477

Basumatary, S., Adhikari, P.P., Das, A.K., Raaman, N., Sharma, G.D., Sarmah, J., Dihingia, A., Baishya, R., Manna, P., Kalita, J., 2024. Antihyperglycemic and antihyperlipidemic effects of fruit extract of *Hodgsonia heteroclita* (Roxb.) Hook. f. & Thomson in diabetic mice. Journal of Ethnopharmacology 328, 118094. https://doi.org/10.1016/j.jep.2024.118094

Behl, T., Kumar, K., Singh, S., Sehgal, A., Sachdeva, M., Bhatia, S., Al-Harrasi, A., Buhas, C., Teodora Judea-Pusta, C., Negrut, N., Alexandru Munteanu, M., Brisc, C., Bungau, S., 2021. Unveiling the role of polyphenols in diabetic retinopathy. Journal of Functional Foods 85, 104608. https://doi.org/10.1016/j.jff.2021.104608

Bijttebier, S., Van der Auwera, A., Foubert, K., Voorspoels, S., Pieters, L., Apers, S., 2016. Bridging the gap between comprehensive extraction protocols in plant metabolomics studies and method validation. Analytica Chimica Acta 935, 136–150. https://doi.org/10.1016/j.aca.2016.06.047

Bonnet, F., Scheen, A., 2017. Understanding and overcoming metformin gastrointestinal intolerance. Diabetes, Obesity and Metabolism 19, 473–481. https://doi.org/10.1111/dom.12854

Chaachouay, N., Zidane, L., 2024. Plant-Derived Natural Products: A Source for Drug Discovery and Development. Drugs and Drug Candidates 3, 184–207. https://doi.org/10.3390/ddc3010011

Chanda, J., Mukherjee, P.K., Biswas, R., Singha, S., Kar, A., Haldar, P.K., 2021. Lagenaria siceraria and it’s bioactive constituents in carbonic anhydrase

inhibition: A bioactivity guided LC–MS/MS approach. Phytochemical Analysis 32, 298–307. https://doi.org/10.1002/pca.2975

Chanda, J., Mukherjee, P.K., Kar, A., Maitra, P.Kr., Singha, S., Halder, P.K., Gajbhiye, R., Vishnuvardh, R., 2020. LC–QTOF–MS-based metabolite profiling and evaluation of α-glucosidase inhibitory kinetics of Coccinia grandis fruit. Biomedical Chromatography 34, e4950. https://doi.org/10.1002/bmc.4950

Chandran, U., 2015. Network pharmacology: An emerging tool for natural product drug discovery and evidence-based Ayurveda. Proceedings of the Indian National Science Academy 81. https://doi.org/10.16943/ptinsa/2015/v81i3/48229

Chanu, K.D., Sharma, N., Kshetrimayum, V., Chaudhary, S.K., Ghosh, S., Haldar, P.K., Mukherjee, P.K., 2023. Ageratina adenophora (Spreng.) King & H. Rob. Standardized leaf extract as an antidiabetic agent for type 2 diabetes: An in vitro and in vivo evaluation. Front. Pharmacol. 14. https://doi.org/10.3389/fphar.2023.1178904

Chhikara, N., Devi, H.R., Jaglan, S., Sharma, P., Gupta, P., Panghal, A., 2018. Bioactive compounds, food applications and health benefits of Parkia speciosa (stinky beans): a review. Agriculture & Food Security 7, 46. https://doi.org/10.1186/s40066-018-0197-x

Choi, J.-H., Lee, E.-B., Jang, H.-H., Cha, Y.-S., Park, Y.-S., Lee, S.-H., 2021. Allium hookeri Extracts Improve Scopolamine-Induced Cognitive Impairment via Activation of the Cholinergic System and Anti-Neuroinflammation in Mice. Nutrients 13, 2890. https://doi.org/10.3390/nu13082890

Choi, S.-I., Men, X., Oh, G., Im, J.-H., Choi, Y.-E., Yang, J.-M., Cho, J.-H., Lee, O.-H., 2024. Identification of marker compounds in fermented *Benincasa hispida* and validation of the method for its analysis. Food Chemistry: X 21, 101208. https://doi.org/10.1016/j.fochx.2024.101208

Dadheech, N., Srivastava, A., Paranjape, N., Gupta, Shivika, Dave, A., Shah, G.M., Bhonde, R.R., Gupta, Sarita, 2015. Swertisin an Anti-Diabetic Compound Facilitate Islet Neogenesis from Pancreatic Stem/Progenitor Cells via p-38 MAP Kinase-SMAD Pathway: An In-Vitro and In-Vivo Study. PLOS ONE 10, e0128244. https://doi.org/10.1371/journal.pone.0128244

Dagawal, D., Kumar, S., Supriya Devi, R., Saradar, B., Rani, M., Chavan, R., 2023. Houttuynia cordata Thunb.: A potential nutraceutical herb, in: Medico-Biowealth of India. APRF Publisher, pp. 25–30. https://doi.org/10.5281/zenodo.8404557

Das, B., Bhardwaj, P.K., Chaudhary, S.K., Pathaw, N., Singh, H.K., Tampha, S., Singh, K.K., Sharma, N., Mukherjee, P.K., 2024. Bioeconomy and ethnopharmacology – Translational perspective and sustainability of the bioresources of northeast region of India. Journal of Ethnopharmacology 330, 118203. https://doi.org/10.1016/j.jep.2024.118203

Das, B., Keithellakpam, O.S., Chanu, T.B., Pathaw, N., Rai, S., Singh, O.S., Nanjappan, S.K., Soibam, A., Sharma, N., Bhardwaj, P.K., Mukherjee, P.K., 2025a. Metabolomic study of *Polygonum posumbu* Buch. Ham. Ex D. Don integrating with anti-diabetic potential - a mechanistic approach. Fitoterapia 182, 106440. https://doi.org/10.1016/j.fitote.2025.106440

Das, B., Somkuwar, B.G., Chaudhary, S.K., Kharlyngdoh, E., Pakyntein, C.L., Basor, K., Shukla, J.K., Bhardwaj, P.K., Mukherjee, P.K., 2025b. Therapeutics of bitter plants from Northeast region of India and their pharmacological and phytochemical perspectives. Pharmacological Research 212, 107626. https://doi.org/10.1016/j.phrs.2025.107626

Das Gupta, B., Kar, A., Gayen, S., Jana, S., Sarkar, S., Mukherjee, M., Sarkar, G., Sinha, S., Sharma, N., Haldar, P.K., 2025a. UPLC-QTOF-MS/MS driven network pharmacology approach for elucidating antiproliferative pharmacological mechanisms of *Plumbago zeylanica* L. Industrial Crops and Products 225, 120441. https://doi.org/10.1016/j.indcrop.2024.120441

Das Gupta, B., Kar, A., Singha, S., Gayen, S., Jana, S., Sharma, N., Haldar, P.K., Mukherjee, P.K., 2025b. Metabolite Profiling and Integrated Network Pharmacology Based Mechanism of Benincasa hispida (Thunb.) Cogn. Fruit Against Non-insulin-Dependent Diabetes Mellitus. Phytochemical Analysis 36, 884–895. https://doi.org/10.1002/pca.3476

Das, M.J., Banerjee, D., Banerjee, A., Muchahary, S., Sinha, A., Gogoi, D., Chattopadhyay, P., Dasgupta, S., Deka, S.C., 2024. Safety and antidiabetic activity of *Lagenaria siceraria* (Molina) Standl. juice in streptozotocin - induced diabetic rats. Journal of Ethnopharmacology 319, 117111. https://doi.org/10.1016/j.jep.2023.117111

Deb, C.R., Sharma, T.I., Jamir, N., 2023. Ethno-medicinal anti-diabetics plants of Northeast India: A review. J Pharmacogn Phytochem 12, 86–110. https://doi.org/10.22271/phyto.2023.v12.i3b.14668

Deka, B., Barge, S.R., Bharadwaj, S., Kashyap, B., Manna, P., Borah, J.C., Talukdar, N.C., 2021. Beneficial effect of the methanolic leaf extract of *Allium hookeri* on stimulating glutathione biosynthesis and preventing impaired glucose metabolism in type 2 diabetes. Archives of Biochemistry and Biophysics 708, 108961. https://doi.org/10.1016/j.abb.2021.108961

Deka, B., Manna, P., Borah, J.C., Talukdar, N.C., 2022. A review on phytochemical, pharmacological attributes and therapeutic uses of *Allium hookeri*. Phytomedicine Plus 2, 100262. https://doi.org/10.1016/j.phyplu.2022.100262

Dent, R., Blackmore, A., Peterson, J., Habib, R., Kay, G.P., Gervais, A., Taylor, V., Wells, G., 2012. Changes in Body Weight and Psychotropic Drugs: A Systematic Synthesis of the Literature. PLOS ONE 7, e36889. https://doi.org/10.1371/journal.pone.0036889

Devi, L.V., Basumatary, K., 2017. Study on medicinal value of herbs and vegetables commonly used in Manipuri cuisine through ayurvedic perspective. Ayushdhara 4, 1019–1025.

Devi, S., Sinha, R., Sibi G, 2022. Northeast India: A treasury of medicinal plants. elsr 08, 05–21. https://doi.org/10.31783/elsr.2022.810521

Dolinoy, D.C., Weidman, J.R., Waterland, R.A., Jirtle, R.L., 2006. Maternal Genistein Alters Coat Color and Protects Avy Mouse Offspring from Obesity by Modifying the Fetal Epigenome. Environmental Health Perspectives 114, 567–572. https://doi.org/10.1289/ehp.8700

Du, R., Wei, Y., Liu, Z., Wang, M., Wang, Z., 2025. In-depth analysis of Pseudotargeted metabolomics strategy: Multi-field applications, advantages and challenges. Microchemical Journal 215, 114414. https://doi.org/10.1016/j.microc.2025.114414

Ebrahimi, E., Shirali, S., Afrisham, R., 2015. Effect and Mechanism of Herbal Ingredients in Improving Diabetes Mellitus Complications (Review Article No. 12). Jundishapur Journal of Natural Pharmaceutical Products.

Fatima, L.A., Campello, R.S., Santos, R. de S., Freitas, H.S., Frank, A.P., Machado, U.F., Clegg, D.J., 2017. Estrogen receptor 1 (ESR1) regulates VEGFA in adipose tissue. Sci Rep 7, 16716. https://doi.org/10.1038/s41598-017-16686-7

Floresta, G., Patamia, V., Zagni, C., Rescifina, A., 2022. Adipocyte fatty acid binding protein 4 (FABP4) inhibitors. An update from 2017 to early 2022. European Journal of Medicinal Chemistry 240, 114604. https://doi.org/10.1016/j.ejmech.2022.114604

Gayen, S., Jana, S., Das Gupta, B., Ghosh, A., Kar, A., Bala, A., Mukherjee, P.K., Haldar, P.K., 2024. Exploration of anti-diabetic activity and metabolite profiling of Bruguiera cylindrica (l.) Bl.—in vivo anti-diabetic activity, exploration of molecular mechanism, and network pharmacological analysis. J Pharm Pharmacol 76, 798–812. https://doi.org/10.1093/jpp/rgae030

Ge, S.X., Jung, D., Yao, R., 2020. ShinyGO: a graphical gene-set enrichment tool for animals and plants. Bioinformatics 36, 2628–2629. https://doi.org/10.1093/bioinformatics/btz931

George Thompson, A.M., Iancu, C.V., Nguyen, T.T.H., Kim, D., Choe, J., 2015. Inhibition of human GLUT1 and GLUT5 by plant carbohydrate products; insights into transport specificity. Sci Rep 5, 12804. https://doi.org/10.1038/srep12804

Ghosh, D., 2022. Chapter 17 - Obesity and lifestyle-related disorders beyond the stethoscope: Role of botanicals, in: Mukherjee, P.K. (Ed.), Evidence-Based Validation of Herbal Medicine (Second Edition). Elsevier, pp. 423–430. https://doi.org/10.1016/B978-0-323-85542-6.00007-X

Gill, N.S., Dhiman, K., Bajwa, J., Sharma, P., Sood, S., 2010. Evaluation of Free Radical Scavenging, Anti-inflammatory and Analgesic Potential of Benincasa hispida Seed Extract. IJP 6, 652–657. https://doi.org/10.3923/ijp.2010.652.657

Government of India, 2004. Kusmanda (Fruit), in: The Ayurvedic Pharmacopoeia of India (Part -I, Volume IV). Government of India, Ministry of Health and Family Welfare, Department of Ayush, India, pp. 55–57.

Government of India, 2001a. Citraka (Root), in: The Ayurvedic Pharmacopoeia of India (Part -I, Volume I). Government of India, Ministry of Health and Family Welfare, Department of Ayush, India, pp. 50–51.

Government of India, 2001b. Tumbini (Fresh Fruit), in: The Ayurvedic Pharmacopoeia of India (Part -I, Volume III). Government of India, Ministry of Health and Family Welfare, Department of Ayush, India, pp. 312–314.

Grover, J.K., Adiga, G., Vats, V., Rathi, S.S., 2001. Extracts of *Benincasa hispida* prevent development of experimental ulcers. Journal of Ethnopharmacology 78, 159–164. https://doi.org/10.1016/S0378-8741(01)00334-8

Gupta, B.D., Kar, A., Narayan, S., Thakur, C.P., Mukherjee, P.K., Haldar, P.K., 2023. Ultra-performance liquid chromatography-Quadrupole time-of-flight tandem mass spectrometry-based metabolite profiling, quality evaluation, and marker analysis of Trachyspermum ammi (L.) Sprague by high-performance thin-layer chromatography. Journal of Separation Science 46, 2200872. https://doi.org/10.1002/jssc.202200872

Gurumayum, S., Bharadwaj, S., Sheikh, Y., Barge, S.R., Saikia, K., Swargiary, D., Ahmed, S.A., Thakur, D., Borah, J.C., 2023. Taxifolin-3-O-glucoside from *Osbeckia nepalensis* Hook. mediates antihyperglycemic activity in CC1 hepatocytes and in diabetic Wistar rats *via* regulating AMPK/G6Pase/PEPCK signaling axis. Journal of Ethnopharmacology 303, 115936. https://doi.org/10.1016/j.jep.2022.115936

Hafizur, R.M., Hameed, A., Shukrana, M., Raza, S.A., Chishti, S., Kabir, N., Siddiqui, R.A., 2015. Cinnamic acid exerts anti-diabetic activity by improving glucose tolerance *in vivo* and by stimulating insulin secretion *in vitro*. Phytomedicine 22, 297–300. https://doi.org/10.1016/j.phymed.2015.01.003

Haldar, P.K., Ghosh, S., Debnath, M., Das Gupta, B., Jana, S., 2024. *Benincasa hispida* (Thunb.) Cogn., in: Haldar, P.K. (Ed.), Traditional Medicinal Plants of India: Unveiling Ethnopharmacological Wonders Vol. 1. Current Books International.

Hood, L., Perlmutter, R.M., 2004. The impact of systems approaches on biological problems in drug discovery. Nat Biotechnol 22, 1215–1217. https://doi.org/10.1038/nbt1004-1215

Hopkins, A.L., 2008. Network pharmacology: the next paradigm in drug discovery. Nat Chem Biol 4, 682–690. https://doi.org/10.1038/nchembio.118

Hopkins, A.L., 2007. Network pharmacology. Nat Biotechnol 25, 1110–1111. https://doi.org/10.1038/nbt1007-1110

Huang, R., Zhang, W., Hu, Y., Xu, J., Dong, Z., Liu, J., Zhou, L., 2025. *Houttuynia cordata* polysaccharides ameliorate atopic dermatitis in mice through modulation of skin immune barrier and lipid metabolism. International Journal of Biological Macromolecules 314, 144264. https://doi.org/10.1016/j.ijbiomac.2025.144264

Hussain, T., Tan, B., Murtaza, G., Liu, G., Rahu, N., Saleem Kalhoro, M., Hussain Kalhoro, D., Adebowale, T.O., Usman Mazhar, M., Rehman, Z.U., Martínez, Y., Akber Khan, S., Yin, Y., 2020. Flavonoids and type 2 diabetes: Evidence of efficacy in clinical and animal studies and delivery strategies to enhance their therapeutic efficacy. Pharmacol Res 152, 104629. https://doi.org/10.1016/j.phrs.2020.104629

Imran, M., Alshrari, A.S., Hafiz, M.N., Jawad, M.M., Khan, A., Alanazi, F.J., Asdaq, S.M.B., 2025. Exploring therapeutic paradigm focusing on genes, proteins, and pathways to combat leprosy and tuberculosis: A network medicine and drug repurposing approach. Journal of Infection and Public Health 18, 102763. https://doi.org/10.1016/j.jiph.2025.102763

Islam, M.T., Quispe, C., El-Kersh, D.M., Shill, M.C., Bhardwaj, K., Bhardwaj, P., Sharifi-Rad, J., Martorell, M., Hossain, R., Al-Harrasi, A., Al-Rawahi, A., Butnariu, M., Rotariu, L.S., Suleria, H.A.R., Taheri, Y., Docea, A.O., Calina, D., Cho, W.C., 2021. A Literature-Based Update on Benincasa hispida (Thunb.) Cogn.: Traditional Uses, Nutraceutical, and Phytopharmacological Profiles. Oxidative Medicine and Cellular Longevity 2021, 6349041. https://doi.org/10.1155/2021/6349041

Jana, S., Gayen, S., Gupta, B.D., Singha, S., Mondal, J., Kar, A., Nepal, A., Ghosh, S., Rajabalaya, R., David, S.R., Balaraman, A.K., Bala, A., Mukherjee, P.K., Haldar, P.K., 2024. Investigation on Anti-diabetic Efficacy of a Cucurbitaceae Food Plant from the North-East Region of India: Exploring the Molecular Mechanism through Modulation of Oxidative Stress and Glycosylated Hemoglobin (HbA1c). http://www.eurekaselect.com 24, 220–234. https://doi.org/10.2174/1871530323666230907115818

Janapati, Y.K., Junapudi, S., 2024. Progress in experimental models to investigate the in vivo and in vitro antidiabetic activity of drugs. Animal Models and Experimental Medicine 7, 297–309. https://doi.org/10.1002/ame2.12442

Jang, J.-Y., Lee, M.-J., You, B.-R., Jin, J.-S., Lee, S.-H., Yun, Y.-R., Kim, H.J., 2017. Allium hookeri root extract exerts anti-inflammatory effects by nuclear factor-κB down-regulation in lipopolysaccharide-induced RAW264.7 cells. BMC Complementary and Alternative Medicine 17, 126. https://doi.org/10.1186/s12906-017-1633-3

Jiang, Y., Li, X., Zhao, W.-J., Liu, F.-J., Yang, L.-L., Li, P., Li, H.-J., 2024. Integration of untargeted and pseudotargeted metabolomics reveals specific markers for authentication and adulteration detection of Fritillariae Bulbus using tandem mass spectrometry and chemometrics. Journal of Pharmaceutical and Biomedical Analysis 242, 116013. https://doi.org/10.1016/j.jpba.2024.116013

Jing, Y., Chen, W., Qiu, X., Qin, S., Gao, W., Li, C., Quan, W., Cai, K., 2024. Exploring Metabolic Characteristics in Different Geographical Locations and Yields of Nicotiana tabacum L. Using Gas Chromatography–Mass Spectrometry Pseudotargeted Metabolomics Combined with Chemometrics. Metabolites 14, 176. https://doi.org/10.3390/metabo14040176

Joshi, C.P., Baldi, A., Kumar, N., Pradhan, J., 2025. Harnessing network pharmacology in drug discovery: an integrated approach. Naunyn-Schmiedeberg’s Arch Pharmacol 398, 4689–4703. https://doi.org/10.1007/s00210-024-03625-3

Ju, L., Wen, X., Wang, C., Wei, Y., Peng, Y., Ding, Y., Feng, L., Shu, L., 2017. Salidroside, A Natural Antioxidant, Improves β-Cell Survival and Function via Activating AMPK Pathway. Front. Pharmacol. 8. https://doi.org/10.3389/fphar.2017.00749

Junejo, J.A., Gogoi, G., Islam, J., Rudrapal, M., Mondal, P., Hazarika, H., Zaman, K., 2018. Exploration of antioxidant, antidiabetic and hepatoprotective activity of *Diplazium esculentum* - A wild edible plant from North Eastern India. Future Journal of Pharmaceutical Sciences 4, 93–101. https://doi.org/10.1016/j.fjps.2017.10.005

Kadiri, S.K., Tiwari, P., 2025. Integrating traditional medicine with network pharmacology for Alzheimer's treatment. Health Sciences Review 15, 100223. https://doi.org/10.1016/j.hsr.2025.100223

Kala, C.P., 2005. Ethnomedicinal botany of the Apatani in the Eastern Himalayan region of India. Journal of Ethnobiology and Ethnomedicine 1, 11. https://doi.org/10.1186/1746-4269-1-11

Kanagali, S.N., Patil, B.M., Khanal, P., Unger, B.S., 2022. *Cyperus rotundus* L. reverses the olanzapine-induced weight gain and metabolic changes-outcomes from network and experimental pharmacology. Computers in Biology and Medicine 141, 105035. https://doi.org/10.1016/j.compbiomed.2021.105035

Kanehisa, M., Furumichi, M., Sato, Y., Ishiguro-Watanabe, M., Tanabe, M., 2021. KEGG: integrating viruses and cellular organisms. Nucleic Acids Research 49, D545–D551. https://doi.org/10.1093/nar/gkaa970

Kar, A., Banerjee, S., Banerjee, R., Sharma, N., Katiyar, C.K., Mukherjee, P.K., 2024. Chapter Three - Antidiabetic natural products: Roadmap 2024, in: Seley-Radtke, K. (Ed.), Annual Reports in Medicinal Chemistry, Annual Reports in Medicinal Chemistry: Roadmaps. Academic Press, pp. 53–102. https://doi.org/10.1016/bs.armc.2024.09.001

Kazeem, M.I., Ashafa, A.O.T., 2015. *In-vitro* antioxidant and antidiabetic potentials of *Dianthus basuticus* Burtt Davy whole plant extracts. Journal of Herbal Medicine 5, 158–164. https://doi.org/10.1016/j.hermed.2015.06.003

Kim, H.J., Lee, M.-J., Jang, J.-Y., Lee, S.-H., 2019. Allium hookeri Root Extract Inhibits Adipogenesis by Promoting Lipolysis in High Fat Diet-Induced Obese Mice. Nutrients 11, 2262. https://doi.org/10.3390/nu11102262

Kim, S., Kim, D.-B., Lee, S., Park, J., Shin, D., Yoo, M., 2016. Profiling of organosulphur compounds using HPLC-PDA and GC/MS system and antioxidant activities in hooker chive (Allium hookeri). Natural Product Research 30, 2798–2804. https://doi.org/10.1080/14786419.2016.1164700

Kim, S., Lee, S., Shin, D., Yoo, M., 2015. Validation of a High-Performance Liquid Chromatography Photo-Diode Array Method for the Temperature Effects on Alk(En)yl Sulfides in Garlic Extracts. Journal of Liquid Chromatography & Related Technologies 38, 1608–1615. https://doi.org/10.1080/10826076.2015.1084317

Kimani, C.N., Reuter, H., Kotzé, S.H., Muller, C.J.F., 2023. Regeneration of Pancreatic Beta Cells by Modulation of Molecular Targets Using Plant-Derived Compounds: Pharmacological Mechanisms and Clinical Potential. Current Issues in Molecular Biology 45, 6216–6245. https://doi.org/10.3390/cimb45080392

Kitano, H., 2002. Computational systems biology. Nature 420, 206–210. https://doi.org/10.1038/nature01254

Kripasana, K., Xavier, J., 2020. Phytochemical analysis and antioxidant activity of leaf extracts of some selected plants of the family Acanthaceae. Plant Science Today 7, 264–274. https://doi.org/10.14719/pst.2020.7.2.717

Kshetrimayum, V., Chanu, K.D., Biona, T., Kar, A., Haldar, P.K., Mukherjee, P.K., Sharma, N., 2024. *Paris polyphylla* Sm. characterized extract infused ointment accelerates diabetic wound healing in In-vivo model. Journal of Ethnopharmacology 331, 118296. https://doi.org/10.1016/j.jep.2024.118296

Kshetrimayum, V., Chanu, K.D., Ghosh, S., Haldar, P.K., Mukherjee, P.K., Sharma, N., 2023. *Paris polyphylla Sm.* extract enriched with diosgenin as

an antidiabetic agent: *In vitro* and *in vivo* study. Phytomedicine Plus 3, 100497. https://doi.org/10.1016/j.phyplu.2023.100497

Kumar, M., Prasad, S., Hemalatha, S., Hemalatha, S., 2014a. A Current Update on the Phytopharmacological Aspects of Houttuynia cordata Thunb. Pharmacognosy Reviews 8, 22–35. https://doi.org/10.4103/0973-7847.125525

Kumar, M., Prasad, S.K., Krishnamurthy, S., Hemalatha, S., 2014b. Antihyperglycemic Activity of Houttuynia cordata Thunb. in Streptozotocin-Induced Diabetic Rats. Advances in Pharmacological and Pharmaceutical Sciences 2014, 809438. https://doi.org/10.1155/2014/809438

Kumari, R., Goldar, W.A., Mondal, S., Patra, S., Bhattacharya, S., Haldar, P.K., 2021. Protective effect of Basella alba leaf against diabetic nephropathy in rats. ADV TRADIT MED (ADTM) 21, 111–119. https://doi.org/10.1007/s13596-020-00458-2

Kumkar, P.S., Chakraborty, R., Upadhyay, A.M., Das, J.M., Bala, A., 2024. Preliminary study on cytotoxicity of selegiline on different cancer cell lines: exploration of the induction of ROS-independent apoptosis in breast cancer cells. Med Oncol 41, 204. https://doi.org/10.1007/s12032-024-02451-0

Lee, D., Imm, J.-Y., 2018. Antiobesity Effect of Tricin, a Methylated Cereal Flavone, in High-Fat-Diet-Induced Obese Mice. J. Agric. Food Chem. 66, 9989–9994. https://doi.org/10.1021/acs.jafc.8b03312

Lee, K.-M., Jeon, J.-Y., Lee, B.-J., Lee, H., Choi, H.-K., 2017. Application of Metabolomics to Quality Control of Natural Product Derived Medicines. Biomolecules & Therapeutics 25, 559–568. https://doi.org/10.4062/biomolther.2016.249

Lee, W.-H., Najjar, S.M., Kahn, C.R., Hinds, T.D., 2023. Hepatic insulin receptor: new views on the mechanisms of liver disease. Metabolism 145, 155607. https://doi.org/10.1016/j.metabol.2023.155607

Li, L., Yang, Lele, Yang, Liuqing, He, C., He, Y., Chen, L., Dong, Q., Zhang, H., Chen, S., Li, P., 2023. Network pharmacology: a bright guiding light on the way to explore the personalized precise medication of traditional Chinese medicine. Chinese Medicine 18, 146. https://doi.org/10.1186/s13020-023-00853-2

Long, Y.C., Zierath, J.R., 2006. AMP-activated protein kinase signaling in metabolic regulation. J Clin Invest 116, 1776–1783. https://doi.org/10.1172/JCI29044

Luo, W., Brouwer, C., 2013. Pathview: an R/Bioconductor package for pathway-based data integration and visualization. Bioinformatics 29, 1830–1831. https://doi.org/10.1093/bioinformatics/btt285

Ma, Q., Wei, R., Wang, Z., Liu, W., Sang, Z., Li, Y., Huang, H., 2017. Bioactive alkaloids from the aerial parts of *Houttuynia cordata*. Journal of Ethnopharmacology 195, 166–172. https://doi.org/10.1016/j.jep.2016.11.013

Maity, S., Acharyya, A., Sankar Chakraborti, A., 2022. Flavonoid-based polymeric nanoparticles: A promising approach for cancer and diabetes treatment. European Polymer Journal 177, 111455. https://doi.org/10.1016/j.eurpolymj.2022.111455

Mangal, P., Khare, P., Jagtap, S., Bishnoi, M., Kondepudi, K.K., Bhutani, K.K., 2017. Screening of six Ayurvedic medicinal plants for anti-obesity potential: An investigation on bioactive constituents from *Oroxylum indicum* (L.) Kurz bark. Journal of Ethnopharmacology, Special issue on Ayurveda 197, 138–146. https://doi.org/10.1016/j.jep.2016.07.070

Mani, A., Kushwaha, K., Khurana, N., Gupta, J., 2022. p-Coumaric acid attenuates high-fat diet-induced oxidative stress and nephropathy in diabetic rats. Journal of Animal Physiology and Animal Nutrition 106, 872–880. https://doi.org/10.1111/jpn.13645

Markley, J.L., Brüschweiler, R., Edison, A.S., Eghbalnia, H.R., Powers, R., Raftery, D., Wishart, D.S., 2017. The future of NMR-based metabolomics. Current Opinion in Biotechnology, Analytical biotechnology 43, 34–40. https://doi.org/10.1016/j.copbio.2016.08.001

Miao, L., Zhang, H., Cheong, M.S., Zhong, R., Garcia-Oliveira, P., Prieto, M.A., Cheng, K.-W., Wang, M., Cao, H., Nie, S., Simal-Gandara, J., Cheang, W.S., Xiao, J., 2023. Anti-diabetic potential of apigenin, luteolin, and baicalein via partially activating PI3K/Akt/Glut-4 signaling pathways in insulin-resistant HepG2 cells. Food Science and Human Wellness 12, 1991–2000. https://doi.org/10.1016/j.fshw.2023.03.021

Moller, D.E., Berger, J.P., 2003. Role of PPARs in the regulation of obesity-related insulin sensitivity and inflammation. International Journal of Obesity 27, S17–S21. https://doi.org/10.1038/sj.ijo.0802494

Moselhy, S.S., Razvi, S.S., ALshibili, F.A., Kuerban, A., Hasan, M.N., Balamash, K.S., Huwait, E.A., Abdulaal, W.H., Al-Ghamdi, M.A., Kumosani, T.A., Abulnaja, K.O., Al-Malki, A.L., Asami, T., Ismail, I.M., 2018. m-Coumaric acid attenuates non-catalytic protein glycosylation in the retinas of diabetic rats. Journal of Pesticide Science 43, 180–185. https://doi.org/10.1584/jpestics.D17-091

Mukherjee, K., Biswas, R., Chaudhary, S.K., Mukherjee, P.K., 2015. Chapter 18 - Botanicals as Medicinal Food and Their Effects against Obesity, in: Mukherjee, P.K. (Ed.), Evidence-Based Validation of Herbal Medicine. Elsevier, Boston, pp. 373–403. https://doi.org/10.1016/B978-0-12-800874-4.00018-0

Mukherjee, P., Roy, S., Ghosh, D., Nandi, S.K., 2022. Role of animal models in biomedical research: a review. Laboratory Animal Research 38, 18. https://doi.org/10.1186/s42826-022-00128-1

Mukherjee, P.K., 2019a. Chapter 17 - Plant Metabolomics and Quality Evaluation of Herbal Drugs, in: Mukherjee, P.K. (Ed.), Quality Control and Evaluation

of Herbal Drugs. Elsevier, pp. 629–653. https://doi.org/10.1016/B978-0-12-813374-3.00017-X

Mukherjee, P.K., 2019b. Chapter 3 - Quality Evaluation of Herbal Medicines: Challenges and Opportunities, in: Mukherjee, P.K. (Ed.), Quality Control and Evaluation of Herbal Drugs. Elsevier, pp. 53–77. https://doi.org/10.1016/B978-0-12-813374-3.00003-X

Mukherjee, P.K., Banerjee, S., Das Gupta, B., Kar, A., 2022a. Chapter 1 - Evidence-based validation of herbal medicine: Translational approach, in: Mukherjee, Pulok K. (Ed.), Evidence-Based Validation of Herbal Medicine (Second Edition). Elsevier, pp. 1–41. https://doi.org/10.1016/B978-0-323-85542-6.00025-1

Mukherjee, P.K., Banerjee, S., Kar, A., Chaudhary, S.K., Bhardwaj, P.K., Haldar, P.K., Sharma, N., 2022b. Chapter 21 - Synergy and network pharmacology—Establishing the efficacy of herbal medicine, in: Mukherjee, Pulok K. (Ed.), Evidence-Based Validation of Herbal Medicine (Second Edition). Elsevier, pp. 501–510. https://doi.org/10.1016/B978-0-323-85542-6.00017-2

Mukherjee, P.K., Harwansh, R.K., Bahadur, S., Biswas, S., Kuchibhatla, L.N., Tetali, S.D., Raghavendra, A.S., 2016. Metabolomics of Medicinal Plants - A Versatile Tool for Standardization of Herbal Products and Quality Evaluation of Ayurvedic Formulations. Current Science 111, 1624. https://doi.org/10.18520/cs/v111/i10/1624-1630

Mukherjee, P.K., Harwansh, R.K., Bahadur, S., Chanda, J., Biswas, S., Banerjee, S., 2020. Chapter 31 - Enzyme inhibition assay for metabolic disorders—exploring leads from medicinal plants, in: Verma, A.S., Singh, A. (Eds.), Animal Biotechnology (Second Edition). Academic Press, Boston, pp. 631–653. https://doi.org/10.1016/B978-0-12-811710-1.00033-1

Mukherjee, S., Bera, S., Banerjee, S., Mitra, A., Mukherjee, P.K., 2022. Chapter 19 - Ayurveda—Translational approaches towards validation as sustainable healthcare practices, in: Mukherjee, Pulok K. (Ed.), Evidence-Based

Validation of Herbal Medicine (Second Edition). Elsevier, pp. 463–485. https://doi.org/10.1016/B978-0-323-85542-6.00016-0

Nafie, M.S., Abu-Elsaoud, A.M., Diab, M.K., 2025. A comprehensive review on computational metabolomics: Advancing multiscale analysis through in-silico approaches. Computational and Structural Biotechnology Journal 27, 3191–3215. https://doi.org/10.1016/j.csbj.2025.07.016

Nahar, L., Sarker, S.D., 2022. Chapter 18 - Advances in applications of high-performance liquid chromatography in the analysis of herbal products, in: Mukherjee, P.K. (Ed.), Evidence-Based Validation of Herbal Medicine (Second Edition). Elsevier, pp. 431–461. https://doi.org/10.1016/B978-0-323-85542-6.00012-3

Neveu, V., Perez-Jiménez, J., Vos, F., Crespy, V., du Chaffaut, L., Mennen, L., Knox, C., Eisner, R., Cruz, J., Wishart, D., Scalbert, A., 2010. Phenol-Explorer: an online comprehensive database on polyphenol contents in foods. Database (Oxford) 2010, bap024. https://doi.org/10.1093/database/bap024

Noor, F., Tahir ul Qamar, M., Ashfaq, U.A., Albutti, A., Alwashmi, A.S.S., Aljasir, M.A., 2022. Network Pharmacology Approach for Medicinal Plants: Review and Assessment. Pharmaceuticals 15, 572. https://doi.org/10.3390/ph15050572

Oliveros, J.C., 2007. Venny. An interactive tool for comparing lists with Venn's diagrams [WWW Document]. Venny 2.1. URL https://bioinfogp.cnb.csic.es/tools/venny/index.html (accessed 6.25.25).

Pan, S., Yin, L., Liu, J., Tong, J., Wang, Z., Zhao, J., Liu, X., Chen, Y., Miao, J., Zhou, Y., Zeng, S., Xu, T., 2024. Metabolomics-driven approaches for identifying therapeutic targets in drug discovery. MedComm 5, e792. https://doi.org/10.1002/mco2.792

Pandey, A., Gupta, S.P., 2024. Personalized Medicine: A Comprehensive Review. Oriental Journal of Chemistry 40, 933–944.

Patil, R.N., Patil, R.Y., Ahirwar, B., Ahirwar, D., 2011. Evaluation of antidiabetic and related actions of some Indian medicinal plants in diabetic rats. Asian Pacific Journal of Tropical Medicine 4, 20–23. https://doi.org/10.1016/S1995-7645(11)60025-4

Patil, S.B., Kuvalekar, M.B., Yaraguppi, D.A., Prasanth, D.S.N.B.K., Halkavatagi, S.G., Tennalli, G.B., Javali, M.A., Khan, T.M.Y., 2025. Exploring the efficacy of Benincasa hispida extract on obesity linked inflammatory bowel disease by integrating computational analysis and experimental validations. Sci Rep 15, 14426. https://doi.org/10.1038/s41598-025-99256-6

Patra, S., Bhattacharya, S., Bala, A., Haldar, P.K., 2020. Antidiabetic effect of Drymaria cordata leaf against streptozotocin–nicotinamide-induced diabetic albino rats. Journal of Advanced Pharmaceutical Technology & Research 11, 44. https://doi.org/10.4103/japtr.JAPTR_98_19

Paul, A., Sarkar, A., Banerjee, T., Maji, A., Sarkar, S., Paul, S., Karmakar, S., Ghosh, N., Maity, T.K., 2023. Structural and molecular insights of protein tyrosine phosphatase 1B (PTP1B) and its inhibitors as anti-diabetic agents. Journal of Molecular Structure 1293, 136258. https://doi.org/10.1016/j.molstruc.2023.136258

Pi-Sunyer, X., 2003. A Clinical View of the Obesity Problem. Science 299, 859–860. https://doi.org/10.1126/science.1082319

Poornima, P., Kumar, J.D., Zhao, Q., Blunder, M., Efferth, T., 2016. Network pharmacology of cancer: From understanding of complex interactomes to the design of multi-target specific therapeutics from nature. Pharmacological Research 111, 290–302. https://doi.org/10.1016/j.phrs.2016.06.018

Pradhan, S., Rituparna, S., Dehury, H., Dhall, M., Singh, Y.D., 2023. Nutritional profile and pharmacological aspect of *Houttuynia cordata* Thunb. and their therapeutic applications. Pharmacological Research - Modern Chinese Medicine 9, 100311. https://doi.org/10.1016/j.prmcm.2023.100311

Putri, S.P., Ikram, M.M.M., Sato, A., Dahlan, H.A., Rahmawati, D., Ohto, Y., Fukusaki, E., 2022. Application of gas chromatography-mass spectrometry-based metabolomics in food science and technology. Journal of Bioscience and Bioengineering 133, 425–435. https://doi.org/10.1016/j.jbiosc.2022.01.011

Qing-Song, X., Sai-Jin, W., 2015. Highly Efficient Extraction of Decanoyl Acetaldehyde from Houttuynia cordata by a Cryogenic Grinding Technique. Chem Nat Compd 51, 359–360. https://doi.org/10.1007/s10600-015-1283-y

Ragasa, C., Cabrera, E., Torres, O., Buluran, A., Espineli, D., Raga, D., Shen, C.-C., 2015. Chemical Constituents and Bioactivities of Glinus Oppositifolius. Pharmacognosy Research 7, 138–147. https://doi.org/10.4103/0974-8490.150520

Rapaka, D., Bitra, V.R., Ummidi, R., Akula, A., 2021. *Benincasa hispida* alleviates amyloid pathology by inhibition of Keap1/Nrf2-axis: Emphasis on oxidative and inflammatory stress involved in Alzheimer's disease model. Neuropeptides 88, 102151. https://doi.org/10.1016/j.npep.2021.102151

Rhyu, D.Y., Park, S.H., 2013. Characterization of alkyl thiosulfinate in Allium hookeri root using HPLC-ESI-MS. J Korean Soc Appl Biol Chem 56, 457–459. https://doi.org/10.1007/s13765-013-3069-x

Safaei, M., Sundararajan, E.A., Driss, M., Boulila, W., Shapi'i, A., 2021. A systematic literature review on obesity: Understanding the causes & consequences of obesity and reviewing various machine learning approaches used to predict obesity. Computers in Biology and Medicine 136, 104754. https://doi.org/10.1016/j.compbiomed.2021.104754

Sarkar, S., Kar, A., Shaw, P., DasGupta, B., Keithellakpam, O.S., Mukherjee, P.K., Bhardwaj, P.K., Sharma, N., Haldar, P.K., Sinha, S., 2023. Hydroalcoholic root extracts of *Houttuynia cordata* (Thunb.) standardized by UPLC-Q-TOF-MS/MS promotes apoptosis in human hepatocarcinoma cell HepG2 via GSK-3β/β-catenin/PDL-1 axis. Fitoterapia 171, 105684. https://doi.org/10.1016/j.fitote.2023.105684

Shahwan, M., Alhumaydhi, F., Ashraf, G.M., Hasan, P.M.Z., Shamsi, A., 2022. Role of polyphenols in combating Type 2 Diabetes and insulin resistance. Int J Biol Macromol 206, 567–579. https://doi.org/10.1016/j.ijbiomac.2022.03.004

Shannon, P., Markiel, A., Ozier, O., Baliga, N.S., Wang, J.T., Ramage, D., Amin, N., Schwikowski, B., Ideker, T., 2003. Cytoscape: A Software Environment for Integrated Models of Biomolecular Interaction Networks. Genome Res. 13, 2498–2504. https://doi.org/10.1101/gr.1239303

Sheikh, Y., Maibam, B.C., Biswas, D., Laisharm, S., Deb, L., Talukdar, N.C., Borah, J.C., 2015. Anti-diabetic potential of selected ethno-medicinal plants of north east India. Journal of Ethnopharmacology 171, 37–41. https://doi.org/10.1016/j.jep.2015.05.030

Singh, K.D., Chetia, D., E., B.D., 2018. New flavonoid compound from Allium Hookeri Thwaites as a gastroprotective agent. Int J Pharm Pharm Sci 10, 24. https://doi.org/10.22159/ijpps.2018v10i5.24341

Singh, K.D., Chetia, D., Gogoi, N., Gogoi, B., Rudrapal, M., 2024. In Vivo and in Silico Based Evaluation of Antidiabetic Potential of an Isolated Flavonoid from Allium hookeri in Type 2 Diabetic Rat Model. Chemistry & Biodiversity 21, e202301299. https://doi.org/10.1002/cbdv.202301299

Singh, S., Gohil, K.J., Singh, M.P., 2024. Pharmacological update on *Benincasa Hispida* (Thunb.): A review. Pharmacological Research - Modern Chinese Medicine 12, 100478. https://doi.org/10.1016/j.prmcm.2024.100478

Singha, S., Das Gupta, B., Sarkar, A., Jana, S., Bharadwaj, P.K., Sharma, N., Haldar, P.K., Mukherjee, P.K., Kar, A., 2024. Chemo-profiling and exploring therapeutic potential of *Momordica dioica* Roxb. ex Willd. for managing metabolic related disorders: *In-vitro* studies, and docking based approach. Journal of Ethnopharmacology 331, 118351. https://doi.org/10.1016/j.jep.2024.118351

Singha, W.R., Kurmi, B., Sahoo, U.K., Sileshi, G.W., Nath, A.J., Das, A.K., 2021. *Parkia roxburghii*, an underutilized tree bean for food, nutritional and regional climate security. Trees, Forests and People 4, 100065. https://doi.org/10.1016/j.tfp.2021.100065

Sojan, A., Xu, B., 2025. A critical review of Luteolin's effects on glycolipid metabolism and the underlying molecular mechanisms. Journal of Functional Foods 132, 106972. https://doi.org/10.1016/j.jff.2025.106972

Srikanth, N., 2021. Bioinformatics and network pharmacology: Scope and relevance in Ayurveda research. Journal of Drug Research in Ayurvedic Sciences 6, 197. https://doi.org/10.4103/jdras.jdras_28_22

Szklarczyk, D., Morris, J.H., Cook, H., Kuhn, M., Wyder, S., Simonovic, M., Santos, A., Doncheva, N.T., Roth, A., Bork, P., Jensen, L.J., von Mering, C., 2017. The STRING database in 2017: quality-controlled protein–protein association networks, made broadly accessible. Nucleic Acids Research 45, D362–D368. https://doi.org/10.1093/nar/gkw937

Talevi, A., 2015. Multi-target pharmacology: possibilities and limitations of the "skeleton key approach" from a medicinal chemist perspective. Front. Pharmacol. 6. https://doi.org/10.3389/fphar.2015.00205

Tang, C., Prueksaritanont, T., 2010. Use of In Vivo Animal Models to Assess Pharmacokinetic Drug-Drug Interactions. Pharm Res 27, 1772–1787. https://doi.org/10.1007/s11095-010-0157-z

Tawfike, A.F., Viegelmann, C., Edrada-Ebel, R., 2013. Metabolomics and Dereplication Strategies in Natural Products, in: Roessner, U., Dias, D.A. (Eds.), Metabolomics Tools for Natural Product Discovery: Methods and Protocols. Humana Press, Totowa, NJ, pp. 227–244. https://doi.org/10.1007/978-1-62703-577-4_17

Thakur, M.S., Deshmukh, K.N., Dey, A., Ranjan, D., Goyal, A., Jachak, S.M., 2024. An alkaloid enriched fraction from *Murraya koenigii* (L.) Spreng. Leaves ameliorate HFD-induced obesity and metabolic complexities in

C57BL/6J mice. Journal of Ethnopharmacology 333, 118423. https://doi.org/10.1016/j.jep.2024.118423

Tong, T., Wang, Y., Zhang, C.-M., Kang, S.-G., 2021. *In vitro* and *in vivo* antihypertensive and antioxidant activities of fermented roots of *Allium hookeri*. Chinese Herbal Medicines, RCEP Traditional Medicine Research 13, 541–548. https://doi.org/10.1016/j.chmed.2021.08.003

Ullah, A., Ali, N., Ahmad, S., Rahman, S.U., Alghamdi, S., Bannunah, A.M., Ali, R., Aman, A., Khan, J., Hussain, H., Sahibzada, M.U.K., 2023. Glycogen synthase kinase-3 (GSK-3) a magic enzyme: it's role in diabetes mellitus and glucose homeostasis, interactions with fluroquionlones. A mini-review. Braz. J. Biol. 83, e250179. https://doi.org/10.1590/1519-6984.250179

Upton, R., 2022. Chapter 5 - Traditional herbal medicine, pharmacognosy, and pharmacopoeial standards: A discussion at the crossroads in the age of COVID, in: Mukherjee, P.K. (Ed.), Evidence-Based Validation of Herbal Medicine (Second Edition). Elsevier, pp. 109–148. https://doi.org/10.1016/B978-0-323-85542-6.00003-2

Vijh, D., Gupta, P., 2024. GC–MS analysis, molecular docking, and pharmacokinetic studies on Dalbergia sissoo barks extracts for compounds with anti-diabetic potential. Sci Rep 14, 24936. https://doi.org/10.1038/s41598-024-75570-3

Wang, L.-C., Pan, T.-M., Tsai, T.-Y., 2018. Lactic acid bacteria-fermented product of green tea and *Houttuynia cordata* leaves exerts anti-adipogenic and anti-obesity effects. Journal of Food and Drug Analysis 26, 973–984. https://doi.org/10.1016/j.jfda.2017.11.009

Wang, Y., Hu, T., Wei, J., Yin, X., Gao, Z., Li, H., 2022. Inhibitory activities of flavonoids from *Scutellaria baicalensis* Georgi on amyloid aggregation related to type 2 diabetes and the possible structural requirements for polyphenol in inhibiting the nucleation phase of hIAPP aggregation. International Journal of Biological Macromolecules 215, 531–540. https://doi.org/10.1016/j.ijbiomac.2022.06.107

Wei, P., Luo, Q., Hou, Y., Zhao, F., Li, F., Meng, Q., 2024. *Houttuynia Cordata* Thunb.: A comprehensive review of traditional applications, phytochemistry, pharmacology and safety. Phytomedicine 123, 155195. https://doi.org/10.1016/j.phymed.2023.155195

Wen, L., Wu, D., Tan, X., Zhong, M., Xing, J., Li, W., Li, D., Cao, F., 2022. The Role of Catechins in Regulating Diabetes: An Update Review. Nutrients 14, 4681. https://doi.org/10.3390/nu14214681

Wen, X., Zhang, B., Wu, B., Xiao, H., Li, Z., Li, R., Xu, X., Li, T., 2022. Signaling pathways in obesity: mechanisms and therapeutic interventions. Sig Transduct Target Ther 7, 298. https://doi.org/10.1038/s41392-022-01149-x

Williams, D.J., Edwards, D., Hamernig, I., Jian, L., James, A.P., Johnson, S.K., Tapsell, L.C., 2013. Vegetables containing phytochemicals with potential anti-obesity properties: A review. Food Research International 52, 323–333. https://doi.org/10.1016/j.foodres.2013.03.015

World Health Organization, 2023. Integrating Traditional Medicine in Health Care [WWW Document]. World Healh Organization. URL https://www.who.int/southeastasia/news/feature-stories/detail/integrating-traditional-medicine (accessed 7.27.25).

Yang, W., Xing, Z., Wang, X., Xu, Z., Jiang, X., Xia, J., Qiu, L., Xu, J., Wang, J., 2025. Microenvironment-responsive collagen hydrogel with *Houttuynia Cordata* Thunb vesicles for diabetic wound repair. International Journal of Biological Macromolecules 320, 145840. https://doi.org/10.1016/j.ijbiomac.2025.145840

Yang, Y., Chen, Z., Zhao, X., Xie, H., Du, L., Gao, H., Xie, C., 2022. Mechanisms of Kaempferol in the treatment of diabetes: A comprehensive and latest review. Front. Endocrinol. 13. https://doi.org/10.3389/fendo.2022.990299

Yao, Y., Yu, Y., Dai, S., Zhang, C., Xue, X., Zhou, M., Yao, C., Li, Y., 2024. Kaempferol efficacy in metabolic diseases: Molecular mechanisms of action in diabetes mellitus, obesity, non-alcoholic fatty liver disease,

steatohepatitis, and atherosclerosis. Biomedicine & Pharmacotherapy 175, 116694. https://doi.org/10.1016/j.biopha.2024.116694

Ye, H., Wei, J., Tang, K., Feuers, R., Hong, H., 2016. Drug Repositioning Through Network Pharmacology. Current Topics in Medicinal Chemistry 16, 3646–3656. https://doi.org/10.2174/1568026616666160530181328

Yoshida, H., Watanabe, W., Oomagari, H., Tsuruta, E., Shida, M., Kurokawa, M., 2013. Citrus flavonoid naringenin inhibits TLR2 expression in adipocytes. The Journal of Nutritional Biochemistry 24, 1276–1284. https://doi.org/10.1016/j.jnutbio.2012.10.003

Zhang, G., Li, Q., Chen, Q., Su, S., 2013. Network Pharmacology: A New Approach for Chinese Herbal Medicine Research. Evidence-Based Complementary and Alternative Medicine 2013, 621423. https://doi.org/10.1155/2013/621423

Zhang, P., Zhang, D., Zhou, W., Wang, L., Wang, B., Zhang, T., Li, S., 2024. Network pharmacology: towards the artificial intelligence-based precision traditional Chinese medicine. Briefings in Bioinformatics 25, 1–12. https://doi.org/10.1093/bib/bbad518

Zhou, B., Xiao, J.F., Tuli, L., Ressom, H.W., 2012. LC-MS-based metabolomics. Mol. BioSyst. 8, 470–481. https://doi.org/10.1039/C1MB05350G

CHAPTER

1

Evidence-based validation of herbal medicine: Translational approach

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Abbreviations

WHO	World Health Organization
AYUSH	Ayurveda, Yoga, Unani, Siddha and Homeopathy
TM	Traditional medicine
IUPAC	International Union of Pure and Applied Chemistry
USFDA	United States Food and Drug Administration
GACP	Good Agricultural and Collection Practices
CDSCO	Central Drugs Standard Control Organization
CYP450	Cytochrome P450
EMEA	European Medicines Agency
T&CM	Traditional and Complementary Medicine

1 Healthcare through herbal medicine

Herbal medicines attract the interest of both patients and scientists, in all aspects of drug development from natural products and also for validation of traditional medicine (TM). Several developing countries rely on TM because of their accessibility and affordability and scientists all over the world consider herbal species as a source for new chemical entities and used them to isolate compounds, such as digoxin, morphine, taxol, atropine and vinblastine [1]. TM has proven to be a boon for the impecunious who are devoid of the modern treatment facilities [2]. Herbal medicines have an important position in health care systems worldwide, their current assessment and quality control are a major bottleneck. Many adverse events of herbal medicines can be attributed to the poor quality of the raw materials or the finished products. Quality issues of herbal medicines can be classified into two categories, external and internal. External issues include toxic metals, pesticides residues, microbes, adulteration and misidentification of medicinal plants. The internal issues affecting the quality of herbal medicines are complexity and non-uniformity of the ingredients. The Indian subcontinent is always known for its monumental widespread of medicinally active plants. Due to larger forest areas covering most of the villages the adaptive nature of humans have helped certain healthcare practitioners to channel the potential of natural resources into potent medications [2]. The concept of Ayurveda is the living proof of experience based treatment of different ailments through TM. Many Ayurvedic texts and books like Charak Samhita, Sushrut Samhita, Ashtanga Hridaya, Madhava Nidana, Bhava Prakasha and many more have always boosted natural product-based healthcare research [3].

Through the use of modern analytical methods and pharmaceutical techniques, previously unsolved internal issues have become solvable [4]. The increasing search for therapeutic agents derived from plant species is justified by the emergence of diseases. Medicinal plants serve as most valuable source for curing many diseases. Herbal medicines include herbal extracts, herbal drug preparations and herbal drugs. Herbal drugs are unprocessed part of plants or whole plants [5]. Herbs include crude plant material such as leaves, flowers, fruit, seed, stems, wood, bark, roots, rhizomes or other plant parts, which may be entire, fragmented or powdered. Herbal preparations include comminuted or powdered materials or extracts, tinctures and fatty oils of herbal materials, which may be produced by extraction, fractionation, purification, concentration or other physical or biological processes [6].

Modern allopathic medicine has developed from ancient medicine, and it is likely that many important new remedies was discovered and commercialized

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following the leads provided by traditional knowledge and experiences. The study of these traditions not only provides an insight into how the field has developed but it is also a fascinating example of our ability to develop a diversity of cultural practices [7]. The administering of a pure chemical or a plant extract containing the same chemical entity is essentially different. The difference is mainly due to the complexity of a plant extract that introduces many variables to conventional phytomedicinal research, which could possibly contribute to chemical complexity and bioactivity. On administration of plant material of *Artemisia annua* versus pure drug e.g. artemisinin showed that the bioavailability from the leaves was 45 times more than that of the pure drug [8]. Thus the complexity of the plant extract could have contributed to the increased bioavailability and thus the bioactivity. Shift in the paradigm from "single compound single target" to "single compound multiple targets" and "multiple compounds single target" the modern researchers are able to develop novel and therapeutically active drugs [3]. A genuine interest on various traditional practices now exists among practitioners of modern medicine and numbers of practitioners of traditional, indigenous or alternative systems are beginning to accept and use some of the modern technologies. Proper methodologies for the research and development, manufacturing and quality control of the formulations in TM and investigations of the therapeutic potentials of plants used in those systems with support of scientific methods may help to use them with maximum possible efficacy [9,11].

2 Integrated approaches for development of herbal medicine

The international trade in herbal medicine has attracted most of the pharmaceutical companies, including the multinationals. Until a few years ago, only small companies had interest in the marketing of herbal medicines. Currently, several large multinational companies are interested in commercializing herbal drugs [9]. The world market for herbal medicine, including herbal products and raw materials, has been estimated to have an annual growth rate up to 15%. Several integrated approaches in herbal research for promotion and development of natural products are shown in Fig. 1.

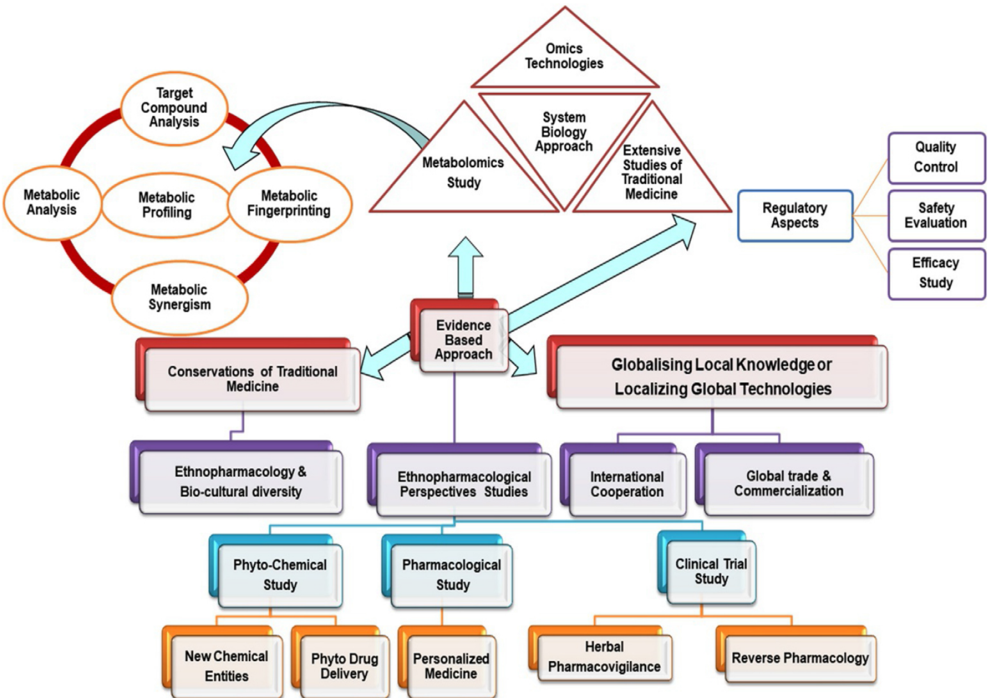

FIG. 1 Integrated approaches in botanical research.

2.1 Opportunities and challenges in herbal medicine

With the global increase in the demand for medicinal plant or plant-derived medicines, there is a call for ensuring the quality and safety of herbal drugs using several modern analytical techniques. Chemical constituents in herbal medicine may vary depending on harvest seasons, plant origins, drying processes and other related factors. Thus, it seems to be necessary to determine most of the phytochemical constituents of herbal products in order to ensure the reliability and repeatability of pharmacological and clinical research, to understand their bioactivities and possible side effects of active compounds and to enhance the quality of the herbal products [10]. The Lack of chemical markers remains a major problem for the quality control of herbal medicines. In many cases, we do not have sufficient chemical and pharmacological data of chemical markers. Further, there are many technical challenges in the production of markers. For example, temperature, light and solvents often cause degradation and/or transformation of purified components; isomers and conformations may also cause changes in the markers. However, a concept of understanding the complex principles of herbal medicine must be developed through marker profiling and related approaches so as to develop evidence-based practice of herbal medicine [11]. It is important to characterize all the phytoconstituents in a plant to ensure a safe and efficacious herbal medicine [2]. Evidence-based submissions for regulatory approval and interlinking of various pharmacopoeial and monographs would be helpful for the herbal manufacturers to regulate markets across the world. A general comparison of the pharmacopoeial standards reveals that there is a wide variation in plant specific parameters, quality standards of the different nations. With respect to South East Asia, India is among the leading countries with respect to development of pharmacopoeial standards as well as modification of existing regulatory guidelines [12].

The major challenges for the development and promotion of TM include the chemo-profiling, safety evaluations, quality control and effective regulatory guidelines for herbal medicines [13]. Wisdom and compassion, enhanced global collaboration and leadership are needed to change the contemporary paradigms and develop new strategies for the enhancement of TMs and dietary supplements. Research through collaboration and cooperation across the nation can help to a high extent in the promotion and development of the TM for the betterment healthcare globally [14]. This would develop a system to bring representatives together to discuss the global issues and implications in new strategic terms, with a new set of goals, a new agenda, but most importantly, a new vigor, and is vital for the global development [2,14]. Development and evaluation of medicinal plant derived products are being controlled and implemented through various agencies in different countries. This provides the unique advantages for researchers and the pharmaceutical industry to enhance drug discovery and development [15].

2.2 Several aspects for revitalization of medicinal plants

In order to revitalize the herbal medicine in line with the modern medicine, various strategic areas in medicinal plant research are being considered. Scientists are convinced that the integration of herbal medicine with modern tools would not only benefit their own development, but also help to fight against many complex diseases through development of new entities [16,17]. Numerous methods exist in order to evaluate the quality of either natural or synthetic substances. Several *in vitro*, *in vivo*, and high throughput screening methods are currently involved in the traditional drug discovery approaches [18]. During the past decades, public interest in natural therapies, namely herbal medicine, has increased dramatically not only in developing countries but mainly in industrialized countries [19]. This has increased the international trade in herbal medicine enormously and has attracted most of the pharmaceutical companies, including the multinationals. India is one of the few countries that are capable of producing most of the important plants used in modern as well as traditional systems of medicine. In modern era the combinatorial chemistry and high throughput screening are very useful method and so many new drug molecules are coming out from herbal resources. The traditional use of medicinal plants needs to be systematically investigated and standardized in the prospective of the quality, safety and efficacy [15]. One of the most important issues involved in any research study is the quality of the test material. A study cannot be considered scientifically valid if the material tested was not authenticated and characterized such that the material can be reproduced. In the case of botanicals, there may be misidentification of the collected plant, adulteration with other species, or contamination with extraneous ingredients [7].

3 Use of herbs in TM

TM generally refers to those medical and health care systems that are practiced in a traditional manner from ancient times, and this discipline is not considered to be a part of conventional modern medicine. Over several years, this system has evolved on the basis of religious

beliefs and social edifices of several indigenous peoples by exploiting the natural resources and more recently by developing a scientific method for validating therapeutic and preventive approaches [20]. However, TM is not always documented properly through evidenced based scientific validation as in conventional modern medicine. TMs are more easily accepted by most people due to their strong belief, faith, practical benefits, economical advantage, easy access, and many other reasons that have regional, religious, and social bases, etc. [21].. The Indian material medica includes approximately 2000 drugs of natural resources, nearly all of which are derived from different traditional systems of medicine and Indian folklore practices. Many conventional modern drugs originate from different natural sources especially medicinal plants: a century ago, most of the effective drugs were plant based [22]. Drug development from medicinal plants continues, with drug manufacturing companies engaged in large-scale pharmacological screening of herbs. In TM, some popular herbs such as Turmeric, Neem, Ginger, Holi Basil, Ashwagandha, and Rauwolfia., create a revival of interest in herbal products at a global level [23]. Around 60% of the global health care product market is dominated by medicinally useful formulations and other health products, derived or developed from botanicals. In India, around 25,000 traditional and folk medicinal effective plant-originated formulations are used. In India, more than 1.5 million consultants are using traditional medicinal systems for health care, and more than 7800 manufacturing units are involved in the production of natural health products (NHP) and traditional plant originated formulations [12]. There is worldwide emerging interest in executing traditional practices in the health care system by exploring their therapeutic as well as preventive potential. In TM, various regulations and control on the use of botanicals have come up, which will not only help to cure different ailments through indigenous natural resources but will also help in the screening and evaluation of the medicinal plants in a better way to use them in traditional healthcare systems [24].

4 Globalization of TM

TM has been defined as skills and a practice based on the theories, beliefs, and experiences that are indigenous to different cultures. It is used in the maintenance of health care as well as in the prevention, diagnosis, and treatment of physical and mental illnesses [25]. Scientists around the world are highly emphasizing on medicinal plants as alternative medicine and their commercial potential in health care. Globalization of TM is necessary for the establishment of evidence-based health care, based on TM in consideration of its safety, efficacy, therapeutic, and clinical evidence [26]. Modern technology and science have developed many techniques and systems for core disciplines including ethnomedicine, ethnobotany, ethnopharmacology, and medical anthropology to promote TM compounds globally [11]. Establishment of global and/or regional regulatory harmonization is obligatory for its development and promotion through scientific validation. The development of TM and natural products requires the convergence of modern techniques and integrated approaches related to their evidence-based research in various fields of science through coordination and cooperation [27]. To combat the growing market demand, there is an urgency to expeditiously utilize and scientifically validate more medicinally useful plants globally, which needs globalizing local knowledge and localizing global technologies, through international collaboration and cooperation. The major limits for the globalization of TMs are due to having different standards of TM products and practices, including varied terminology and philosophical approaches. Development of effective guidelines for safety, efficacy, and quality is regarded as a fundamental requirement in order to establish the evidence base for TM [28]. The International Union of Pure and Applied Chemistry (International of Pure and Applied Chemistry (IUPAC)) has published a series of protocols on quality control, safety, efficacy, standardization, and documentation of herbal medicine in which various significant aspects and features of phytochemistry and analytical chemistry have been described. If these strategies are fully implemented by the IUPAC, the World Health Organization (WHO) will explore TM from its pessimistic view to modern medicine [29].

4.1 Strategies for globalization of TM

The term "globalization" means the increased mobility of individuals, information, goods, services, labor, technology, and capital throughout the world. There are huge databases of TM, which are used by ancient people as folk medicine, and this evidence was found in many written textbooks [30]. There are several strategies for the expansion of TM such as (1) addition in the health care system, (2) promotion of secure and valuable use, (3) increasing its access, (4) increasing communication, and (5) cooperation in generation and distribution of TM-related information. These strategies based on information, botany, chemistry, and biology of medicinal plant validation and quality control are essential [31]. In the era of modern research, some new drug molecules are emerging with the help of combinatorial chemistry and high-throughput screening from herbal resources. A study cannot be considered scientifically valid if the material tested was not authenticated and characterized such that the material

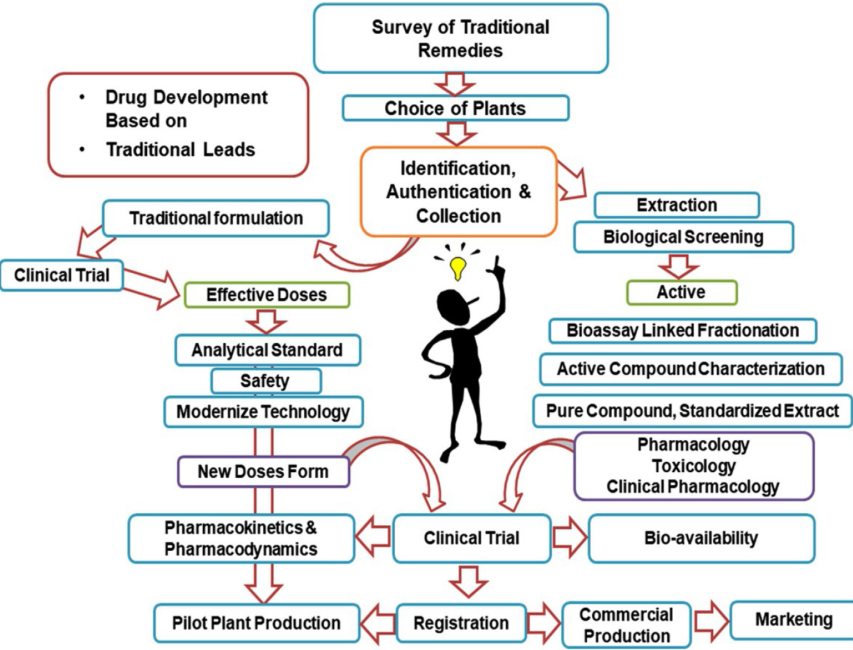

FIG. 2 Drug development based on traditional claims.

can be reproduced. In the case of botanicals, there may be misidentification of the collected plant, adulteration with other species, or contamination with extraneous ingredients. From the perspective of a regulatory action, these cases may range from simple misleading labeling to frank poisoning due to toxic contaminants. It can often be difficult to compare reported efficacy or toxicity studies even when "standardized" material has been used. Many studies refer to the use of standardized botanical material, which usually implies a chemical standardization [32]. Inter disciplinary approach of work on TM is to be explored for the discovery of novel bioactive compounds. Issues related to the appropriateness of conventional biomedical and clinical models for evaluating the efficacy of TM are sometimes very crucial. A holistic approach based on systems biology seems much more suited to study the therapeutic efficacy and pharmacodynamics of TM-based drug development [11]. Approaches for drug development based on traditional leads are described in Fig. 2.

Most of the Indian and Chinese herbal formulations contain a mixture of herbs, and several methods are available to classify them. When two or more herbs with their bioactive compounds are combined to prepare formulations, they can be observed to have the following effects [33]:

- Synergistic significance
- Synergistic improvement
- Co-counteraction
- Mutual repression
- Communal aggression
- Communal inappropriateness

The main ingredient is a component that provides the main therapeutic action; the second ingredient potentiates the therapeutic actions of the other, which is known as synergistic reaction, and the component is synergism. The rest serve one of the following functions:

- Treat accompanying symptoms,
- Moderate the ruggedness or toxicity of the primary ones,
- Target the medicine to the proper organs,
- Exert a complementary effect.

In some cases, standardized bioactive compounds are subjected to animal models to correlate the presence of certain phytoconstituents with a pathophysiological condition of the human body [34]. In the context of modern biomedical research, there should also be necessary prerequisites for clinical trials.

5 TM inspired drug discovery and drug development

In order to revitalize herbal medicine in line with modern medicine, various strategic areas in medicinal plant

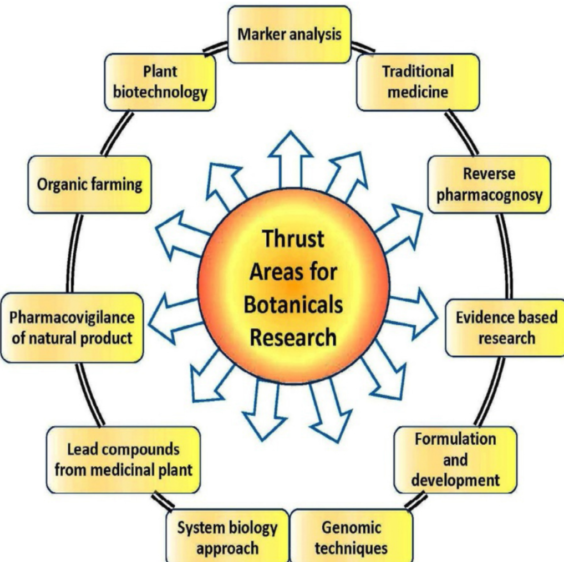

FIG. 3 Thrust areas for botanical research.

research are to be considered of global importance [35]. Integration of herbal medicine and modern tools would not only benefit their own development but will also help to fight against many complex diseases through the development of new entities. Such dedicated research would be beneficial only with support from advanced approaches and novel strategies [36]. There are various thrust areas that play a very significant role for research and development of natural products as represented in Fig. 3.

TM is helpful in all aspects of drug development from natural resources. A few examples of drugs from natural products would better explain the history of its own tradition. Several approaches on drug discovery and development from TM had been practiced by scientists for many years. Several therapeutically potential constituents were isolated from plants such as artemisinin (antimalaria), vincristine, vinblastine, camptothecin podophyllotoxin, etoposide, teniposide, and paclitaxel (anticancer) [37]. The development of drugs from ayurvedic plants is ongoing, with pharmaceutical companies engaged in large-scale pharmacologic screening of herbs. "Sushruta-Samhita," a Sanskrit text on Ayurveda written in 600 BC noted that the plant *Commiphora mukul* Hook was useful in the treatment of obesity and related diseases. In recent years, a confluence of spectacular advances in chemistry, molecular biology, genomics, and chemical technology and the cognate fields of spectroscopy, chromatography, and crystallography may influence several therapeutically potent leading compounds from TM [12].

There are many approaches for the search of new biologically active principles from botanicals. One can simply look for new chemical constituents and find a biologist who will test the substance pharmacologically. This is not considered to be a very valid approach. A second approach is simply to collect every readily available plant, prepare extracts, and test each extract for one or more types of pharmacological activity. This testing will help in the standardization of extracts and the bioassay-guided isolation of the active constituents. The phytoconstituents obtained can then be taken further for structure-activity relationship studies [38]. Once all these factors are determined, the constituent/extract obtained can be further examined for its toxicity and safety evaluation, followed by clinical trials. This random collection and extensive screening method is a reasonable and the most effective approach that eventually should produce useful drugs, which can be well produced and formulated in industries. The classic method of pharmacologic screening involves sequential testing of herbal extracts or phytoconstituents from biological materials in isolated organs followed by testing in whole animals, mostly in rats and mice. Most of the drugs in use today as therapeutic agents have been found and evaluated with these methods [39]. However, for the evaluation of TM, we should not follow the reductionist approach, but go back to the holistic in vivo approach. This can be done in two different ways: one is through clinical trials; the other is through animal experiments. Besides the classic

physiologic observations that can be made by in vivo experiments, for example, blood pressure, analgesic activity, and sedation, nowadays, it is also possible to measure gene expression, the proteome, and the metabolome. These methods open up a completely new world of possibilities with several new technologies now giving a much better insight into the possible changes in the organism, in a holistic way. It will give us the possibility to better understand the mode of action by comparing the changes in the transcriptome, proteome, and metabolomic patterns when compared with those observed with known drugs. Such an approach is now known as the systems biology approach. The metabonomic approach requires the statistical analysis of large data sets by methods such as multivariate and principle component analysis to extract the information from these data [40]. Moreover, by using the systems biology approach for the organism combined with metabolomic data for the different extracts of the medicinal plant or fractions thereof, it should be feasible to make correlations between the occurrence of certain compounds in the extract and the activity.

Evidence-based medicine research should be conducted with the involvement of patients and funding bodies to establish a role of medical practitioners in decision making [41]. A widespread revolution in phytochemistry has been observed through strengthening its importance with the application of new technologies to enhance the original link between phytochemistry and TM. Evidence-based research includes developing policies, regulatory criteria, and technical guidelines that would ensure and provide the continued availability of quality, safety, and effective traditional medicinal products, which could support inclusion in health care systems, insurance programs, and on essential medicine lists [29]. Evidence based submissions for regulatory authorization and interlinking of various pharmacopoeia and monographs would make it easy for herbal manufacturers and they will gain greater access to regulated markets across the world [42]. It is under these circumstances that some of the rationalists, scientists, scholars, and protagonists of alternative medicines dedicated themselves to the development of these alternative systems for drug development from natural resources, which required to be harmonized through international coordination.

6 Quality control and quality assurance of herbal medicine

Quality control and quality assurance of herbal medicines are very important to protect the integrity of the herbal extracts/products for the management of pharmaceutical quality. They have an important role for the reproducibility of the effect of the active ingredients from batch-to-batch uniformity. To maintain and comply with standard conditions with respect to quality, safety, and efficacy of herbal medicine, it is required to follow some important steps for the standardization [43]. This includes the (1) proper authentication and taxonomic assignment, such as through DNA fingerprinting and DNA bar coding; (2) structural elucidation of all isolated compounds of the medicinal plant; (3) identification and characterization of the bioactive constituents for the pharmacological activity; (4) standardization of the single extracts through spectroscopic analyses in the multi-compound extracts; (5) international harmonization of specific standardization processes under the umbrella of the International Federation of Pharmaceutical Manufacturers Associations. Therefore, it is very clear that major requisites for standardization of herbal products comply with international standards. There are several variables that can influence the standardization process. Therefore, it is compulsory to optimize all aspects of cultivation, harvesting, sample preparation, and sample processing to ensure reproducibility and eventually standardization of the herbal dugs. There are various new hyphenated technologies present such as chromatographic and spectroscopic analyses, which need to be effectively incorporated to ensure that sufficient quality control measures are implemented. By using several chromatographic and spectroscopic techniques, it is possible to analyze the full herbal product and thus generate a standardized "metabolic fingerprint" of specific herbal dugs. Metabolic profiling can then be incorporated to identify all the constituents [44]. The chemical fingerprints obtained from chromatographic or spectroscopic techniques should be similar in different samples. Spectroscopic and chromatographic techniques are now being used together, which leads to effective chemometric approaches. When these approaches are used in combination with chemometrics profiles, more precise data can be obtained that will be helpful in the establishment of the integrity of the herbal product and similarities and differences of the observed data will be produced [31].

Generally, it is believed that the risk associated with herbal drugs is very less, but reports on serious reactions indicate the need for the development of effective marker systems for isolation and identification of the individual components [45]. Standardization of herbal medicine includes the authentication of genuine drugs, harvesting of the best quality raw material, assessment of intermediate and finished product, and detection of harmful and toxic ingredients [46]. Several markers such as taxonomic, chemical, genomic, proteomic markers aid in the identification of herbal drug components. Chemical markers help in the identification of adulterants, confirmation of collection site, and quality evaluation and diagnosis of herbal intoxication. As per the WHO definition, there are three

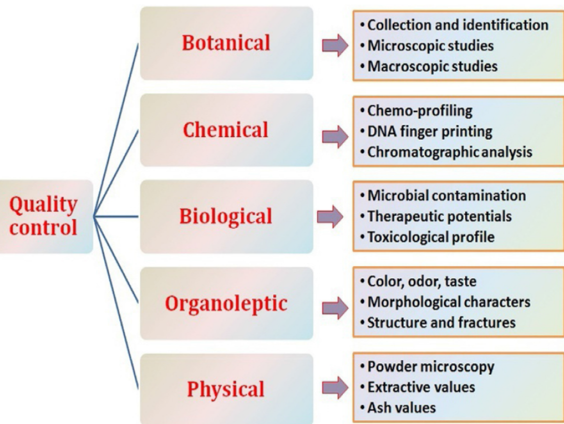

FIG. 4 Important steps for quality control of herbs.

kinds of herbal medicines that are obtained from raw plant material, processed plant material, and medicinal herbal products [40]. Herbal medicine products are dietary supplements that people take to improve their health and are marketed as tablets, capsules, powders, extracts, and fresh or dried plants. Herbals are traditionally considered harmless and increasingly being consumed by people, without any prescription. The evidence for the therapeutic actions of herbal drugs is documented in Indian, Chinese, European, and African systems of medicine [24]. There are several important aspects for quality control of herbal medicine that are shown in Fig. 4.

The WHO has recognized the importance of the quality control of herbal medicine and developed a series of guidelines to assist several nations to develop their strategies for the quality control of herbal medicines and for conducting research on TMs [47]. The WHO had published the "Quality Control Methods for Medicinal Plant Materials," a collection of recommended test procedures for assessing the identity, purity, and content of medicinal plant materials to assist national laboratories engaged in drug quality control [48]. The WHO published the "Guidelines on good agricultural and collection practices (GACP) for medicinal plants" and in 2007, a new guideline "WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues" were formulated. The European Union, China, and Japan have developed regional and national guidelines for good agricultural and collection practices for medicinal plants that ensure that soil and irrigation water used for herbal material cultivation and propagation are within the limits or are free from harmful heavy metals, pesticides, herbicides, and toxicologically hazardous substances. The certification for this is based on parameters such as identification, water content, and chemical assay of active ingredients, in organic impurities (toxic metals), microbial limits, mycotoxins, pesticides, and others [49]. From the cultivation to the final herbal product development of herbal products, there are so many significant factors that can influence the quality of herbal products. Some significant issues related to the quality control of herbal medicine are being described briefly in the subsequent section.

6.1 Contamination

There are so many contaminants mostly found in medicinal herbs including pesticides, heavy metals, microbes, and mycotoxins. Contaminations also present serious obstacles for the trade of herbal medicines [50]. Heavy metals have been found in herbal medicines with some regularity. Three most commonly detected toxic metals are mercury, arsenic, and lead. These contaminations may occur due to (1) the accumulation of heavy metals in the environment (e.g., from contaminated soil or atmosphere); (2) unintentional pollution during the production process; (3) deliberate addition. In some of the herbal products, residues of pesticides including their metabolites and depredated products remained in plants, and such residues have become a notable source of contamination for herbal medicines [51].

6.2 Adulteration

Adulteration in herbal medicine increases the impurity by adding some extraneous, improper, or inferior ingredients. Herbal medicines are adulterated with conventional drugs, and plant materials have repeatedly been documented. Adulterations can be done in the following way including addition of orthodox drugs, substitution of fake or inferior plant materials, and addition of foreign materials [36].

6.3 Misidentification

Conflicting to adulteration or substitutions, misidentification of herbal medicine mostly happens unintentionally. False identification can occur when an importer or retailer mistakes one herb for another, due to incorrect labeling and similar appearance of the herbal materials. Confusing nomenclature can be one of the reasons, because one herb may be known by many names: one or more common names, a Latin name, local names, and the brand name. Some different medicinal herbs of different plant species with different constituents may have similar names. The problem becomes even more complex through confusing terminologies and the use of different languages in different countries [52]. The common names of herbs usually do not reflect differences in scientific taxonomy; and the description and microscopic identification of an herb cannot identify its constituents. Thus, a study of ancient documents and the use of modern analysis techniques are often necessary to properly authenticate herbal materials.

6.4 Nonuniform chemical constituents

The chemical composition of herbal products varies and depends on the growing conditions and geographic region. Several environmental factors that include atmospheric humidity, rainfall pattern, soil, altitude, seasonal variation, temperature, length of day light, may affect the concentration of chemical constituents in medicinal plants. Some other relevant factors, such as genetic make-up, seeding time, use of pesticides and fertilizers, planting density, also play a significant role. Various processing steps of raw materials can also change the pharmacological activity of the plant extract. Therefore, batch-to-batch standardization is very essential to maintain the uniformity of active constituents [53].

6.5 Pharmacopoeial standards for evaluation of herbal products

Safety and efficacy assessment for any pharmaceutical must be taken into account for the quality of the prepared formulation. Minimum standards for acceptable quality are generally laid down in pharmacopoeial monographs, which provide all the details of the acceptable substance and give the niceties of significant tests to determine its identity and purity. One type of pharmacopoeial monograph is found in the British or European pharmacopoeias, which give only details of the tests to be used to establish quality, with very concise notes about its therapeutic application. Another type of monograph is more concerned with the complete information about a medicinal plant and consists of all the information about its chemical constituents, pharmacology, toxicology, clinical studies and usage [54].

Pharmacopoeial monographs for the medicinal herbs deal with all types of pharmaceuticals and plant materials which have been included since the earliest editions with authorization at a national or international level. Itis interesting to trace the evolution of a monograph for one particular medicinal plant because it reflects developments in analytical techniques, the increasing knowledge of the chemical compounds present, and the growing body of knowledge that links the compounds present to the desired biological or clinical effect [55]. More recent editions of the British Pharmacopoeia and European Pharmacopoeia have included monographs for many more herbal drugs and more sophisticated chromatographic methods, especially liquid chromatography (LC), have been introduced for both identity tests, impurities tests, and for assay procedures. Therefore, more attention should be given for the biological activity relevant to the reputation and claims for treating particular diseases associated with herbal medicines [36].

The Indian Pharmacopoeia 2007 includes pharmacopoeial specifications with monographs for some medicinal plants being most commonly used as therapeutic agents. The specifications include the name of the drug (along with its common name), its biological source (Latin name), the part of the plant under consideration, its description, macroscopic and microscopic study, identification, several quality control parameters, and assays with respect to the phytochemical reference standards or botanical reference standards [56]. The Ayurvedic Pharmacopoeia of India is another official compendium published by the Ministry of Health and Family Welfare, Government of India. This describes different methods for quality control and standardization of medicinal plants and herbal preparations. Several specifications for quality evaluation of natural products as prescribed in the Ayurvedic Pharmacopoeia include morphological study, determination of quantitative data (e.g., extractive values and foreign matter), limit tests, and different physical tests (e.g., boiling range, refractive index, and pH) [57].

7 Marker analysis and standardization of botanicals

Chemo profiling of NHP helps in identifying the major metabolites and is useful to assess biological effects. The development of marker-based medicines requires a comprehensive understanding of plant systems including biological, chemical, genetic, and agronomic aspects. Chemical consistency at all stages of manufacturing processes is most important to ensure medicinal efficacy and consumer safety. This includes

all the stages such as extraction, stability, shelf life, and purity of herbal medicines. Different methods for characterization of herbal drugs such as morphological identification, anatomical identification, and chemical analysis, such as thin layer chromatography (TLC), high-performance thin layer chromatography (HPTLC), high-performance liquid chromatography (HPLC), capillary electrophoresis (CE), Liquid chromatography-mass spectrometry (LC-MS), and protein analysis are extensively used [28].

According to the European Medicines Agency (EMEA), markers may be defined as chemical constituents or groups of constituents of a herbal medicinal product that are very important for quality control purposes regardless of whether they possess any therapeutic effect. Chemical markers are basically categorized into the analytical markers and active markers. Analytical markers are the constituents or groups of constituents that serve solely for analytical purposes, whereas active markers are the constituents or group of constituents that contribute to therapeutic activities [58]. Secondary metabolites as markers have been widely used in quality control and standardization of herbal medicines. Herbal products derived from botanicals are mostly obtained from wild sources and have the greatest challenges for ensuring consistent product quality. These are used for making medicines where the standardization and quality control with proper integration of scientific techniques and traditional knowledge is vital requirement [30]. Marker compound selection is generally based upon a variety of different factors including stability, ease of analysis, time and cost of analysis, relevance to therapeutic effect and indicator of product quality or stability. Chemical markers are frequently used for assuring quality consistency of natural products derived from botanical sources [59]. An ideal chemical marker for a natural product should not be only a characteristic constituent but also the therapeutic constituent. Marker compounds are not necessarily pharmacologically active all the time but their presence is well established in products with characteristic chemical features. Marker components may be classified as active principles, active markers and analytical makers, while biomarkers may be defined as pharmacologically active [60]. Herbal manufacturers and researchers need to address these critical questions to aid in the harmonization of specifications and analytical methodologies for natural products. Usually, determination of single or several marker compounds by a developed method is required for quality control purpose [61]. Standardization methods through chemical fingerprinting should take into account for all the aspects that contribute the quality of the herbal medicine, including correct identification of sample, pharmacognostic evaluation, organoleptic evaluation, volatile matter, quantitative evaluation (ash values, extractive value, foreign matter), phytochemical evaluation, xenobiotics testing, toxicity testing, microbial load testing and biological activity determination [41]. Medicinal plants contain several phyto-constituents in certain ratios and in standardized extracts. The ratio of these chemical constituents must be constant within narrow limits from one batch to another [62]. Chemical fingerprints obtained by chromatographic, spectroscopic, thermogravimetric analysis, capillary electrophoresis and polarography techniques have become the most important tools for quality control and standardization herbal medicines [13].

For ensuring consistent quality, the use of markers, standardization, chemical and DNA fingerprinting, bioassays, and the emerging field of phytomics are very important [63]. Some medicinally important plants are listed in Table 1. Marker selection may be based upon a variety of different factors including stability, ease of analysis, time and cost of analysis, relevance to therapeutic effect, indicator of product quality, or stability or previous use by other manufacturers or researchers [60].

A list of several therapeutically potent phyto-markers from plant species has been shown in Table 1. Development of lead compounds from these medicinal plants and their evaluation may help to promote natural products based on their quality efficacy and safety. Marker analysis of several herbal drugs including polyherbal formulations from Indian system of medicine has been performed. The fingerprint profiles of Emodin (1) from *Aloe vera*, Gallic acid (2) from *Terminalia chebula*, Boswellic acids (3) from *Boswellia serrata*, Capsaicin (4) from *Capsicum annum*, Glycyrrhizin (5) from *Glycyrrhiza glabra*, epicatechin (6) from *Camellia sinensis*, Eugenol (7) from *Eugenia caryophyllata*, Ferulic acid (8) from *Coffea Arabica*, Garlicin (9) from *Allium sativum*, Genistein (10) from *Glycine* max, Ellagic acid (11) from *Punica granatum* and Piperine (12) from *Piper betel,* Syringic acid (13) from *Tagetes erecta,* Anthocyanidin (14) from *Paullinia cupana,* Apigenin (15) from *Matricaria recutita* and *Stereospermum suaveolens,* Ascorbic acid (16) from *Citrus sinensis,* Berberine (17) from *Berberis aristata,* Curcumin (18) from *Curcuma longa*, Gingerol (19) from *Zingiber officinale,* Naringenin (20) from *Citrus lemon,* Resveratrol (21) from *Vitis vinifera,* Lapachol from *Stereospermum suaveolen,* and their pharmacological activities have been reported. Marker analysis of Glycyrrhizin from *G. glabra* has been reported through HPTLC densitometry. This is a validated method as per the International Conference on Harmonization guideline where the amount of glycyrrhizin was determined in the extract of *G. glabra* through HPTLC. The method was validated in terms of specificity, linearity, precision, detection limit, and quantification limit [64,65].

Chlorogenic acid (22) was quantified from the medicinal plants of the cucurbitaceae family like *Sechium edule, Trichosanthes cucumerina, Luffa acutangula and Trichosanthes dioica* with a suitable solvent system of ethyl

TABLE 1 Some medicinally important plants and their known phyto-markers.

Scientific name	Family	Parts used	Marker compound
Aloe vera	Liliaceae	Leaves	Emodin (1)
Terminalia chebula	Combretaceae	Fruit	Gallic acid (2)
Amaranthus tricolor	Amaranthaceae	Aerial parts	
Boswellia serrata	Burseraceae	Resin	Boswellic acids (3)
Capsicum annum	Solanaceae	Fruits	Capsaicin (4)
Glycyrrhiza glabra	Leguminaceae	Root	Glycyrrhizin (5)
Camellia sinensis	Theaceae	Leaves	Epicatechin (6)
Eugenia caryophyllata	Myrtaceae	Flower bud	Eugenol (7)
Coffea arabica	Rubiaceae	Seed	Ferulic acid (8)
Hemidesmus indicus	Apocynaceae	Whole plant	
Allium sativum	Amaryllidaceae	Bulb	Garlicin (9)
Glycine max	Fabaceae	Seed	Genistein (10)
Punica granatum	Punicaceae	Fruit	Ellagic acid (11)
Piper betel	Piperaceae	Leaves	Piperine (12)
Tagetes erecta	Asteraceae	Leaves	Syringic acid (13)
Paullinia cupana	Sapindaceae	Seed	Anthocyanidin (14)
Matricaria recutita	Asteraceae	Flowering head	Apigenin (15)
Stereospermum suaveolens	Bignoniaceae	Bark	
Citrus sinensis	Rutaceae	Fruit	Ascorbic acid (16)
Berberis aristata	Berberidaceae	Berries	Berberine (17)
Curcuma longa	Zingiberacease	Rhizome	Curcumin (18)
Zingiber officinale	Zingiberacease	Rhizome	Gingerol (19)
Citrus lemon	Rutaceae	Fruit	Naringenin (20)
Vitis vinifera	Vitaceae	Fruit	Resveratrol (21)
Sechium edule	Cucurbitaceae	Fruit	Chlorogenic acid (22)
Trichosanthes cucumerina			
Luffa acutangula			
Trichosanthes dioica			
Inula racemose	Asteraceae	Whole plant	
Lagenaria siceraria	Cucurbitaceae	Fruit	Cucurbitacin E (23)
Benincasa hispida			
Momordica charantia			
Coccinia grandis			
Cucurbita pepo			
Luffa acutangula			
Cultivars of *Cucumis sativus*			
Myristica fragrans	Myristicaceae	Whole plant	Quercetin (24)
Amaranthus tricolor	Amaranthaceae	Aerial parts	Quercetin, Rutin (25)
Ayapana triplinervis	Compositae	Leaves	Ayapanin (26)

Continued

TABLE 1 Some medicinally important plants and their known phyto-markers—cont'd

Scientific name	Family	Parts used	Marker compound
Dillenia indica	Dilleniaceae	Fruits	Betulinic acid (27)
Swertia chirata	Gentianaceae	Leaves	Ursolic acid (28)
Andrographis paniculata	Acanthaceae	Aerial parts	Andrographolide (29)
Bacopa monnieri	Scrophulariaceae	Root	Bacoside A (30)
Centella asiatica	Mackinlayaceae	Whole plant	Asiaticoside (31)

Emodin (**1**)

Gallic acid (**2**)

Boswellic acid (**3**)

Capsaicin (**4**)

Glycyrrhizin (**5**)

Epicatechin (**6**)

Eugenol (**7**)

Ferulic acid (**8**)

Garlicin (**9**)

Genistein (**10**)

Ellagic acid (**11**)

Piperine (**12**)

TABLE 1 Some medicinally important plants and their known phyto-markers—cont'd

Syringic acid (**13**)

Anthocyanidin (**14**)

Apigenin (**15**)

Ascorbic acid (**16**)

Berberine (**17**)

Curcumin (**18**)

Gingerol (**19**)

Naringenin (**20**)

Resveratrol (**21**)

Chlorogenic acid (**22**)

Cucurbitacin E (**23**)

Quercetin (**24**)

Continued

TABLE 1 Some medicinally important plants and their known phyto-markers—cont'd

Rutin **(25)**

Ayapanin **(26)**

Betulinic acid **(27)**

Ursolic acid **(28)**

Andrographolide **(29)**

Bacoside A **(30)**

Asiaticoside **(31)**

Tinosporaside **(32)**

Trigonelline **(33)**

acetate:chloroform:formic acid (6:4:0.5 V/V) using HPTLC. Sensitivity, linearity, accuracy, precision and specificity were considered as per the ICH guidelines to validate the method. The proposed method offers a novel approach for the quality control and standardization of the medicinal food plants of the Cucurbitaceae family. *{**163**}* Cucurbitacin E (23) was also reported in the medicinal plants of cucurbitaceae family through validated reverse phase high performance liquid chromatography (RP-HPLC) using acetonitrile: water (1% glacial acetic acid) in the ratio of 70:30. Significant amount of cucurbitacin E content was found in *Lagenaria siceraria*, *Benincasa hispida*, *Momordica charantia*, *Coccinia grandis*, *Cucurbita pepo* and *Luffa acutangula* [66].

Traditional formulations from Ayurveda and Unani include herbs which provide nutrition to the skin, promote skin brightening and improve skin health. These are known as Ayurvedic "Varnya" formulations which require modern chromatographic techniques to explore the quality of the constituents and determine the safety for human use. Varnya formulation containing *Myristica fragrans*, *Hemidesmus indicus*, and *Inula racemose* is said to promote skin health are rich in polyphenolic compounds. Quercetin (24), ferulic acid and chlorogenic acid was quantified in significant amount using validated HPTLC and RP-HPLC methods [67]. Densitometric scanning via HPTLC showed simultaneous estimation of quercetin, rutin (25) and gallic acid in *Amaranthus tricolor* using a mobile phase of toluene-ethyl acetate-formic acid in a ratio of 7:5:1 (v/v) [68]. *Ayapana triplinervis* also known as "Visalyakarani" is a traditional wound healing plant. Standardization with validated HPTLC methods revealed the presence of Ayapanin (26), a potent haemostatic [69]. Biswas and co-researchers studied standardized RP-HPLC of *Dillenia indica* to characterize Betulinic acid (27) as the most prominent secondary metabolite [70]. Ethanolic leaf extract of *Swertia chirata* was standardized by validated HPLC method to yield urosolic acid (28) as the major phytoconstituent [71]. Methanolic extracts of *Andrographis paniculata*, *Bacopa monnieri* and *Centella asiatica* was subjected to standardization through RP-HPLC with optimized solvent system to get Andrographolide (29), Bacoside A (30) and Asiaticoside (31) respectively at 210 nm. Marker compound present in the extracts were 3.51 g/Kg, 0.14 g/Kg and 0.35 g/Kg respectively [72]. Hydroalcoholic extract of *Tinospora cordifolia* (aerial parts) was standardized through reverse phase HPLC with an optimized isocratic mobile phase composed of acetonitrile and Milli-Q water in the ratio of 25:75 (v/v) with flow rate of 1 ml/min. The percentage amounts of standard constituent, tinosporaside (32) present in the crude extract were found to be 1.64% (w/w) [73]. *Trigonella foenum-graecum* seeds were subjected to hydroalcoholic extraction using 70% methanol. RP-HPLC analysis of the standardized extract with optimized mobile phase revealed the concentration of the standard phytomarker Trigonelline (33) to be 3.38% (w/w) [74].

7.1 Applications of marker profiling

Identification, authentication, and quality evaluation of medicinal plants are fundamental requirements of industries and other organizations dealing with herbal health products. The fact must be taken into account that the plant material to be examined has a complex and inconsistent composition based on its content of secondary breakdown products or metabolites [19]. It is an accepted fact that the qualitative and quantitative analysis of major bioactive marker components of plant material is an important and reliable part of a quality control protocol because any change in the quality of the plant material directly affects the constituents. Medicinal plant materials that qualified within the requirements of the WHO guidelines and other regulatory affairs can be used to develop reference fingerprints of phytoconstituents. The presence of marker compounds may be detected by the densitometric scanning of the chromatograms [75]. The presence of these marker compounds in plant materials may be useful for quantifying the plant materials in formulations or herbal medicinal products and will be helpful for the quality control of single and polyherbal formulations. The marker profiling system is helpful as a tool in the quality control and standardization of the raw plant materials and finished herbal formulations. Marker analysis of phytoconstituents may also be helpful in phytoequivalence studies, including issues such as pharmacokinetics and other related parameters that can be recognized by studying the absorption, distribution, and metabolism of herbal drugs [17].

Further, medicinal plants do not have a constant chemical composition to their different parts such as roots, leaves, stems, flowers, and fruits. Therefore, each part needs individual chemoprofiling based on their different phytoconstituents. The use of marker profiling, standardization, DNA fingerprinting, bioassays, and related metabolomics studies, which is the new emerging field of phytomics can provide mechanisms for ensuring consistent quality. Marker profiling plays a vital role in several ways to evaluate quality control parameters so as to ensure the efficacy and safety of the herbal products Fig. 5 [24].

There are numerous challenges in the isolation and identification of marker components of medicinal plants. Herbal manufacturers and researchers need to address these critical questions to aid in the harmonization of specifications and analytical methodologies for the development of natural products [76]. Also

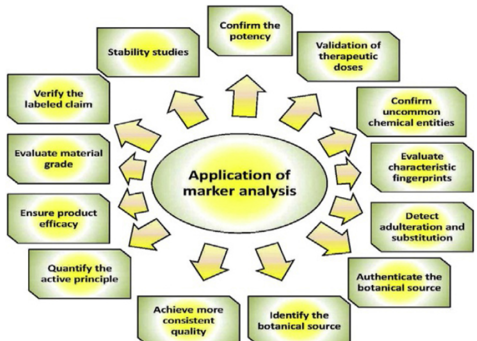

FIG. 5 Application of marker analysis.

nutraceutical industries can develop validated methods for standardization and quality control of medicinal food plants through HPLC and HPTLC for safe human consumption [66].

8 Pharmacovigilance of herbal medicine

Pharmacovigilance is the process of monitoring, evaluating, and communicating drug safety with profound implications that depend on the integrity and collective responsibility of all parties such as consumers, health professionals, researchers, academic, media, pharmaceutical industry, drug regulators, governments, and international organizations. The main objective of pharmacovigilance is to extend the safety monitoring and detect any adverse drug reactions that have previously been unrecognized in the evaluation of clinical trials. There is an ongoing problem with unexpected toxicity of herbal products due to quality issues, including the use of poor quality herbal materials, incorrect or misidentified herbs, incorrect processing methods, and supply of adulterated or contaminated herbs or products. The Medicines and Health care Products Regulatory Agency define some significant problems in the regulation of herbal medicines in the United Kingdom [77]. These include (1) Lack of knowledge about the products being used, (2) Limited use of yellow card adverse drug reporting-scheme, which represents underreporting rather than indicating an absence of adverse reactions, (3) Uniform manufacturing standards mostly of unlicensed products, and (4) Herb-drug interactions of herbal medicines.

These quality issues can be addressed to some degree by improved regulation requiring good manufacturing practice (GMP) standards for manufacturing. Pharmacovigilance is a very essential tool for developing reliable information on the safety of herbal medicines. The existing systems were developed for synthetic medicines and require some modification to address the specific differences of herbal medicine. Systematic pharmacovigilance is essential to build up reliable information on the safety of herbal medicines for the development of appropriate guidelines for their safe and effective use [78].

8.1 Why pharmacovigilance for herbal drugs?

The importance and significance of the pharmacovigilance of herbal medicines are increasing day by day. Presently, accessible surveillance system for herbal drug monitoring is not enough, and various cases of herbal toxicity are likely to be significantly under reported. There is no discrimination between chemically defined drugs and herbal drugs in the filing of procedure of adverse events. The recent information on "phytovigilance" (the term used for pharmacovigilance of herbal drugs) raises the suspicion that there is a tendency to unequal treatment of herbal medicine [79]. Botanicals are complex mixtures of multiple components or unknown active ingredients. This can change pharmacokinetic characteristics through various mechanisms of action. Because the process defines the product, extrapolation of scientific data across products from different manufacturers or sources is not possible. Defining the herb-drug interaction lies in the proper identification of plants, which includes Latin binominal and authority, identification of the plant and part(s) used in the preparation of herbal products, and the processes used to extract and isolate the desired active from plant resources [78].

Concomitant administration of herbal medicine with approved conventional medications can result in therapeutic failures or in adverse effects. Several research reports have suggested that St John's Wort decreases plasma levels of various other drugs [80]. There are no strict regulatory guidelines, and there are gaps in the inefficient regulatory processes that have allowed entry of unsafe products in the market. With prescription medicines, self-medication is a long-time practice that is unsafe and yet difficult to control, due to public assumptions that herbs are generally safe because of the long tradition of their usage and the concept of being natural. It is surprising that these are not recognized and if ever observed are attributed to the remedy's beneficial healing effect rather than harmful effects. Because of the scarcity of local data and lack of rigorous investigations on herbal traditional remedies, the promotion of the use of such products focused on claims of the beneficial effects and ignored the possible adverse effects. Therefore, there is now a need to revise the registration procedure for herbal products. The WHO has recognized the importance of the use of herbal medicines and developed some guidelines for monitoring herbal safety within the existing pharmacovigilance framework [79].

Herbal medicines are promoted in the market as natural and therefore as being safe and harmless. However,

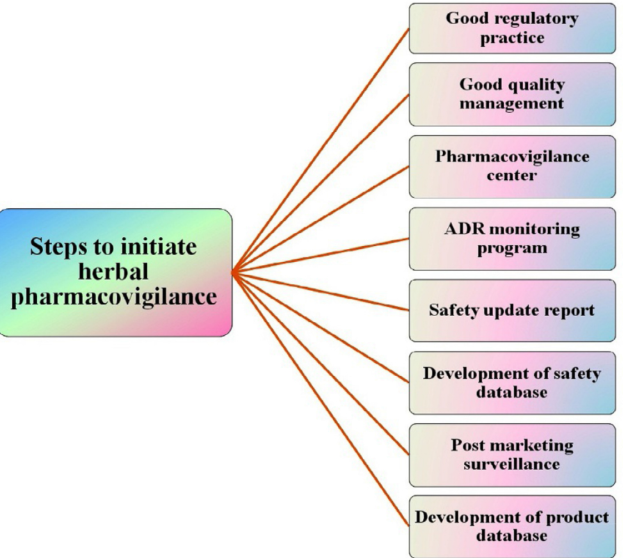

FIG. 6 Steps to initiate herbal pharmacovigilance.

there is very less regulation control in the manufacturing of such products; consequently, quality control issues such as misidentification of herbs, mislabeling, contamination, standardization of dose, method of processing, product uniformity, batch-to-batch variation, and toxicity are the major problems in herbal drugs [81]. Manufacturing botanicals to meet analytical standards for marker compounds does not necessarily ensure product efficacy or generic equivalence with the products that have shown efficacy. Herbal medicines are complex mixtures of more than one active ingredient. Many times, it is unclear as to which or how many constituents are responsible for pharmacologically activity. This multitude of active ingredients increases the possibilities of interactions between conventional medicines and herb-herb interactions. The interaction of drugs with herbal medicine is a significant safety concern, especially for drugs with a narrow therapeutic index such as warfarin and digoxin [82]. In view of establishing the safety of herbs, initiating the pharmacovigilance program will assist in the understanding and prevention of adverse effects or any other possible drug-related problems. The strength and potency of herbal products are not easily quantified, and impurity and stability are habitually not easy to examine. Therefore, botanicals should be regulated as in western countries, and the necessities include labeling, GMP, packaging, marketing, and adverse effects reporting requirements, etc. [78]. Researchers, manufacturers, and regulatory agencies must apply precise systematic methodologies and clinical trials to ensure the quality, safety, and consistency of the herbal products, to gain public faith and confidence and to bring herbal products into the mainstream of health care systems.

8.2 Steps to initiate herbal pharmacovigilance

Due to an increasing awareness at several levels of herbal medicine, it is compulsory to develop pharmacovigilance practices. Several models of pharmacovigilance and its associated tools have been developed in relation to synthetic drugs, and applying these methods to monitoring the safety of herbal medicines presents unique challenges in addition to those described for conventional medicines [83]. Several steps to initiate herbal pharmacovigilance to monitor the safety profiles of herbal medicines are shown in Fig. 6.

9 Safety issues on herbal medicine-cytochrome P450 study

It is a common belief that modern drugs are dangerous and produce side effects, while herbal medicines are natural and very safe. In fact, some herbs can also be unsafe and even cause serious adverse effects leading to death, if used inappropriately. The complexity of herbal medicine preparations and the interpretation of bibliographic data on safety and efficacy reflecting the experience gathered during long-term use are best addressed by involving

specific expertise and experience. Without the knowledge of the prescriber, the consumer tends to consume the herbal products along with prescription medicine, which may lead to herb-drug interaction [84]. The use of complementary and substitute medical therapies has now become a very common trend, and their use has been documented widely during 2004–14. The increased usage of herbs as dietary supplements and over-the-counter products suggested the need for the development of clinical and scientific data for quality and safety evaluation [85].

Toxicity may be due to the interaction of the herbal material with conventional drugs. Besides there are large number of clinical drugs reported to have potential hepato, renal, cardio toxicity, etc. during epidemiological and other prospective studies. Other agents, such as excipients present in formulations and herbal medicines that are consumed and often not disclosed should also be considered for safety evaluation [86]. Several phytoconstituents have been identified as inhibitors or inducers of cytochrome resulting in herb-drug interaction. Interaction of active compounds including allicin, quercetin, silymarin compounds, etc., has also been reported. Conventional pharmacokinetic literature generally deals with drug-drug interactions, but recently, such interactions between phytoconstituents and prescription drugs have drawn attention, because of increasing physician awareness of the widespread adverse effects of undisclosed herbal use by the patients [12].

Cytochrome P450 (CYP450) enzymes are the major drug-metabolizing enzymes and responsible for the metabolism of a variety of xenobiotics including therapeutic drugs and some important endogenous substances, and most of the herb-drug interactions occur due to either induction or inhibition of these enzymes. CYP450 enzymes are necessary for the production of cholesterol, steroids, prostacyclins, and thromboxane [87]. These enzymes also play an important role in the detoxification of foreign chemicals and the metabolism of drugs. There are more than 50 CYP450 enzymes, but the CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4,and CYP3A5 enzymes metabolize about 90% of drugs. These isozymes are predominantly found in the liver, and are also present in the small intestine, placenta, lungs, and kidneys [88]. An abundance of several cytochrome isoforms has been determined as 30% CYP3A4, 20% CYP2C, 13% CYP1A2, 7% CYP2E1, 4% CYP2A6, 2% CYP2D6, and 1% CYP2B6, which appears to be most relevant for the metabolism of drugs [89]. Repeated administration of drugs can induce CYP enzymes by enhancing the rate of enzyme synthesis. Induction of enzymes leads to an increase in the rate of metabolite production and hepatic biotransformation and decreases in serum drug half-life, response and can also lead to alteration of the pharmacokinetic profile of the drug. CYP enzyme inhibition can be classified into reversible inhibition and irreversible inhibition based on the enzymatic mechanism. Reversible inhibition occurs as a result of direct competition for the binding site on a CYP enzyme between a substrate and an inhibitor, whereas irreversible inhibition is caused by reactive metabolites generated from CYP-catalyzed reactions. Thus, modulation of CYP450 enzyme metabolism is the key to change systemic drug concentrations.

With the increasing consumption of herbal extracts along with prescription medicine, issues related to the safety of herbs have become a key concern. The medical and scientific literature is abundant in in vitro and in vivo reports; this suggests that the concomitant oral administration of natural products and prescription drugs or over-the-counter products may affect human drug metabolism and significantly increase the risk of serious (clinical) adverse reactions. When conventional drug substances are co-administered along with dietary substances or herbal components, herb-drug interaction may occur by CYP450 isozymes in several ways [87].

- A herbal constituent can be a substrate of one or several isoforms of CYP enzymes. Therefore, one substrate can compete for another substrate for metabolism by the same CYP isoenzyme, resulting in higher plasma concentrations of the drug due to competitive inhibition.
- A herbal constituent can also be an inducer of one or several CYP isoforms, and thereby lower plasma concentrations of the drug due to a higher rate of metabolism. Such interactions may produce subtherapeutic plasma drug concentrations.
- A compound can also be an inhibitor of CYP450enzymes and result in reduced activity of one or several isoforms of CYP450.

Several attempts have been made to evaluate the inhibitory effects of medicinal plants on CYP enzymes. The potential for herbal remedies to induce or inhibit CYP levels has been examined. By using in vitro and in vivo methods, several herbs have been studied for their potential inhibitory effects on human liver microsomes, rat liver microsomes, and cDNA expressed human liver microsomes [90]. Studies on drug metabolizing enzymes enhance our understanding of the possibilities for herb-drug interactions. Several existing reports on the role of drug metabolism enzymes, mainly CYP450, in herb-drug interactions are summarized in Table 2 with reference to individual herbs.

Several therapeutically active plant extracts have been reported to interact with drugs leading to clinically relevant adverse drug reactions. Interaction potentials of some medicinal plants are being described in the following section.

TABLE 2 Effects of herbal extracts on CYP450 isozymes [94].

Name of the plant	Part used in the study	Type of extract	Study method	CYP isoforms used	Effects on CYP450	IC_{50} value/percentage inhibition	Reference
Acorus calamus	Root	Hydro-alcohol	Fluorimetry	Human CYP3A4 and CYP2D6	Inhibition	46.84 μg/mL and 36.81 μg/mL	[97]
Capsicum annuum	Fruit	Methanolic	Fluorimetry	Human CYP3A4, CYP2D6, CYP2C9, and CYP2C19	Inhibition	99.69 μg/mL, 68.25 μg/mL, 88.03 μg/mL, and 84.16 μg/mL	[98]
Centella asiatica	Whole plant	Aqueous	HPLC	Human CYP2C9, CYP2D6, CYP3A4	Inhibition	599.0 μg/mL, 413.1 μg/mL, 229.5 μg/mL	[99]
Centella asiatica	Whole plant	Ethanolic	HPLC	CYP2C9, CYP2D6, and CYP3A4	Inhibition	28.3 μg/mL, 418.9 μg/mL, and 465.8 μg/mL	[99]
Curcuma longa	Rhizome	Aqueous	HPLC	Human CYP2C9, CYP2C19, CYP2D6, CYP3A4	Inhibition	82.3%, 92.7%, 48.6%, 92.8%	[100]
Cymbopogon nardus	Aerial part	Methanolic	Radiometry	Rat CYP3A4	Inhibition	370 μg/mL	[101]
Emblica officinale	Fruit	Hydroalcohol	Fluorimetry	Human CYP3A4 and CYP2D6	Inhibition	152.11 μg/mL and 109.96 μg/mL	[102]
Origanum vulgare	Leaves	Aqueous	HPLC	Human CYP2C9, CYP2C19, CYP2D6, CYP3A4	Inhibition	35.4%, 80.2%, 94.6%, 98.6%	[100]
Piper longum	Fruit	Methanolic	Fluorimetry	Human CYP3A4 and CYP2D6	Inhibition	164.81 μg/mL and 210.60 μg/mL	[103]
Piper nigrum	Fruit	Methanolic	Fluorimetry	Human CYP3A4 and CYP2D6	Inhibition	178.34 μg/mL and 234.90 μg/mL	[103]
Rheum palmatum	Root	Methanolic	Radiometry	Rat CYP3A4 and CYP2D6	Inhibition	467 μg/mL and 385 μg/mL	[101]
Rhodiola rosea	Root	Ethanolic	Spectro fluorimetry	CYP2D6 and CYP3A4	Inhibition	32 μg/mL and 67%	[104]
Rhododendron groenlandicum	Leaf	Ethanolic	Spectro fluorimetry	Human CYP3A4	Inhibition	48%	[104]
Salvia officinalis	Leaves	Aqueous	HPLC	Human CYP2C9, CYP2C19, CYP2D6, CYP3A4	Inhibition	97.2%, 99.9%, 99.8%, 97.0%	[100]
Sanatalum album	Wood	Methanolic	Radiometry	CYP3A4 and CYP2D6	Inhibition	337 μg/mL and 886 μg/mL	[101]
Strychnos ligustriana	Wood	Methanolic	Radiometry	Rat CYP2D6	Inhibition	637 μg/mL	[101]
Strychnos ligustrina	Leaf	Methanolic	Radiometry	Rat CYP2D6	Inhibition	302 μg/mL	[101]
Syzygium aromaticum	Flower	Methanolic	Radiometry	Rat CYP3A4 and CYP2D6	Inhibition	219 μg/mL and 249 μg/mL	[101]
Tanacetum parthenium	Leaves	Hydroalcohol	HPLC	Human CYP2C9, CYP2C1, CYP2D6, and CYP3A4	Inhibition	51.5%, 46.2%, 54.1%, and 64.7%	[100]
Terminalia bellerica	Fruit	Hydroalcohol	Fluorimetry	Human CYP3A4 and CYP2D6	Inhibition	77.94 μg/mL and 90.20 μg/mL	[105]
Terminalia chebula	Fruit	Hydroalcohol	Fluorimetry	Human CYP3A4 and CYP2D6	Inhibition	95.52 μg/mL and 102.35 μg/mL	[102]
Thonningia sanguinea	Root	Aqueous	Spectro photometry	Rat CYP1A1, CYP2B1, CYP2B2, CY1A2	Inhibition	19%, 18%, 18%, 40%	[106]

Continued

TABLE 2 Effects of herbal extracts on CYP450 isozymes—cont'd

Name of the plant	Part used in the study	Type of extract	Study method	CYP isoforms used	Effects on CYP450	IC_{50} value/percentage inhibition	Reference
Tinospora crispa	Stem	Methanolic	Radiometry	Rat CYP3A4 and CYP2D6	Inhibition	428 μg/mL, 488 μg/mL	[101]
Zingiber aromaticum	Rhizome	Ethanolic	Radiometry	Rat CYP3A4 and CYP2D6	Inhibition	102 μg/mL and 693 μg/mL	[101]
Zingiber officinale	Fruit	Methanolic	Fluorimetry	Human CYP3A4 and CYP2D6	Inhibition	286.49 μg/mL and 249.52 μg/mL	[103]
Plumbago zeylanica	Roots	Hydroalcohol	Fluorimetry	Human CYP3A4, CYP2D6, CYP1A2, and CYP2C9	Inhibition	167.81 ± 2.06 μg/mL, 149.60 ± 1.37 μg/mL, 166.12 ± 2.98 μg/mL, 143.02 ± 1.01 μg/mL	[107]
Cyperus rotundus	Rhizomes	Hydroalcohol	Fluorimetry	Human CYP3A4, CYP2D6, CYP1A2, and CYP2C9	Inhibition	197.49 ± 2.19 μg/mL, 171.52 ± 3.16 μg/mL, 159.21 ± 2.93 μg/mL, 167.51 ± 1.59 μg/mL	[107]
Embelia ribes	Seeds	Hydroalcohol	Fluorimetry	Human CYP3A4, CYP2D6, CYP1A2, and CYP2C9	Inhibition	202.73 ± 2.88 μg/mL, 248.46 ± 1.67 μg/mL, 229.71 ± 2.17 μg/mL, 199.19 ± 1.97 μg/mL	[107]
Berberis aristata	Bark	Ethanolic	Fluorimetry	Human CYP1A2, CYP2C9, CYP2D6, and CYP3A4	Inhibition	138.88 ± 1.93 μg/mL, 169.13 ± 11.03 μg/mL, 146.57 ± 5.66 μg/mL, 181.27 ± 5.97 μg/mL	[108]
Andrographis paniculata	Aerial parts	Methanolic	Fluorimetry	Human CYP3A4, CYP2D6, CYP2C9, CYP1A2	Inhibition	63.06 ± 1.35 μg/mL, 88.80 ± 3.32 μg/mL, 122.46 ± 3.81 μg/mL, 107.14 ± 2.14 μg/mL	[72]
Bacopa monnieri	Root	Methanolic	Fluorimetry	Human CYP3A4, CYP2D6, CYP2C9, CYP1A2	Inhibition	143.23 ± 2.61 μg/mL, 116.47 ± 4.27 μg/mL, 72.97 ± 1.16 μg/mL, 135.59 ± 1.46 μg/mL	[72]
Centella asiatica	Whole plant	Methanolic	Fluorimetry	Human CYP3A4, CYP2D6, CYP2C9, CYP1A2	Inhibition	225.71 ± 2.26 μg/mL, 139.99 ± 1.73 μg/mL, 184.68 ± 3.79 μg/mL, 288.83 ± 1.61 μg/mL	[72]
Tinospora cordifolia	Aerial part	Hydroalcohol	Fluorimetry	Human CYP3A4, CYP2D6, CYP2C9, CYP1A2	Inhibition	136.45 ± 1.37 μg/mL, 144.37 ± 1.06 μg/mL, 127.55 ± 1.26 μg/mL, 141.82 ± 1.37 μg/mL	[72]
Swertia chirata	Leaves	Ethanolic	Fluorimetry	Human CYP3A4, CYP2D6	Inhibition	197.49 ± 2.68 μg/mL, 211.45 ± 3.54 μg/mL	[71]
Aegle marmelos	Fruits	Ethanolic	Fluorimetry	Human CYP3A4, CYP2D6, CYP2C9, CYP1A2	Inhibition	149.62 ± 2.22 μg/mL, 159.73 ± 1.43 μg/mL, 137.45 ± 2.16 μg/mL, 128.49 ± 1.27 μg/mL	[109]
Morus alba	Leaves	Hydroalcohol	Fluorimetry	Human CYP3A4, CYP2D6, CYP2C9, CYP1A2	Inhibition	132.96 ± 1.39 μg/mL, 138.22 ± 3.18 μg/mL, 157.23 ± 1.42 μg/mL, 122.65 ± 2.14 μg/mL	[110]
Moringa oleifera	Leaves	Methanolic	Fluorimetry	Human CYP3A4, CYP2D6	Inhibition	127.36 ± 2.98 μg/mL, 133.73 ± 1.79 μg/mL	[111]
Trigonella foenum graceum	Seeds	Methanolic	Fluorimetry	Human CYP3A4, CYP2D6	Inhibition	142.23 ± 2.61 μg/mL, 116.47 ± 4.27 μg/mL	[74]

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9.1 *Cimicifuga racemose* (family: Ranunculaceae)

A commercially available dietary supplement made from black cohosh was identified as CYP3A4 inhibitor [91]. The polar fraction from the extract showed 44% inhibition at 5 μg/mL, which was as potent as the inhibition produced by ketoconazole 58% at 5 μg/mL. The IC_{50} values of these six compounds ranged from 0.10 to 7.78 mM [87].

9.2 *Boswellia serrata* (family: Burseraceae)

The main constituents of salai guggal are volatile oil, polysaccharides, triterpene acids such as α, β boswellic acids **(34, 35).** *Boswellia carteri, Boswellia frereana, Boswellia sara,* and *Boswellia serrata* (Family: Burseraceae) herbal extracts were studied for their potential inhibitory activity on CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and 3A4 [92]. The aqueous methanolic (MeoH 80% v/v) extracts of Boswellia species showed potential inhibitory activities. CYP1A2 and CYP2D6 were inhibited at a concentration 10 μg/mL. CYP2C8/9/19 and 3A4 were strongly inhibited at this concentration (IC_{50} values between 1 and 10 μg/mL). In order to evaluate the contribution of boswellic acids, selected boswellic acids were evaluated for their inhibition activity. From the study, boswellic acids were identified as moderate to potent inhibitors of the CYP enzymes (IC_{50} values between 5 and 120 μM) [87].

9.3 *Gardenia jasminoids* (family: Rubiaceae)

The effects of *Gardenia jasminoids* extract on liver CYP-450 dependent monooxygenases, glutathione and glutathione S-transferase were investigated using rats treated orally with the iridoid glycoside (0.1 g/kg body weight/day) or the crude extract of its fruits (2 g/kg/day) for 4 days [93]. *G. jasminoides* decreased CYP450 content in liver microsomes and demonstrate that geniposide from *G. jasminoides* has the ability to inhibit a CYP3A4 monooxygenase and increase glutathione content in rat liver. Further the immunochemical data using immunoblotting studies using antibodies revealed that geniposide treatment markedly decreased the protein immunorelated to CYP3A in rat liver [87].

9.4 *Matricaria recutita* (family: Asteraceae)

The inhibitory effect of *Matricaria recutita* essential oil and its major constituents such as chamazulene (36) and α-bisabolol (4) on four selected human CYP450 enzymes (CYP1A2, CYP2C9, CYP2D6, and CYP3A4) demonstrated the inhibition of these enzymes, with CYP1A2being more sensitive than the other isoforms. Chamazulene ($IC_{50} = 4.41$ μM), cis-spiro ether ($IC_{50} = 2.01$ μM), and trans-spiroether ($IC_{50} = 0.47$ μM) were shown to be potent inhibitors of this enzyme and also active towards CYP3A4, CYP2C9, and CYP2D6. Chamazulene ($IC_{50} = 1.06$ μM) and a-bisabolol ($IC_{50} = 2.18$ μM) caused a significant inhibition of CYP2D6. As indicated by these in vitro data, chamomile preparations contain constituents inhibiting the activities of major human drug-metabolizing enzymes [94].

9.5 *Echinacea purpurea* (family: Asteraceae)

In vitro CYP3A4 inhibition profiles of *Echinacea purpurea* and ketoconazole were evaluated by different substrates and showed a much lower CYP3A4 inhibition by *E. purpurea* ($IC_{50} = 5394$ μg/mL) compared to that by fluorescent substrates (IC_{50} 354 and 452 μg/mL, respectively). From the study, it was suggested that the substrate/assay-dependent effects may occur due to the complex nature of *E. purpurea* constituents, involving different CYP3A4 substrate binding sites. A weak inhibition potential of *E. purpurea* towards CYP3A4-mediated metabolism in vitro was confirmed by the use of three different substrates [95].

9.6 *Ginkgo biloba* (family: Ginkgoaceae)

Ginkgo is believed to improve cerebral and peripheral blood flow through nitric oxide-induced vasodilation and possesses antioxidant activity [96]. Certain components of *Ginkgo biloba* are potent in vitro inhibitors of human CYP2C9. Another study was undertaken to clarify the influence of repeated oral administration of ginkgo extract on CYP2C9 and CYP3A4. A combination of *G. biloba* and tolbutamide is to be administered cautiously in terms of potential interactions, especially in elderly patients or patients treated with drugs exerting relatively narrow therapeutic windows. Greenblatt et al. (2006) investigated the effect of *G. biloba* on the activity of CYP2C9 when administered with warfarin in humans [97].

9.7 *Evodia rutaecarpa* (family: Rutaceae)

Rutaecarpine (38), a quinazolinocarboline alkaloid that is a major constituent of Evodia fruit, caused the most dramatic decrease in residual CYP3A4 activity. It was further identified as a mechanism-based inhibitor of CYP3A4. Rutaecarpin also showed potent inhibition to CYP1A1 and CYP1A2 (IC_{50} 0.90 and 0.06 μM). An analysis showed that methanol extract increased the levels of CYP1A1, CYP1A2, CYP2B, and glutathione-*S*-transferase Yb immunoreactive proteins and aqueous extract increased CYP1A2 protein level. Three major bioactive alkaloids, that is, rutaecarpine, evodiamine (39), and dehydroevodiamine (40) at 25 mg/kg increased hepatic ethoxyresorufin-*O*-deethylase (EROD) activity. These results demonstrated that *Evodia rutaecarpa* methanol and aqueous extracts could affect drug metabolizing enzyme activities [87].

9.8 *Hydrastis canadensis* (family: Ranunculaceae)

This contains the alkaloids berberine (17) and hydrastine (41), hydrastinine (42), and canadine (43). Its extract contains approximately equal concentrations (~17 μM) of two ethylenedioxyphenyl alkaloids, berberine and hydrastine, inhibited with increasing potency the isoform CYP2C9 (diclofenac 4b-hydroxylation), CYP2D6 (bufuralol 1b-hydroxylation), and CYP3A4 (testosterone 6β-hydroxylation) activities in human hepatic microsomes with interpolated IC_{50} values of 0.98%, 0.66%, and 0.18%, respectively [87].

9.9 *Piper methysticum* (family: Piperaceae)

Whole kava extract (normalized to 100 1Mtotal kavalactones) caused concentration-dependent decreases in CYP450 activities, with significant inhibition of the activities of CYP1A2 (56% inhibition), CYP2C9 (92%), CYP2C19 (86%), CYP2D6 (73%), CYP3A4 (78%), and CYP4A9/11 (65%) following preincubation for 15 min. These data indicated that kava has a high potential for causing drug interactions through inhibition of CYP450 enzymes. Jennifer and Ramzan (2004) [98] reported that several kavalactones are potent inhibitors of CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4, and CYP4A9/11 and studied the effect of kava rhizome extracts and kava alkaloids, pipermethystine (44) in rat liver microsomes. Pipermethystine alone demonstrated a non-significant increase in CYP1A2, while kava rhizome extracts alone and in combination with pipermethystine increased hepatic CYP1A2 protein levels by 98%. From the study, it is understood that kavakava may have a potential to produce drug interactions. Zou et al. (2002) [99] investigated the influence of isolated kavalactones kavain (45), dihydrokavain (46), methysticin (47), yangonin (48), and desmethoxyyangonin (49) on recombinant human CYP isoforms such as CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4.They calculated IC_{50} values between 0.43 and 153.20 μM from the mean of four determinations for the potent inhibitory active compounds.

9.10 *Radix pueraria* (family: Fabaceae)

This possesses a high content of flavonoids, coumarins, and isoflavones such as daidzein (50) and puerarin (51). Crude extracts containing puerarin inhibited in adose-dependent fashion. Although both CYP content and reduced nicotinamide adenine dinucleotide phosphate-(CYP)-c-reductase activity were significantly increased, a complex pattern of CYP modulation was observed, including both induction (puerarin: CYP2A1, CYP1A1/2, CYP3A1, CYP2C11; Ge-gen: CYP1A2, CYP3A1, CYP2B1) and inactivation (Ge-gen and puerarin: CYP3A, CYP2E1, CYP2B1) [100].

9.11 *Phyllanthus amarus* (family: Euphorbiaceae)

All the CYP450 enzymes were significantly inhibited by the *Phyllanthus amarus* extract in a concentration dependent manner. The concentrations needed for 50%inhibition of CYP1A1, CYP1A2, and CYP2B1/2 were 4.6 μg/mL, 7.725 μg/mL, and 4.18 μg/mL, respectively. Oral administration of *P. amarus* (250 mg/kg) was found to reduce the activity of these enzymes and an increased concentration of the extract (750 mg/kg) [101].

9.12 *Valeriana officinalis* (family: Valerianaceae)

Its essential oil contains terpenoid-like hydroxylvalerenic acid (52), and valerenic acid (53). CYP3A4-mediated metabolism tended to be slightly lower in aqueous extracts and slightly higher in those extracted with acetonitrile. In most cases, the ethanolic extracts were slightly less active than the corresponding aqueous or acetonitrile extracts. From the study, it was concluded that valerian extracts exhibited a marked inhibition of CYP3A4-mediated metabolism [87].

9.13 Triphala

"Triphala" is a well-known polyherbal formulation from Ayurveda. It consists of dried fruits of *Emblica officinalis*, *Terminalia belerica*, and *Terminalia chebula* in equal proportions (1:1:1). Traditionally this formulation has been prescribed for several ailments such as laxative, detoxifying agent of the colon, digestive and rejuvenator [102]. It has been reported that *E. officinale* extract showed highest IC_{50} value (152.11 ± 2.18 μg/mL) and *T. bellerica* showed the lowest IC_{50} value (69.89 ± 2.50 μg/mL) against CYP3A4. The inhibitory activity against CYP3A4 with IC_{50} values <0.1 mg/mL were found with the three fruit extract and the formulation, while gallic acid **(2)** was found to produce inhibitory activity with approximately 0.09 mg/mL. Likewise all the fruit extracts showed more IC_{50} values (<0.1 mg/mL) compared to gallic acid (0.09 mg/mL) against CYP2D6. On the other hand, quinidine and ketoconazole showed relatively strong inhibition with an IC_{50} value of <0.007 mg/mL. Results indicated that the test substances have much less interaction potential with co-administered drug than the known inhibitors. Triphala formulation has less interaction potential when compared with individual plant extracts and bioactive molecule. Triphala formulation and its individual ingredients may likely to interact with drug metabolizing enzymes, but less likely to produce significant drug interactions [103].

9.14 Trikatu

Trikatu is a very well known "Rasayana" in Ayurveda and widely used as a poly herbal ayurvedic formulation in India. Trikatu means the mixture of three acrids. It consists of three well known plants, viz., *Piper longum*, *Piper nigrum* and *Zingiber officinale* in equal ratio. Trikatu has been prescribed for cough, cold, fever, asthma, respiratory problems and improvement of digestive disorders. It is reported that the trikatu and its bio-markers have very less inhibitory effect on cytochrome P450 enzymes. Different concentrations of the trikatu formulation and its individual components showed significantly ($P < 0.001$) less in hibitory activity on individual isoenzymes as compared to the positive control [104].

9.15 *Murraya koenigii* (family: Rutaceae)

Murraya koenigii extract has a higher IC_{50} value (160.47 ± 5.45, 206.63 ± 1.99, and 156.56 ± 3.77 μg/mL of CYP3A4, 2D6, and 2C9 isozymes, respectively) than do the standard biomarkers. *M. koenigii* extract and its bioactive compounds have an inhibitory effect on drug metabolism enzymes when consumed along with conventional medicine. The IC_{50} values were higher than those of the positive control and indicated that the test extracts and constituents have moderate interaction in drug metabolism [105].

9.16 *Glycyrrhiza glabra* (family: Fabaceae)

The major bioactive constituents of *G. glabra* are glycyrrhizin, glabranin A and B, glycyrrhetol, glabridin, formononetin, glabrone, etc. [63]. A research report on the CYP450 interaction profiles of *G. glabra* and its bioactive compound glycyrrhizin showed that the extract and glycyrrhizin have higher interaction potential with CYP2D6 compared with CYP3A4. The licorice extract showed a comparably higher IC_{50} value with both of the isozymes, which may be related to the synergistic effects for the presence of other constituents in the extract. The higher IC_{50} values than the positive inhibitors indicated that the test samples have only a weak interaction potential in drug metabolism. A lower IC50 value of the extract than the pure compound indicate that care should be taken when administering the extract with other cytochrome P450-interacting compounds, particularly those with a low therapeutic index [106].

9.17 Trimada

Trimada is a recognized polyherbal vinous Ayurvedic rasayana consisting of three herbs namely *Plumbago zeylanica* Linn. (Chitraka), *Cyperus rotundus* Linn. (Musta) and *Embelia ribes* Burm. F. (Vidanga) mixed in equal portions. Trimada has been traditionally used for better functioning of the digestive system and also helps in reducing cholesterol levels. The study reported lower toxicity of Trimada towards HepG2 cell line. The individual herbs Chitraka, Musta and Vidanga exhibited very less interaction with CYP3A4, CYP2D6, CYP1A2 and CYP2C9 than their respective standard inhibitors. CYP-CO complex assay using liver microsomes showed less interaction potential with individual herbs than the standard inhibitors. Thus, the individual herbs and Trimada formulation exhibited low cytotoxicity and has negligible interaction with CYP450 isozymes. So, the formulation may be considered to be safe for its therapeutic management without any potential drug interaction involving CYP 450 isozymes [107].

9.18 *Berberis aristate* (family: Berberidaceae)

Ayurvedic excerpts proclaimed the herb *Berberis aristata* DC (Family: Berberidaceae). in the management of diabetes in India and other Asian countries. The study portrayed a possible enzyme inhibition potential of the anti-diabetic herb *Berberis aristata* and its combination with glimepiride and gliclazide as the standard oral hypoglycaemic agents and berberine as the phytomarker against CYP450 isoforms such as CYP3A4, CYP2D6, CYP2C9 and CYP1A2 isozymes. Fluorescence based HTS assay predicted the plant extract to have lower interaction potential compared to the standard inhibitors. The combination index (CI)—isobologram method showed that *Berberis aristata* and berberine in the binary combinations with glimepiride and gliclazide also showed significantly less ($P < 0.001$, $P < 0.01$) interaction potential than known CYP450 inhibitors. Thus, the herb is safe for human consumption as a hypoglycaemic agent [108].

9.19 *Andrographis paniculata* (family: Acanthaceae)

Andrographis paniculata Nees (Family: Acanthaceae) is an important medicinal food plant in India. Traditional system of medicine used it for its ability in lowering body temperature and to eliminate toxins. It can also be used as a potent analgesic, anti-inflammatory, to treat neurodegenerative disorders and ant-allergic. *Andrographis paniculata* has a potent antiviral activity and can be used to stimulate immunogenic responses. Andrographolide (29), the major phytoconstituent of *Andrographis paniculata* was studied for evaluating its interaction potential against pooled CYP450 as well as human CYP3A4, CYP2D6, CYP2C9 and CYP1A2 isozymes by CYP-CO complex assay and HTS based fluorogenic assay. Less inhibition potential was recorded than the standard inhibitor ketoconazole ($P < 0.05$ and above) in CYP-CO complex assay. Fluorogenic assay revealed *Andrographis paniculata* extract showed higher IC50 value and less interaction potential than the positive inhibitors. *Andrographis paniculata* showed highest inhibition for

CYP3A4 (63.06 ± 1.35 mg/mL) and lowest in CYP2C9 (122.46 ± 3.81 mg/mL) whereas the bioactive compound Andrographolide showed lesser inhibition potential with CYP3A4 (106.27 ± 2.92 mg/mL) and CYP2C9 (288.62 ± 2.07 mg/mL). Thus, *Andrographis paniculata* may not possess any harmful effect with regards to its therapeutic application [72].

9.20 *Bacopa monnieri* (family: Scrophulariaceae)

Bacopa monnieri (Family: Scrophulariaceae) also known as Bramhi is an important medicinal food plant in the Indian subcontinent. Bramhi is used for enhancement of memory, intelligence and to treat neurodegenerative disorders. Pharmacological activities of *Bacopa monnieri* include anti-epileptic, anticancer, anti-ulcer and anti-inflammatory. *Bacopa monnieri* and its bioactive constituent Bacoside A (30) was studied for evaluating its interaction potential against pooled CYP450 as well as human CYP3A4, CYP2D6, CYP2C9 and CYP1A2 isozymes by CYP-CO complex assay and HTS based fluorogenic assay. Less inhibition potential was recorded than the standard inhibitor ketoconazole ($P < 0.05$ and above) in CYP-CO complex assay. Fluorogenic assay revealed *Bacopa monnieri* extract showed higher IC50 value and less interaction potential than Bacoside A and the positive inhibitors. *Bacopa monnieri* showed highest inhibition for CYP2C9 (72.97 ± 1.16 mg/mL) and lowest in CYP3A4 (143.23 ± 2.61 mg/mL) whereas the bioactive compound Bacoside A showed lesser inhibition potential with CYP2C9 (155.69 ± 1.54 mg/mL) and CYP3A4 (373.90 ± 2.49 mg/mL). Thus, *Bacopa monnieri* may not possess any harmful effect with regards to its therapeutic application. [72]

9.21 *Centella asiatica* (family: Mackinlayaceae)

The food plant *Centella asiatica* (Family: Mackinlayaceae) commonly known as Gotu Kola has a plethora of pharmacological activities like increasing mental activity and regulate high blood pressure, reduce rheumatism, decrease high body temperature and treat nervous disorders. The whole plant of *Centella asiatica* was used to assess the interaction potential against CYP3A4, CYP2D6, CYP2C9 and CYP1A2 isozymes. *Centella asiatica* showed highest inhibition for CYP2D6 (139.99 ± 1.73 mg/mL) and lowest in CYP1A2 (288.83 ± 1.61 mg/mL) whereas the bioactive compound Asiaticoside (31) showed lesser inhibition potential with CYP2D6 (235.35 ± 2.63 mg/mL) and CYP1A2 (504.02 ± 4.45 mg/mL). The plant extract showed significantly lesser interaction potential than both the positive inhibitors Quinidine (5.61 ± 0.94 mg/mL) and α-Naphthoflavone (19.64 ± 2.11 mg/mL). Thus, *Centella asiatica* may not possess any harmful effect with regards to its therapeutic application [72].

9.22 *Tinospora cordifolia* (family: Menispermaceae)

Tinospora cordifolia (Family: Menispermaceae), locally known as guduchi, is a widely distributed and essential Ayurvedic in the Indian subcontinent. Traditionally Guduchi has been used as an anti-diabetic, regulate bile secretion, reduce burning sensation and to treat several urinary tract infections. The major phytoconstituents responsible for the pharmacological actions are tinosporaside (32), tinosporone, tinosporic acid and cordifolisides A to E. The plethora of pharmacological actions include anticancer, antiulcer, anti-ischemic, cardiotonic, antiinflammatory, antispasmodic, antiarthritic, analgesic, diuretic and antidepressant. Guduchi also helps in enhancing memory and is used as an immunomodulatory agent. The study reported the content of the major constituent tinosporaside standardized through HPLC to be 1.64% (w/w). CYP450-CO complex assay method with pooled rat liver microsome was used to evaluate the metabolism related interaction potential of the standardized extract. 96-well microplate-based fluorescence assay was done with CYP3A4, CYP2D6, CYP2C9, and CYP1A2 isozymes and respective IC50 values were determined. Ethanolic extract of *Tinospora cordifolia* showed highest IC50 value of 144.37 μg/mL for CYP2D6 compared to tinosporaside having IC50 value of 178.88 ± 1.97 μg/mL whereas the lowest IC50 value of 127.55 ± 1.26 μg/mL and 240.17 ± 1.69 μg/mL was recorded for *Tinospora cordifolia* and tinosporaside respectively against CYP2C9 isozyme. The extract showed concentration dependent inhibition and both the extract and bioactive compound significantly lesser interaction potential than the positive inhibitors concluding its safe consumption for human use [73].

9.23 *Swertia chirata* (family: Gentianaceae)

Swertia chirata (Family: Gentianaceae) commonly known as Chirata is used as a bitter tonic stomachic, febrifuge, hypoglycaemic, anti-leishmaniasis, anti-malarial and anthelmintic. It is also used to lower inflammation and relax uterine wall in pregnant women. The study investigated the metabolism related inhibition potential of the standardized hydro-alcoholic extract and the bioactive compound ursolic acid (28) against CYP3A4 and CYP2D6 isozymes. The inhibitory potential of the hydroalcoholic extract on pooled rat liver microsome was found to be 23.64 ± 1.80%. The IC_{50} values of the hydroalcoholic extract and the standard compound urosolic acid against CYP3A4 was 197.49 ± 2.68 mg/mL and 229.25 ± 2.52 mg/mL respectively whereas for CYP2D6 was 211.45 ± 3.54 mg/mL and 212.66 ± 1.26 mg/mL respectively. The results showed that *Swertia chirata* hydroalcoholic extract has significantly ($P < 0.001$) less inhibition potential than both the positive inhibitors

ketoconazole (IC_{50}: 6.84 ± 1.02 mg/mL) and quinidine (IC_{50}: 3.06 ± 0.80 mg/mL) and may be considered safe for human consumption [71].

9.24 *Aegle marmelos* (family: Rutaceae)

Aegle marmelos L. (Family: Rutaceae) commonly known as Bael in Hindi is an essential food plant of India. Traditionally the fruit was used to treat diabetes, respiratory problem, inflammation, dysentery and diarrhea. The fruits of *Aegle marmelos* are rich in flavonoids, terpenoids, carotenoids and coumarins. The major bioactive constituents include imperatorin (54), aegelin, lupeol, eugenol (7), cineol, citronellal etc. The CYP450-CO assay to evaluate the inhibitory potential of the fruit extract and the standard phytoconstituent imperatorin revealed dose dependent inhibition of the *Aegle marmelos* extract. The fruit extract and imperatorin showed lesser inhibition ($P < 0.001$) than the standard ketoconazole. The 96-well fluorogenic assay showed lowest inhibition for *Aegle marmelos* extract against CYP2D6 (159.73 ± 1.43 μg/mL) and highest inhibition against CYP1A2 (128.49 ± 1.27 μg/mL). The higher IC50 values of the *Aegle marmelos* extract than the positive controls showed less or moderate interaction in drug metabolism [109].

9.25 *Morus alba* (family: Moraceae)

Morus alba L. (Family: Moraceae) is an important dietary supplement, general tonic and used to enhance heath. The plant is used as a potent anti-diabetic, hypolipidemic, anti-hypertensive and antimicrobial. In this study the standardized extract of *Morus alba* leaves have been screened for their inhibitory potential against CYP450 isoforms with respect to its bioactive compound, chlorogenic acid (22). The content of chlorogenic acid was found to be 1.12% (w/w). CYP450-CO complex assay indicated the extract has higher inhibition potential than chlorogenic acid, suggesting synergistic effect of other phytoconstituents present. Fluorometric assay indicated highest inhibition of the ethanolic extract against CYP1A2 (122.65 ± 2.14 μg/mL) and lowest against CYP2C9 (157.23 ± 1.42 μg/mL). The findings suggested the plant extract and its bioactive compound contributed to insignificant herb drug interaction [110].

9.26 *Moringa oleifera* (family: Moringaceae)

Moringa oleifera (Family: Moringaceae) known as drumstick, has been reported as potent hypoglycemic, hypolipidemic, nootropic and anti-inflammatory. The leaves were traditionally used in paralysis, fever, wound, cough, enlarged liver and spleen etc. *Moringa oleifera* leaf extract and the standard phytomarker chlorogenic acid was tested against drug metabolizing CYP450 enzyme isoforms CYP3A4 and CYP2D6 for any interaction. CYP-CO complex assay done on 96-well microplate showed lesser inhibition of 23.45% on rat liver microsome than positive inhibitor ketoconazole. Inhibition potential of the extract and chlorogenic acid (22) dissolved in DMSO was comparatively higher than the ethanolic extract. HTS based fluorescence assay was done using ketoconazole and quinidine as the positive inhibitors of CYP3A4 and CYP2D6 isozymes respectively. Inhibition potential was reported in the order chlorogenic acid < *Moringa oleifera* < positive inhibitors. The extract showed higher inhibition than the standard making way for synergistic effects from other phytoconstituents present. The screened results suggested both *Moringa oleifera* ethanolic as well as DMSO extract and chlorogenic acid showed less inhibition than the positive inhibitors. In other words, the interaction potentials of both the standard inhibitors (Ketoconazole and Quinidine) are much higher than the two extracts as well as the marker compound [111].

9.27 *Trigonella foenum-graceum* (family: Leguminosae)

Trigonella foenum-graecum (Family: Leguminosae) commonly known as methi in Hindi is a widely distributed spice and seasoning in India. Pharmacological activities of methi include antiulcer, antidiabetic, antioxidant, anticancer, anti-inflammatory, and antipyretic. It can also be used as a stimulant for CNS, as a wound healing agent and mediate immune response. *Trigonella foenum-graecum* seeds have a wide range of phytoconstituents like trigonelline (33), protodioscin, trigoneoside, diosgenin, yamogenin, 4-hydroxyisoluecine, and galactomannans. The major compound trigonelline can be used as antidiabetic, CNS stimulant and can be beneficial in Alzheimers disease. Fluorescence based assay was performed against CYP3A4 and CYP2D6 isozymes. The screened results showed concentration-dependent inhibitory effect in both ethanolic and DMSO extract of *Trigonella foenum-graecum* seeds and trigonelline. Both the extract and standard bioactive compound showed significantly lesser inhibition potential than the positive inhibitors ketoconazole and quinidine. IC_{50} values of trigonelline ethanolic and DMSO extract in CYP3A4 (180.90 ± 2.49 μg/mL and 176.23 ± 3.24 μg/mL) was found higher than the ethanolic and DMSO extract of methi (142.23 ± 2.61 μg/mL and 131.00 ± 1.86 μg/mL) suggesting inhibitory activity of rest of the phytoconstituents in the extract. Similarly for CYP2D6, IC_{50} values of trigonelline ethanolic and DMSO extract (172.35 ± 2.63 μg/mL and 168.73 ± 4.03 μg/mL) was found higher than the ethanolic and DMSO extract of methi (116.47 ± 4.27 μg/mL and 102.65 ± 2.63 μg/mL). Thus results suggest *Trigonella foenum-graecum* seeds may be safe for human consumption [74].

10 HERB-drug interactions

The plant material as an active ingredient in herbal products may also be related to health risks, because it also

contains some toxic constituents or constituents that are known to affect the bioavailability and pharmacokinetic or pharmacodynamic interaction of other compounds or drugs [112]. Another problem associated with the use of herbal products is underreporting of observed adverse reactions and herb-drug interactions. In a study, it was found that 58% of users do not inform their physician when they buy any herbal medicinal products. It has been reported that 69% of Britishers and 61.7% of Italians that use herbal product do not consult their physicians [113]. Herb drug interaction has been displayed in Fig. 7.

Many medicinal herbs have a long history of use, and this may have generated a significant amount of published toxicological information including scientifically accepted monographs, clinical experience, and epidemiological studies, as well as data from postmarketing surveillance programs. This information may be used as a basis for a simplified registration procedure and may serve as a substitute for animal experiments and reduce the number of clinical trials in humans [114]. Prescribers and consumers of herbal products will be able to recognize and report on major acute adverse events, such as dermatological reactions, nausea, and disturbances of the gastrointestinal tract. Consequently, data on traditional use are unlikely to provide information on chronic toxicity and carcinogenic, mutagenic, and teratogenic effects [87]. Several regulatory bodies have acknowledged this problem and have given the right to national authorities to demand such supplementary safety testing when bibliographic evidence is deemed to be insufficient to prove safety before marketing authorization of the herbal products [89].

Consumption of herbal products that are capable of modulating CYP metabolism can cause clinically relevant herb-drug interactions and can alter drug bioavailability. Any inhibitory effect of herbal extracts on CYP may result in enhanced plasma and tissue concentrations leading to toxicity, while any inductive effect may cause reduced drug concentrations leading to decreased drug efficacy and treatment failure [113]. The complexity of herbal medicine preparations and the interpretation of bibliographic data on safety and efficacy reflecting the experience gathered during long-term use are best addressed by involving specific expertise and experience.

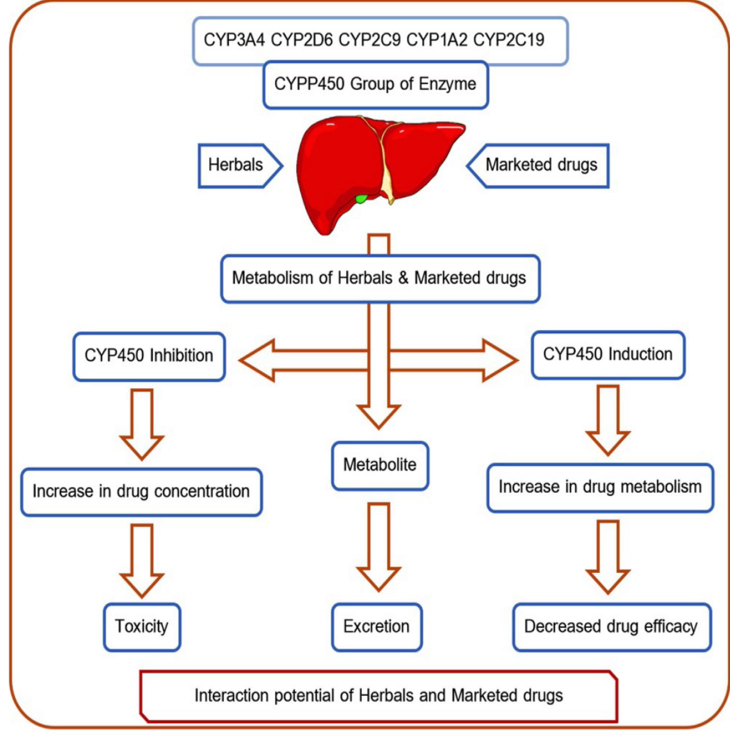
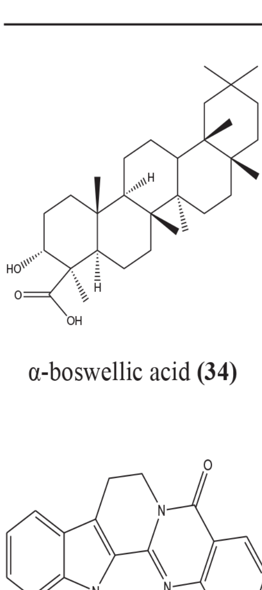
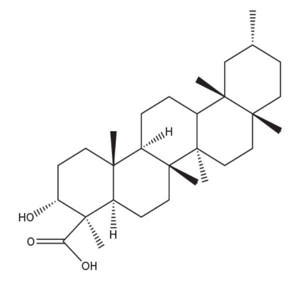


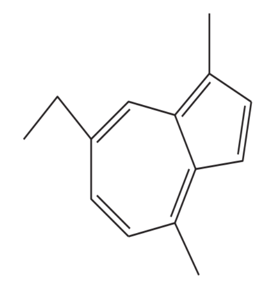
FIG. 7 Herb-drug interaction potential with CYP450 group of enzymes. *Reproduced from Mukherjee. Quality evaluation of herbal medicine. 1st ed. Elsevier; 2019. p. 655–82.*



α-boswellic acid **(34)**



β – boswellic acid **(35)**



Chamazulene **(36)**

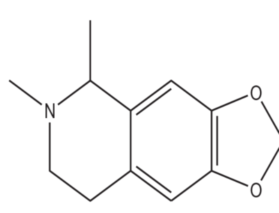
α – bisabolol **(37)**

Rutaecarpine **(38)**

Evodiamine **(39)**

Dehydroevodiamine **(40)**

Hydrastine **(41)**



Hydrastinine **(42)**

Canadine **(43)**

Pipermethystine **(44)**

Kavain **(45)**

Dihydrokavain **(46)** Methysticin **(47)** Yanogonin **(48)**

Desmethoxyyanogonin **(49)** Daidzein **(50)** Puerarin **(51)**

Hydroxyvalerenic acid **(52)** Valerenic acid **(53)** Imperatorin **(54)**

10.1 Synergistic effects of herbs

The mechanisms of synergistic actions of herbal ingredients can be explored for designing new multitarget drugs and drug combinations and for discovering potent drug combinations that are individually subtherapeutic but efficacious in combination. Synergistic actions involve interactions with multiple sites, targets, and pathways that are sensitively influenced by genetic, environmental, behavioral, and scheduling profiles [115]. It is claimed that combinations of herbs have synergistic effects. There is much in vitro and/or in vivo evidence to support the occurrence of synergism between constituents in certain herbal extracts. Synergy is also taken to mean an attenuation of undesirable effects, another key theory of herbalism being that the toxicity of plant extracts is less than that of a single isolated constituent.

Synergism has a major role in therapeutic efficacy of medicinal plants and plant-derived formulation. This cannot be easily distinguished from additive effects and usually relies on high margins of variation. In fact, the mechanism of action of many phytomedicines is still unknown, and there are several instances of a total herb extract showing a better effect than an equivalent dose of an isolated compound [116]. Generally, synergistic effects are considered to be positive, with the low doses used perceived as a benefit, although itis obvious that there may also be negative aspects. Pepper contains the alkaloid piperine, which is known to increase the

bioavailability of a number of clinically used drugs. Unwanted interactions, for example, would be the presence of tannins in herbal drugs, which may hinder the absorption of proteins and alkaloids, or the induction of enzymes, such as CYP450, which may accelerate drug metabolism resulting in blood levels of actives too low for a therapeutic effect [102]. The issues concerning the safety of herbs with increasing consumption of herbal extracts along with prescription medicine have become a major concern. This leads to several studies on evaluation of their effects on drug metabolizing enzymes. These studies will help to understand and ensure the possibilities for herb-drug interactions.

11 System biology and metabolomics

System biology intends to recognize the biological complexity of different measurements without any hypothesis. The major focus of systems biology is to enquire the dynamics of all genetic, regulatory, and metabolic processes in a cell and to understand the complexity of cellular networks [117]. Adoption of the systems biology approach would be more helpful for exploring the scientific implication of herbal medicine and the modernization of TM.

There are several technological platforms of system biology, such as genomics, proteomics, and metabolomics, that provide powerful tools for the study on the essence and function of herbal drugs (Fig. 8). Scientifically and technologically validated herbal products can be explored on a fast track using various innovative approaches such as reverse pharmacology and systems biology, which are based on a knowledge of TM. TM discover practice transforming techniques. The methods for carrying out metabolic modeling by means of collecting, storing, and analyzing metabolomic data are considerably different, which will generally be performed by individuals or in laboratories with different skill sets, and yet necessarily will deal with the same molecules [118]. It is therefore very essential to timely bring

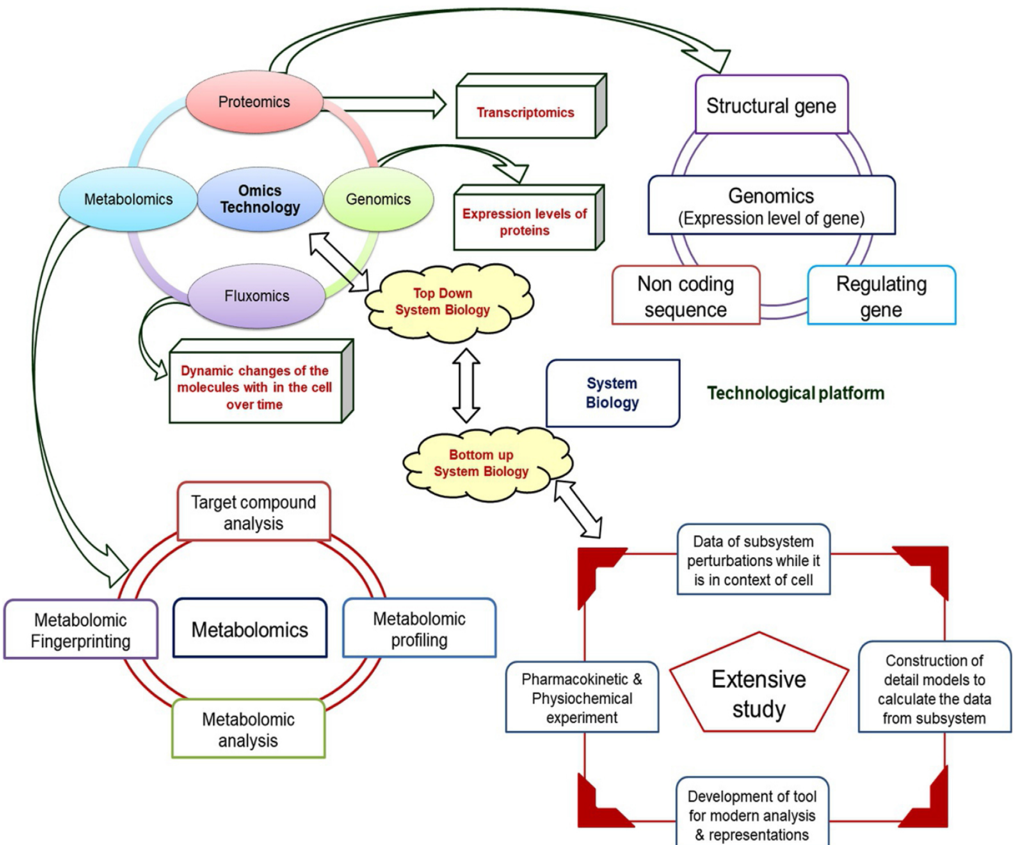


FIG. 8 System biology in drug discovery.

together the known or conditional metabolic maps of suitable organisms with measurements of their metabolomes to provide a system level understanding of the metabolic fluxes and metabolite concentration in these organisms, and their way of changing under different conditions [119].

LC-MS is an analytical technique that combines the physical separation capabilities of LC with the mass analysis capabilities of MS. LC-MS is a powerful technique used for many applications and has a very high sensitivity and selectivity. LC-MS consists of an ultra high LC combined with a mass spectrometer that contributes to achieve high-throughput studies in metabolomic analysis. LC-MS is frequently used in drug development at many different stages including natural product dereplication, metabolomic stability screening, metabolomic identification, impurity identification, quantitative analysis, and quality control. Generally, its application is oriented towards the specific detection and potential identification of phytoconstituents in a plant extract. LC-MS becomes a powerful approach for the rapid identification of phytochemical constituents in botanical extracts, and it can avoid the time-consuming isolation of all compounds to be identified. LC-MS is most commonly used for metabolomic analysis of plant extract where secondary metabolite masses may overlap even with a high-resolution mass spectrometer. Profiling of secondary metabolites in plants or food, such as phenolics, can be achieved with LC-MS [120]. Metabolomics based LC-MS/MS approach for targeted investigation is proven to be crucial for assessing the cluster of phytomolecule present in well known ayurvedic formulations [107].

Metabolomics aims at qualitatively and quantitatively determining as many compounds as possible. This can not only be in extracts of tissues but also in body fluids such as serum or urine in the case of humans. Chromatographic methods in combination with MS, MS with nuclear magnetic resonance (NMR) spectrometry, are used for such analyses [121]. By combining the result of such analyses with other parameters, novel correlations can be found, for example, a relationship between the occurrence of certain compounds in extracts and a biological activity. Analysis of metabolites in urine by means of ^{1}H NMR is already extensively applied for studying the toxicity of drugs [122]. The metabolomics approach is also a very promising tool for the quality control of botanicals. A study on the metabolic profiling of *G. biloba* L. in pharmaceutical preparation by means of ^{1}H NMR has been reported. Besides a recognizable pattern, the quantitative analysis of ginkgolides and bilobalide could be done with a 5-min acquisition time of the spectrum, without the need for any elaborate sample preparation. Also, for other preparations, it was found that this method is very suitable; among other preparations studied were strychnos, ephedra, and cannabis. Such studies are the first steps in the long way to a better understanding of the activity of medicinal plants [123].

Metabolomic study reveals the quantitative and qualitative estimation of "whole" metabolite found in a cellular or organism system. It can be defined as the systemic study of the individual chemical fingerprints that a definite cellular process leaves behind and even more particularly the technique of the metabolite profile of the "whole" small molecules in an organism. The combined data of all the metabolites in a biological system, which are the final products of its gene expression, are known as the metabolome. These approaches deal with the study of genomics, transcriptomics, and proteomics of biological systems. It involves four major steps of analysis of the unknown compound present in the herbal medicine, which includes the following:

- Targeted investigation of the compound: This deals with quantitative estimation of definite metabolites.
- Metabolic documentation: Quantitative and qualitative data for the estimation of the unknown compound or of definite metabolic pathways.
- Metabolomic fingerprinting: This is the process of sample classification by rapid global investigation.
- Metabolomic examination: This pertains to the quantitative and qualitative analysis of "whole" metabolites.

Metabolomic analyses use a particular set of analytical techniques such as Fourier transformed infrared spectroscopy, gas chromatography-mass spectrometry, LC-MS, NMR, CE-MS, and TLC. Recent advances made in analytical chemistry for small mass compound detection and characterization, such as MS and high field NMR, coupled with modern multivariate statistics, have led to a highly efficient system for the comprehensive analysis of the metabolite data matrices generated by metabolomic experiments [10]. In the past decades, several attempts have been made to solve these problems using metabolomics. Metabolomics is a relatively new field of "omics" research concerned with the high-throughput identification and quantification of small-molecule metabolites in the metabolome. Analysis of a large number of samples might facilitate the identification of patterns or metabolite markers that are characteristic of a species, a cultivar, a certain stage of development or conditions, such as disease state, stress, or daily and seasonal changes. Therefore, the high throughput global analysis of a metabolome through hyphenated technologies is a key factor in this field [124]. Data analysis (multivariate and univariate) can be the key to measure the safety and efficacy of herbal products when coupled with metabolomics techniques [125]. Metabolomics based metabolite fingerprinting of nutritional and nutraceutical products has opened doors for development of more and more safe and efficacious products for the betterment of

human health [126]. Thus, metabolomics is now emerging as a powerful tool for the characterization of complex phenotypes affected by both genetic and environmental factors. Nevertheless, metabolomic fingerprinting often lacks robustness, so targeted or profiling approaches may be useful techniques for the validation of herbal medicine with the necessary specificity, precision, accuracy, linearity, sensitivity, recovery, and stability in the presence of potentially interfering compounds [127].

12 Translation of traditional medicine and trend

Traditional medicine has a long history. It is the sum total of the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health, as well as in the prevention, diagnosis, improvement or treatment of physical and mental illnesses. The terms complementary/alternative/non-conventional medicine are used interchangeably with traditional medicine in some countries. The World Health Organization (WHO) recognizes that traditional and complementary medicine (T&CM), are a vital part of the global health care system. With this in mind, WHO recommends that governments harness the potential contribution of T&CM to health and wellness; and promote the safe and effective use of T&CM by regulating, researching and integrating products, practitioners and practice into health systems. In October 2013, WHO released the WHO Traditional Medicine Strategy 2014–23 (the Strategy) to provide guidance for countries as they work to manage priorities, regulations and governance of the T&CM field within their own jurisdiction. In India, the history of health care goes back to 5000 years BC, when health care needs and diseases were noted in ancient literatures like "Rig-Veda" and "Atharva-Veda." Later, the texts like "Charak Samhita" and "Sushruta Samhita" were documented in about 1000 years BC, where use of plants and polyherbal formulations was highlighted for health care. Evolution of Ayurveda and plant-based remedies for health care through day-to-day life experiences is a part of cultural heritage of India. Indian *Materia Medica* includes about 2000 drugs of natural origin almost all of which are derived from different traditional systems and folklore practices [128]. In most countries today, practices of allopathic and traditional systems of medicine crop up side by side in a complimentary way. So the efforts are made to harmonize the process of their evaluation and quality control for their optimum utilization botanicals in relation with drug discovery. In order to ensure the accurate identification and authentication of the herbs is the first crucial step to avoid confusion, admixtures or adulterations in the botanicals. As regards to quality assurance, plant identification is crucial to guarantee that the right plant raw material has been used, and on the other hand to detect possible admixtures, adulterations and confusions. Identification should be carried out combining various methods, including macroscopic and microscopic examination; chemical fingerprinting and DNA based characterization, etc. The useful active compounds in plants for medicinal preparation are among the huge diversity of secondary plant products that are often specific for certain plants or plant groups [35]. These are some vital aspects that should be considered for the correct identification and authentication of traditional medicine herbs [62]. There are several important processes and integrated strategies which need to be considered for the validation is traditional medicine like Ayurveda in each and every step [129]. Global implementations of evidence-based validation are required for traditional medicine that would transform healthcare for all [130]. The development of traditional medicine requires the convergence of modern techniques and integrated approaches related to their evidence based research in various fields. Botanicals are standardized based on the presence of a known active ingredient or specific markers when the active markers are not yet recognized. But this can help in establishing the product's quality depending on the characteristic fingerprints. Plants contain several active substances in certain ratios and in standardized extracts. This ratio must be kept constant, within narrow limits, from one preparation to another. The unique processing methods followed for the manufacturing of traditional medicine products turn herbal ingredients into very complex mixtures, through which the separation, identification and estimation of chemical components become more challenging in some cases. Moreover, herbals are known to contain several components and in many cases the absolute compound responsible for the pharmacological activity is unknown.

India has an ancient system of Ayurvedic medicine which provides a wealth of information on the folklore practices and traditional aspects of therapeutically important natural products. One of the major challenges of Ayurvedic medicine is quality, safety and efficacy issues and these things need to be more emphasized [128]. The history of natural product research is pointing towards its glorious future. Medicinal plants have been clinically explored and used as therapeutic agents since ancient times. It contains secondary metabolites which serve as leads for the development of new drugs. There are continuous quests in the discovery of new lead molecules from medicinal plants due to the fact that synthetic libraries did not yield the expected number of developmental candidates in industry during the last decade. Considering the vast natural entity and the wide variability of the raw materials and the wide complexity of the phyto-constituents in the finished products, the use of High Throughput Screening (HTS) techniques with

de-replication of the extracts can play a lead role for the screening of natural products for a specific diseases which can be further correlated with another disorders to alleviate human suffering [131]. Globalization and reinforcement of Ayurvedic medicine is necessary for the establishment of the evidence based healthcare claims. The scientific validation is a process of documentation on their ethnic uses by traditional healers. These traditional medicine inspired development of new novel drugs, which improved significantly the quality of healthcare. The Chinese identified qinghao (the Chinese name of *Artemisia annua* L.) to be effective against plasmodium parasites found in traditional chinese literature Ge Hong's A Handbook of Prescriptions for Emergencies. Artemisinin was isolated in 1971 and published in 1982 [132]. World Health Organization (WHO) has taken artemisinin based combination therapies (ACTs) to curb out malaria with significant effect. This significant discovery from natural product fetched recognition as Youyou Tu got Noble Prize for it in 2015.

In this context, plant metabolomic is a pioneered approach in medicinal plant research using modern technology likes LC-MS, GC-MS, NMR, HPLC, HPTLC etc. The combination of improved analytical capabilities with newly designed, dedicated statistical, bioinformatics and data mining strategies is beginning to broaden the horizons of our understanding of complex mixture of plant secondary metabolites. Metabolomics is a well known modern research field for quantitative and qualitative estimation of metabolite in the medicinal plants. Advancement in the metabolomic approaches provides the unambiguous value of metabolomic tools in natural products discovery, gene-function analysis, systems biology and diagnostic techniques. Measurement and analysis of metabolites can be a precise and potentially valuable technology for identifying biomarkers. Combining the unique features of identifying biomarkers that are highly conserved across species, metabolomic offers a promising approach to biomarker-driven drug discovery and development. Recent research highlights the various scientific approaches for quality evaluation and validation on Indian medicinal plants as adaptogens for some life style related disorder related to stress [133].

In the past few years, the emerging field of network pharmacology and metabolomics has become an important strategy in natural product based drug discovery by exploring synergy is important to understand the mechanistic pharmacology, safety and re-purposing of medicinal plants which present a multi-molecular matrix with an array of target interactions [128,134]. It has been used for informative, discriminative and predictive purposes associated with quality related safety issues. Metabolomic studies aim at the comprehensive analysis of numerous targeted as well as non-targeted metabolites in plant extracts. There is an emerging need to carry out identification and characterization of phytomolecules to explore secondary metabolite diversity, and also to disclose previously hidden natural molecules from secondary metabolomes.

The concept of traditional drugs and formulations finds its relevance in spite of changes in the environment, lifestyle, culture and disease patterns [128]. Medicinal plants used traditional medicine represent molecular combinations which may achieve more stable "network responses" than single drugs. In order to translate medicinal plants to phytopharmaceuticals, integration of traditional medicine and modern tools will play a major role and also help to develop phytopharmaceuticals to fight against many complex diseases through the development of new entities. Spectacular advances in natural product chemistry, high throughput screening, and the fields of spectroscopy, chromatography which lead to develop several therapeutically potent lead drugs form medicinal plants. The translational phytopharmaceuticals development approaches will be able to reestablish the therapeutic claim more scientifically. This approach will help in the standardization of extracts and the bioassay-guided isolation of the active constituents. The phytoconstituents obtained can then be taken further for structure activity relationship studies and other studies for drug development.

Phytopharmaceuticals developed from Indian medicinal plants represent multi-modal treatments or multi-target approaches are increasingly applied to treat complex diseases in different disciplines of medicine—mainly based on empirical knowledge. There is a need to elucidate and define further the combinatory actions in the context of the theoretical basis, experimental design and quantification of synergy at different dose and effect levels, including optimal combination ratios or sequences and the prevention of potential adverse events. Therefore efforts should be made towards the development of personalized medicine by way of approaching the effect of drug combinations on the patients with a systems approach. Thus exploring synergistic actions/effects of the natural compounds in medicinal plant and phytopharmaceutical formulations, including the "mechanistic" principles of synergy and the use of network analysis and suitable modern bioinformatics approaches which will lead to developing phytopharmaceuticals or fixed dose formulations based on leads from Indian medicinal plants. This will also be greatly helpful to decipher the synergy of potent phyto-constituents of phytopharmaceuticals of medicine for safe, efficacious and affordable healthcare with the metabolomic approach for evaluation of some Indian medicinal plants for various ailments.

This can be understood that plant derived herbal drugs or phytopharmaceuticals can be considered a

natural "model" for combinatory treatments with multi-molecular combinations which has mainly two purpose which are minimizing off-target toxicity by minimizing doses (synergistic potency) and improve outcomes by escalating effect (synergistic efficacy). The traditional system is challenged by the necessity to determine their complex composition and their multi-target mode of action. Recent technological development to investigate the mechanistic principle of synergistic action of some traditional herbs and their phytoconstituents found effective against these diseases. The phytoconstituents of these plants together with a standard drug or antibody, both part of the standard therapy should be explored to understand its synergistic mechanism of action. The functional experiments with the herbal extracts and isolated molecules needs to be done to evaluate their effect on the production of relevant biomarkers and the regulation of the systemic response in human cell lines. Information on the biological activity will be achieved by the determination of cell proliferation and a subsequent application of omics techniques, especially deep sequencing to obtain an overview about the modulated molecules/signal cascades and to evaluate further the effect on related biomarkers and other molecules. Systematic screening of herbal molecule and drug combinations for synergistic effects based on different pharmacological definitions of "synergy" with an emphasis on the combination index based on the combination index theorem of Chou and Talalay together with network pharmacology analysis based on the information gathered from different databases by analyzing compound target, compound-target-pathway networks using molecular annotation of functional significance for the elucidation of their synergistic molecular mode of action [134]. Fig. 9 clearly portrays the flow of traditional knowledge coupled with modern techniques to globalization the traditional medicinal plants.

13 Traditional medicine as sustainable healthcare

The increasing global population is engulfing forests and other resources around the world. They are being rapidly and often irreversibly depleted for energy, food, shelter, material goods, and drugs to meet the immediate needs of the population. Plants are being used in TMs all over the world, which is either in crude or extract form, and represent the basis of primary health care for the foreseeable future [28]. The end of 20th century marked a renewed interest in TM. The resurgence of natural therapies was mainly due to the limitations of modern drugs to cure complex disorders and also the observation of their increasing side effects. Contemporary harvesting methods for medicinal plants are severely depleting these critical indigenous resources [134].

Maintenance of the availability of quality herbal raw materials on a sustainable basis is an yet unappreciated aspect of public health care. In order to achieve these goals for prospective health care, and refurbish the well-being of the Earth, a change in idea is necessary. Irrespective of their source, traditional medicine may be regarded as a sustainable commodity. Several approaches towards enhancing the accessibility of safe and efficacious plant-based medicinal agents include integrated strategies to get information on botany, chemistry, and biology for medicinal plants along with its quality control. Such integrated systems include information involving metabolomics, DNA barcoding, nanotechnology, biotechnology, the application of new detection techniques for in-field analysis of medicinal plants for better quality of safe and reproducible biological agents [135].

13.1 Sustainability in TM practice

Sustainability in TM is found in different sociological domains with different notions. Bioprospecting in general and as practiced in Ayurveda in respect to the usage of animals or plants could be the ultimate key, which can ensure a sustainable biodiversity for our future generations. Bioprospecting, which is defined as sector of pharmacological breakthroughs meant for human utilization conceives and includes, (i) cultivation (ii) the production of secondary metabolites in bioreactors and (iii) chemical synthesis of the compounds as the method to deal with the sustainability issue in regards to the substrates derived from living beings. Sustainability in traditional medical practice of ancient periods is relevant to current practice despite the absence of historical evidence. Theoretical assumptions may be regarded as the ascendants of novel researches in science. With this in mind and with empirical evidence of its efficacy existing information base should be relied upon to take the science of TM further [135]. Natural resources for TM are limited and are not rapidly renewable. Any unfamiliarity of this condition will be injurious to both TM and the global environment [3]. Ideas regarding sustainability is not an assumption and is based upon perception and developments; therefore, new ideas must be respected for the betterment of the global community.

As a consequence of expanding urbanization, rapid reduction in natural resources is leading to global warming and disrupted natural habitat causing a threat to the sustainability of traditional medicine. Traditional medicine being entirely dependent on natural resources like herbs, minerals and animal products, would go extinct if the heritage of an alternative way is not considered well in time, Ayurveda presents some unique sustainable

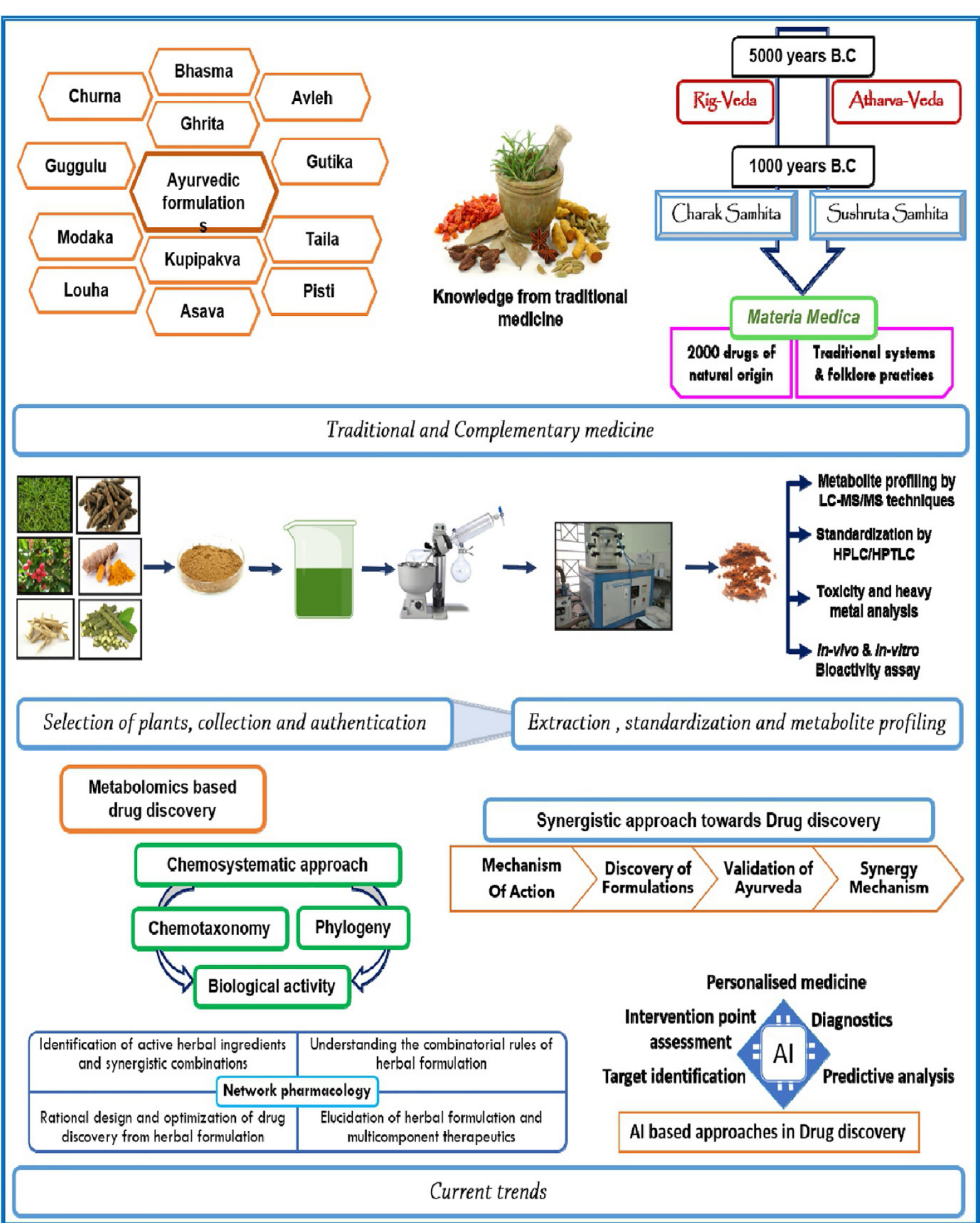


FIG. 9 Approaches for the development of medicinal plants: Tradition to trend.

models where animals and plants are used without posing any threat to their existence. In the current context, these ideas may give us a new insight to refine our outlook at natural resources used in traditional medicine to save natural resources which are facing a threat to their existence.

Sustainability in healthcare can be presented in Ayurveda at multiple levels such as preventive care, primary healthcare, resource utilization and novel applications which is an ecologically sustainable medicinal system. For past several decades, *Ayurveda* and other traditional

medical systems have faced the problem of non-availability of genuine raw material. Manufacturers of Ayurveda based medicines, have unsuccessfully tried to meet the demands by seeking alternatives for the conventional herbs or other ingredients, which are either not available or have lost their identity. Over exploitation, changes in global environment and loss of delicate ecosystems due to urbanization collectively resulted in convergence of gene pool, growth of species with poor endurance and consequent loss of such species has been increasingly seen. As the resources are not easily available in future TM will become more expensive. Producers of TM are going for mass production without considering intricacy of plant cultivation and their utilization, as described traditionally due to demand-oriented market pressures and poor quality checks in the TM. Ayurveda with regard to their utilization of natural products for human welfare in an eco- and bio-friendly way that takes care of the human health with an equal concern for the surrounding environment.

Legally Ayurvedic medicines can be manufactured under license from the formulae or the natural raw materials of plant, animal, mineral or marine origin mentioned in the authoritative books listed in a schedule of the Drugs and Cosmetics Act, 1940. Different kinds of formulations are accordingly manufactured and administered as Ayurvedic drugs. Considering their method of preparation, palatability, bioavailability and therapeutic values formulations are grouped in various dosage forms viz. *Avleh, churna, Asava, Bhasma, Ghrita, Taila, Kupipakva, Gutika, Guggulu Modaka, Louha, Pisti,* etc. as described in the Ayurvedic Formulary of India published by the Government. In persuasion of standardization of Ayurvedic drugs for the purpose of effective quality control, 265 standardized formulations from classical texts are published in four volumes of the National Ayurvedic Formulary and 645 monographs of quality standards of single drugs and 252 monographs of quality standards of multi-ingredient formulations are published in two parts of Ayurvedic Pharmacopoeia in 13 volumes. Pharmacopoeial standards of Ayurvedic drugs are developed on the basis of 12 assessment parameters of identity, purity and strength including confirmed identification, chemical constituents and permissible limits of heavy metals, pesticide residue, aflatoxins and microbial load. Similarly, in order to ensure supply of quality Ayurvedic medicines to the health facilities across the country, an Essential Drug List containing more than 250 medicines is published and the states are supported to procure such medicines for free public distribution to patients through dispensaries and other medical centers. The work of development and revision of standards of Ayurveda drugs is done under the supervision of the Pharmacopoeia Commission of Indian Medicine and Homeopathy and with the responsibility of the Ayurvedic Pharmacopoeia Committee of interdisciplinary experts. Various scientific laboratories and Pharmacopoeial Laboratory for Indian Medicine (PLIM), which is an appellate laboratory under the provisions of Drugs & Cosmetics rules, 1945, are engaged in the work of standardization and SOPs of Ayurvedic drugs using sophisticated equipment and analytical tools. Although significant achievements have been made by the existing pharmacopoeia set up, a unified pharmacopoeial infrastructure is intended for better coordination and outcomes. For this purpose, development of pharmacopoeial standards is proposed to be augmented through studies conducted by laboratories or institutions accredited by Government [136]. This effort will substantiate the existing pharmacopoeial standards of single and multi-ingredient drugs of plant, mineral, metal and animal origin [12]. Standard Operating Procedures (SOP) of manufacturing processes of formulations, assays, atlas of chromatography, Pharmacognosy atlas etc. are being appended to the quality standards of drugs to facilitate the testing procedures and estimation of marker compounds and phytochemical standard materials. Parameters like DNA barcoding or fingerprinting of medicinal plant materials are combined within the framework for quality control of Ayurvedic drugs. Drug Control Cell in the Ministry of AYUSH looks after regulatory and quality control matters of Ayurvedic drugs under the provisions of Drugs and Cosmetics Act, 1940 and rules there under. Drug Control Cell coordinates with the State Licensing Authorities, Drug Controllers and Drug Testing Laboratories for the purpose of enforcement of legal provisions of quality control of Ayurvedic and other traditional medicines. Amendment of regulatory provisions is a continuous process and it is taken up in accordance with the felt needs and emerging trends in the quality control of natural medicinal products. Lot of thrust has been given to check manufacturing companies for compliance to Good Manufacturing Practices, prescribed Shelf-life and evidence of safety and effectiveness of drugs. Government has sanctioned additional senior level posts of regulatory positions and steps taken to set up a vertical structure for AYUSH drugs in the Central Drug Standards Control Organization headed by Drugs Controller General. Financial support is provided to the states to strengthen infrastructural and functional capacities for production, testing and quality enforcement of Ayurvedic and other traditional medicines [128].

India is endowed with 15 agro-climatic zones and 17,000–18,000 species of flowering plants. Out of which 6000–7000 are estimated to have medicinal usage in folk and Ayurveda like documented systems of medicine. There are about 960 species of medicinal plants which are estimated to be in the trade. 178 species of them have annual consumption levels more than 100 metric tons. Cultivation and conservation of medicinal plants through

the development of sustainable management strategies is an absolute requirement. To address these issues the National Medicinal Plants Board (NMPB) under the Ministry of AYUSH was set-up in November 2000 by the Government of India. The board supports policies and programs for the growth of trade, export, conservation, and cultivation of medicinal plants. The NMPB provides support for survey, inventorization, in-situ conservation, ex-situ conservation, herbal gardens and linkage with joint forest management programs, research, and development, etc. This project aims at delivering Ayurvedic health care by medicinal plants to the rural and tribal population of India, at their doorstep, where the conventional modes of treatment don't reach. Department of Biotechnology and Department of Forests undertakes biodiversity conservation projects including cultivation, post-harvest processing and storage of medicinal herbs through various assistance. Threatened, endangered, vulnerable medicinal plants species are being protected in different ways. Restrictions are enforced for rampant deforestation for the collection of raw materials of medicinal value from the wild sources. The Ministry of AYUSH has been focused towards implementing promotional programs of information, education and communication (IEC) to develop awareness among the masses about the efficacy of Ayurveda, their cost effectiveness and dissemination of proven results on research and development work. The AYUSH Ministry provides funding for the organization of national and state level Arogya Fairs. Health fairs or melas or exhibitions organized by government departments, state governments and other reputed organizations and participations therein. Promotional incentives are provided to the Ayurveda industry to participate in Arogya and other fairs/melas/exhibitions/conferences/seminars etc.

The market demand for Ayurvedic medicine has been part of India's socio- cultural heritage and the market is increasing steadily, the world over. Although the Ayurvedic industry is the most traditional form of industry, the emerging market opportunities remain underexploited. In order to address that problem initiative were taken up by the Department of Science & Technology to start the Drugs & Pharmaceuticals Research Programme (DPRP) in 1994–95 for promotion of industry-institutional collaboration in the development of new drugs in pharmaceutical sector. Pharmacy courses like B. Pharm (Ayurveda) and M. Pharm (Ayurveda) in different parts of the country. Emphasis has been laid on Ayurveda in some specific development of herbal drugs. Research regarding process validation and biological evaluation of formulations is made a major thrust area. Pharmaceutical Export Promotion Council (Pharmexcil) constituted a National committee in the field of Ayurvedic medicines to guide the industry to march ahead with the basic objective of promoting exports to developed countries.

14 International harmonization

To ensure homogeny of quality, safety, and efficacy of the herbal medicines across countries, harmonizing efforts have been initiated on pharmacopoeial specifications, standardization, and classification of herbal drugs. Different specifications have been found in pharmacopoeias of Korea, Japan, and China for a single herbal medicine. The same crude plant material may be described, but the family or species of the original plant may be different [137]. The Western Pacific Regional Forum for the Harmonization of herbal medicine tried to harmonize the crude drug monographs in the pharmacopoeias of six Asian countries (Japan, China, Korea, Singapore, Vietnam, and Hong Kong) in order to help in promoting commercialization of safe and effective herbal drugs across countries. Harmonization process and herbal product registration were initiated in 2000 among different countries. International harmonization would help in developing evidence-based clinical practice guidelines on TM. India has nearly 8000 herbal drug companies, among which about 5000 companies have GMP-compliant manufacturing units, and most of them are of small and medium size. Seventy percent of the Indian exports from the herbal sector consist of only raw materials, and 30% consist of finished products including herbal extracts. There are 55 major herbal drugs exporting companies in India [29]. As discussed in the monographs of the American Herbal Pharmacopoeia, the use of single or multiple chemical markers was important for quality control. Protocols and guidance documents on safety and toxicity testing of TMs have been issued by the International Life Sciences Institute, the Institute of Medicine, National Research Council (2004), the Union of Pure and Applied Chemistry, the EMEA (e.g., EMEA, 2009) [138], and by the European Food Safety Authority (EFSA, 2009) [139]. These regulation documents tell us about the assessment of the safety, efficacy, and quality of herbs for food and medicine purposes. Standards for medicinal plants are being developed worldwide, but as yet there is no consensus as to how these should be adopted. There are several publications: US Pharmacopoeia, British Herbal Compendium, British Herbal Pharmacopoeia, Chinese Pharmacopoeia, and Physician's Desk Reference for herbal medicines, Ayurvedic Pharmacopoeia of India have monographs for herbal raw materials [25]. For a single plant, the monograph may vary in different publications, different country standards with respect to a single formulation, which creates difficulties for manufacturers in herbal drug trade. Thus, the need to establish global regulatory mechanisms for regulating herbal drugs seems obvious. An improvement in the processes of regulation and global harmonization is desirable and necessary, which combines scientific data and traditional knowledge [35]. Several challenges and issues on

promotion and development of herbal drugs have been identified, which should be solved through international coordination and collaboration Therefore, there is a need for coordination and harmonization of research and development of medicinal plants as both pharmaceuticals and food supplements.

15 Conclusion

The existing knowledge of herbal medicines needs to be validated and documented through recent regulatory guidelines of quality control, standardization and manufacturing process. Chemical consistency at all stages of manufacturing processes such as extraction, standardization, quality control, quality assurance, stability, shelf-life and purity is of utmost importance to ensure medicinal efficacy and safety. Considering the widespread use and popularity of herbal medicine, proper standardization and validation methods have been developed for promotion of natural products. Medicinal plants are most commonly used in traditional medicines and can potentially influence the bioavailability and pharmacokinetics of some pharmaceuticals by affecting CYP450 drug metabolism. Thus, the bioavailability of conventional medicines can be changed, which leads to therapeutic failure or increased drug concentrations and may cause serious adverse events. Such studies may be helpful for the safety profile evaluation of botanicals. Documentation of their safety profiles and pharmacovigilance study are pioneer areas to develop the standard procedures for herbal products. Evidence based submissions for regulatory approval and interlinking of various pharmacopoeial and monographs would be helpful for the herbal manufacturers to regulate markets across the world. Research through collaboration and cooperation across the nation can help to a high extent in the promotion and development of traditional medicine for the betterment of healthcare globally. This would develop a system to bring representatives together to discuss the global issues and implications in new strategic terms, with a new set of goals, a new agenda, but most importantly, a new vigor is vital for the global development. The different systematic approach of traditional medicines is not about a single science or technique but also amalgamation of these concomitant areas, which are mutually interrelated.

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References

[1] Tistaert C, Dejaegher B, Vander HY. Chromatographic separation techniques and data handling methods for herbal fingerprints: a review. Anal Chim Acta 2011;690:148–61. https://doi.org/10.1016/j.aca.2011.02.023.

[2] Mukherjee PK. Ethnopharmacology and ethnomedicine-inspired drug development. In: Mukherjee PK, editor. Quality control and evaluation of herbal drugs. Elsevier; 2019. p. 29–51.

[3] Mukherjee PK, Banerjee S, Kar A, Chanda J. Drugs from our ancestors: tradition to innovation. In: Sen S, Chakraborty R, editors. Herbal medicine in India: indigenous knowledge, practice, innovation and its value. Singapore: Springer; 2020. p. 263–80. https://doi.org/10.1007/978-981-13-7248-3_19.

[4] Zhang J, Wider B, Shang H, Li X, Ernst E. Quality of herbal medicines: challenges and solutions. Complement Ther Med 2012;20:100–6. https://doi.org/10.1016/j.ctim.2011.09.004.

[5] Mukherjee PK, Sahoo AK, Narayanan N, Kumar NS, Ponnusankar S. Lead finding from medicinal plants with hepatoprotective potentials. Expert Opin Drug Discovery 2009;4:545–76. https://doi.org/10.1517/17460440902911433.

[6] Shinde VM, Dhalwal K, Potdar M, Mahadik KR. Application of quality control principles to herbal drugs. Int J Phytomedicine 2009;1.

[7] Booker A, Johnston D, Heinrich M. Value chains of herbal medicines—research needs and key challenges in the context of ethnopharmacology. J Ethnopharmacol 2012;140:624–33. https://doi.org/10.1016/j.jep.2012.01.039.

[8] Weathers PJ, Arsenault PR, Covello PS, McMickle A, Teoh KH, Reed DW. Artemisinin production in Artemisia annua: studies in planta and results of a novel delivery method for treating malaria and other neglected diseases. Phytochem Rev 2011;10:173–83. https://doi.org/10.1007/s11101-010-9166-0.

[9] Choi DW, Kim JH, Cho SY, Kim DH, Chang SY. Regulation and quality control of herbal drugs in Korea. Toxicology 2002;181–182:581–6. https://doi.org/10.1016/s0300-483x(02)00487-0.

[10] Liang Y-Z, Xie P, Chan K. Quality control of herbal medicines. J Chromatogr B Analyt Technol Biomed Life Sci 2004;812:53–70. https://doi.org/10.1016/j.jchromb.2004.08.041.

[11] Patwardhan B, Mashelkar RA. Traditional medicine-inspired approaches to drug discovery: can Ayurveda show the way forward? Drug Discov Today 2009;14:804–11. https://doi.org/10.1016/j.drudis.2009.05.009.

[12] Pulok M, Venkatesh P, Venkatesh M, Ponnusankar S, Khan M. Strategies for revitalization of traditional medicine. Chin Herb Med 2010;2:1–15. https://doi.org/10.3969/j.issn.1674-6384.2010.01.001.

[13] Mukherjee P. Exploring botanicals in Indian system of medicine—regulatory perspectives. Clin Res Regul Aff 2003;20:249–64. https://doi.org/10.1081/CRP-120023840.

[14] Mukherjee P. Evaluation of Indian traditional medicine. Drug Inf J 2001;35:623–32.

[15] Mukherjee P, Ponnusankar S, Murugan V. Ethno medicine in complementary therapeutics. In: Chattopadhyay D, editor. Ethanomedicine: a source of complementary therapeutics. Trivandrum: Research Signpost; 2010. p. 29–52.

[16] Nema N, Dalai M, Mukherjee P. Ayush herbs and status quo in herbal industries. The pharma review. New Delhi: Kongposh Publications; 2011. p. 141.

[17] Mukherjee PK, Houghton PJ, editors. Evaluation of herbal medicinal products: perspectives on quality, safety, and efficacy. London ; Chicago: Pharmaceutical Press; 2009.

[18] Mukherjee PK. Quality control of herbal drugs: an approach to evaluation of botanicals. 1st ed. India: Business Horizons; 2002.

[19] Mukherjee PK, Venkatesh M, Gantait A. Ayurveda in modern medicine: development and modification of bioactivity. In: Liu

H-W(B), Mander L, editors. Comprehensive natural products II. Oxford: Elsevier; 2009. p. 479–507. https://doi.org/10.1016/B978-008045382-8.00655-9.

[20] Mukherjee P, Bahadur S, Harwansh R, Nema N, Bhadra S. Development of traditional medicines: globalizing local knowledge or localizing global technologies. Pharma Times 2013;45:39–42.

[21] Mukherjee PK, Wahile A. Integrated approaches towards drug development from Ayurveda and other Indian system of medicines. J Ethnopharmacol 2006;103:25–35. https://doi.org/10.1016/j.jep.2005.09.024.

[22] Mukherjee P, Sahu M, Suresh B. Indian herbal medicines. Eastern Pharmacist 1998;42:21–4.

[23] Mukherjee P, Saha B. Quest for GMP for the production of quality botanicals. In: Mukherjee P, Verpoorte R, editors. GMP for botanicalsdregulatory and quality issues on phytomedicine. New Delhi, India: Eastern Publishers, Business Horizons Ltd; 2003. p. 165–90.

[24] Mukherjee PK, Nema NK, Venkatesh P, Debnath PK. Changing scenario for promotion and development of Ayurveda—way forward. J Ethnopharmacol 2012;143:424–34. https://doi.org/10.1016/j.jep.2012.07.036.

[25] Mukherjee PK, Maiti K, Mukherjee K, Houghton PJ. Leads from Indian medicinal plants with hypoglycemic potentials. J Ethnopharmacol 2006;106:1–28. https://doi.org/10.1016/j.jep.2006.03.021.

[26] Bilello JA. The agony and ecstasy of "OMIC" technologies in drug development. Curr Mol Med 2005;5:39–52. https://doi.org/10.2174/1566524053152898.

[27] Sahoo N, Manchikanti P, Dey S. Herbal drugs: standards and regulation. Fitoterapia 2010;81:462–71. https://doi.org/10.1016/j.fitote.2010.02.001.

[28] Cordell GA. Phytochemistry and traditional medicine—a revolution in process. Phytochem Lett 2011;4:391–8. https://doi.org/10.1016/j.phytol.2011.05.005.

[29] Cordell GA, Colvard MD. Natural products and traditional medicine: turning on a paradigm. J Nat Prod 2012;75:514–25. https://doi.org/10.1021/np200803m.

[30] Mukherjee PK, Rai S, Kumar V, Mukherjee K, Hylands P, Hider R. Plants of Indian origin in drug discovery. Expert Opin Drug Discov 2007;2:633–57. https://doi.org/10.1517/17460441.2.5.633.

[31] Guo R, Canter PH, Ernst E. A systematic review of randomised clinical trials of individualised herbal medicine in any indication. Postgrad Med J 2007;83:633–7. https://doi.org/10.1136/pgmj.2007.060202.

[32] Homma M, Oka K, Yamada T, Niitsuma T, Ihto H, Takahashi N. A strategy for discovering biologically active compounds with high probability in traditional Chinese herb remedies: an application of saiboku-to in bronchial asthma. Anal Biochem 1992;202:179–87. https://doi.org/10.1016/0003-2697(92)90225-v.

[33] Mukherjee PK, Kumar V, Mal M, Houghton PJ. In vitro acetylcholinesterase inhibitory activity of the essential oil from Acorus calamus and its main constituents. Planta Med 2007;73:283–5. https://doi.org/10.1055/s-2007-967114.

[34] Mukherjee PK, Verpoorte R. GMP for botanicals: regulatory and quality issues on phytomedicines. New Delhi: Business Horizons; 2003.

[35] Mukherjee P, Houghton P. The worldwide phenomenon of increased use of herbal products: opportunity and threats. In: Mukherjee P, Houghton PJ, editors. Evaluation of herbal medicinal productsdperspectives on quality, safety and efficacy. Pharmaceutical Press, Royal Pharmaceutical Society of Great Britain; 2009. p. 3–12.

[36] Cragg GM, Schepartz SA, Suffness M, Grever MR. The taxol supply crisis. new NCI policies for handling the large-scale production of novel natural product anticancer and anti-HIV agents. J Nat Prod 1993;56:1657–68. https://doi.org/10.1021/np50100a001.

[37] Sharma R, Arora R. Herbal drugs—a twenty first century perspective. vol. 141. New Delhi: Jaypee Brothers, Medical Publishers (P) Ltd; 2006.

[38] Mukherjee P, Wahile A. Perspectives of safety for natural health products. In: Sharma R, Arora R, editors. Herbal drugs: a twenty first century perspective. New Delhi: Jaypee Brothers Medicinal Publishers; 2006. p. 50–9.

[39] Verpoorte R, Choi YH, Kim HK. Ethnopharmacology and systems biology: a perfect holistic match. J Ethnopharmacol 2005;100:53–6. https://doi.org/10.1016/j.jep.2005.05.033.

[40] Mukherjee PK, Ponnusankar S, Venkatesh P, Gantait A, Pal BC. Marker profiling: an approach for quality evaluation of Indian medicinal plants. Ther Innov Regul Sci 2011;45:1–14. https://doi.org/10.1177/009286151104500101.

[41] Evans S. Changing the knowledge base in western herbal medicine. Soc Sci Med 2008;67:2098–106. https://doi.org/10.1016/j.socscimed.2008.09.046.

[42] Ulrich-Merzenich G, Zeitler H, Jobst D, Panek D, Vetter H, Wagner H. Application of the "-Omic-" technologies in phytomedicine. Phytomedicine 2007;14:70–82. https://doi.org/10.1016/j.phymed.2006.11.011.

[43] Tyler VE. Phytomedicines: back to the future. J Nat Prod 1999;62:1589–92. https://doi.org/10.1021/np9904049.

[44] Venkatesh P, Mukherjee PK, Kumar NS, Bandyopadhyay A, Fukui H, Mizuguchi H, et al. Anti-allergic activity of standardized extract of Albizia lebbeck with reference to catechin as a phytomarker. Immunopharmacol Immunotoxicol 2010;32:272–6. https://doi.org/10.3109/08923970903305481.

[45] Mukherjee P, Wahile A, Venkatesan K, Rai S, Saha B. Marker profiling of botanicals used for hepatoprotection in Indian system of medicine. Drug Inf J 2006;40:131–9. https://doi.org/10.1177/009286150604000202.

[46] Raja S, Ahamed H, Venkatesan K, Bandyopadhyay A, Mukherjee P. Exploring the effect of Asclepias curassavica on markers of oxidative stress in rats. Evid Based Integr Med 2005;2:87–93. https://doi.org/10.2165/01197065-200502020-00004.

[47] Organization WH. Quality control methods for medicinal plant materials. World Health Organization; 1998.

[48] Organization WH. Guidelines for assessing quality of herbal medicines with reference to contaminants and residues; 2007.

[49] Xue J, Liu D, Chen S, Liao Y, Zou Z. Overview on external contamination sources in traditional Chinese medicines. World Sci Technol 2008;10:91–6. https://doi.org/10.1016/S1876-3553(09)60001-6.

[50] WHO. Guidelines on good agricultural and collection practices (GACP) for medicinal plants; 2003.

[51] Organization WH. Plants WC on SM, WHO consultation on selected medicinal plants (2nd : 1999 : Ravello-Salerno I), WHO consultation on selected medicinal plants (3rd : 2001 : Ottawa O), WHO consultation on selected medicinal plants (4th : 2005 : Salerno-Paestum I). WHO monographs on selected medicinal plants; 2006.

[52] Bent S, Ko R. Commonly used herbal medicines in the United States: a review. Am J Med 2004;116:478–85. https://doi.org/10.1016/j.amjmed.2003.10.036.

[53] Anonymous. Markers and natural health products. vol. 3; 2006. p. 2–4.

[54] Mukherjee P, Satheesh Kumar N, Heinrich M. Plant made pharmaceuticals (PMPs)—development of natural health products from bio-diversity. Indian J Pharm Educ Res 2008;42:113–21.

[55] Anon. Ayurvedic Pharmacopoeia of India. vol. III. Part I. Ministry of health and family welfare, department of ISM and H; 2001.

[56] Li S, Han Q, Qiao C, Song J, Lung Cheng C, Xu H. Chemical markers for the quality control of herbal medicines: an overview. Chin Med 2008;3:7. https://doi.org/10.1186/1749-8546-3-7.

[57] Choudhary N, Sekhon BS. An overview of advances in the standardization of herbal drugs. J Pharm Educ Res 2011;2:16.
[58] Mukherjee P, Rai S, Bhattacharyya S, Wahile A, Saha B. Marker analysis of polyherbal formulation, Triphala—a well known Indian traditional medicine. Indian J Tradit Know 2008;7:379–83.
[59] Song J-Z, Li S-L, Zhou Y, Qiao C-F, Chen S-L, Xu H-X. A novel approach to rapidly explore analytical markers for quality control of Radix Salviae Miltiorrhizae extract granules by robust principal component analysis with ultra-high performance liquid chromatography-ultraviolet-quadrupole time-of-flight mass spectrometry. J Pharm Biomed Anal 2010;53:279–86. https://doi.org/10.1016/j.jpba.2010.03.022.
[60] Liu Y, Shi X-W, Liu E-H, Sheng L-S, Qi L-W, Li P. More accurate matrix-matched quantification using standard superposition method for herbal medicines. J Chromatogr A 2012;1254:43–50. https://doi.org/10.1016/j.chroma.2012.07.020.
[61] Guliyev VB, Gul M, Yildirim A. Hippophae rhamnoides L.: chromatographic methods to determine chemical composition, use in traditional medicine and pharmacological effects. J Chromatogr B Analyt Technol Biomed Life Sci 2004;812:291–307. https://doi.org/10.1016/j.jchromb.2004.08.047.
[62] Mukherjee PK. Problems and prospects for good manufacturing practice for herbal drugs in Indian systems of medicine. Ther Innov Regul Sci 2002;36:635–44. https://doi.org/10.1177/009286150203600318.
[63] Gantait A, Pandit S, Nema NK, Mukjerjee PK. Quantification of glycyrrhizin in Glycyrrhiza glabra extract by validated HPTLC densitometry. J AOAC Int 2010;93:492–5.
[64] Pulok K, Bahadur S, Harwansh R, Chaudhary S. Shifting paradigm for validation of medicinal plants in indian traditional medicine. Indian Drugs 2014;51:5–14.
[65] Nag M, Mukherjee PK, Chanda J, Biswas R, Harwansh RK, Al-dhabi NA, et al. Plant developed analytical profile of Stereospermum suaveolens in Indian traditional knowledge. Indian J Tradit Know 2015;14(4):590.
[66] Chanda J, Biswas S, Kar A, Mukherjee PK. Determination of cucurbitacin E in some selected herbs of ayurvedic importance through RP-HPLC. J Ayurveda Integr Med 2020;11:287–93. https://doi.org/10.1016/j.jaim.2019.01.002.
[67] Sharma A, Katiyar CK, Banerjee S, Chanda J, Kar A, Biswas S, et al. RP-HPLC and HPTLC methods for analysis of selected herbs used as complexion promoters in Ayurveda and Unani systems of medicine. J AOAC Int 2020;103:692–8. https://doi.org/10.5740/jaoacint.19-0290.
[68] Biswas S, Harwansh RK, Kar A, Mukherjee PK. Validated high-performance thin-layer chromatographic method for the simultaneous determination of quercetin, rutin, and gallic acid in Amaranthus tricolor L. JPC-J Planar Chromat 2019;32:121–6. https://doi.org/10.1556/1006.2019.32.2.7.
[69] Biswas S, Mukherjee PK. Validated high-performance thin-layer chromatographic–densitometric method for the isolation and standardization of ayapanin in Ayapana triplinervis. JPC-J Planar Chromat-Modern TLC 2019;32:41–6. https://doi.org/10.1556/1006.2019.32.1.5.
[70] Biswas R, Chanda J, Kar A, Mukherjee PK. Tyrosinase inhibitory mechanism of betulinic acid from Dillenia indica. Food Chem 2017;232:689–96. https://doi.org/10.1016/j.foodchem.2017.04.008.
[71] Ahmmed SM, Mukherjee PK, Bahadur S, Harwansh RK, Kar A, Bandyopadhyay A, et al. CYP450 mediated inhibition potential of Swertia chirata: an herb from Indian traditional medicine. J Ethnopharmacol 2016;178:34–9. https://doi.org/10.1016/j.jep.2015.11.046.
[72] Kar A, Pandit S, Mukherjee K, Bahadur S, Mukherjee PK. Safety assessment of selected medicinal food plants used in Ayurveda through CYP450 enzyme inhibition study. J Sci Food Agric 2017;97:333–40. https://doi.org/10.1002/jsfa.7739.
[73] Bahadur S, Mukherjee PK, Milan Ahmmed SK, Kar A, Harwansh RK, Pandit S. Metabolism-mediated interaction potential of standardized extract of Tinospora cordifolia through rat and human liver microsomes. Indian J Pharmacol 2016;48:576–81. https://doi.org/10.4103/0253-7613.190758.
[74] Ahmmed SM, Mukherjee PK, Bahadur S, Kar A, Mukherjee K, Karmakar S, et al. Interaction potential of Trigonella foenum graceum through cytochrome P 450 mediated inhibition. Indian J Pharmacol 2015;47:530–4. https://doi.org/10.4103/0253-7613.165179.
[75] Busse W. The significance of quality for efficacy and safety of herbal medicinal products. Ther Innov Regul Sci 2000;34:15–23. https://doi.org/10.1177/009286150003400102.
[76] Srinivasan VS. Challenges and scientific issues in the standardization of botanicals and their preparations. United States Pharmacopeia's dietary supplement verification program—a public health program. Life Sci 2006;78:2039–43. https://doi.org/10.1016/j.lfs.2005.12.014.
[77] Kayne S. Problems in the pharmacovigilance of herbal medicines in the UK. Pharm J 2006;276:543–5.
[78] Huang S-M, Hall SD, Watkins P, Love LA, Serabjit-Singh C, Betz JM, et al. Drug interactions with herbal products and grapefruit juice: a conference report. Clin Pharmacol Ther 2004;75:1–12. https://doi.org/10.1016/j.clpt.2003.07.002.
[79] Organization WH. WHO guidelines on safety monitoring of herbal medicines in pharmacovigilance systems. World Health Organization; 2004.
[80] Bauer S, Störmer E, Johne A, Krüger H, Budde K, Neumayer H-H, et al. Alterations in cyclosporin A pharmacokinetics and metabolism during treatment with St John's wort in renal transplant patients. Br J Clin Pharmacol 2003;55:203–11. https://doi.org/10.1046/j.1365-2125.2003.01759.x.
[81] Ponnusankar S, Venkatesh P, Venkatesh M, Mandal SC, Mukherjee PK. Herbal drugs require pharmacovigilance study-need and reality. Pharma Rev 2007;12:113–20.
[82] Zhou S-F, Zhou Z-W, Li C-G, Chen X, Yu X, Xue CC, et al. Identification of drugs that interact with herbs in drug development. Drug Discov Today 2007;12:664–73. https://doi.org/10.1016/j.drudis.2007.06.004.
[83] Barnes J. Pharmacovigilance of herbal medicines: a UK perspective. Drug Saf 2003;26:829–51. https://doi.org/10.2165/00002018-200326120-00001.
[84] Isnard Bagnis C, Deray G, Baumelou A, Le Quintrec M, Vanherweghem JL. Herbs and the kidney. Am J Kidney Dis 2004;44:1–11. https://doi.org/10.1053/j.ajkd.2004.02.009.
[85] Mukherjee P, Ponnusankar S, Bhadra S, Pandit S, Venkatesh M. Confluence of strategies for the development of botanicals. Pharm Rev 2008;12.
[86] Larrey D, Pageaux G-P. Drug-induced acute liver failure. Eur J Gastroenterol Hepatol 2005;17:141–3. https://doi.org/10.1097/00042737-200502000-00002.
[87] Mukherjee PK, Ponnusankar S, Pandit S, Hazam PK, Ahmmed M, Mukherjee K. Botanicals as medicinal food and their effects on drug metabolizing enzymes. Food Chem Toxicol 2011;49:3142–53. https://doi.org/10.1016/j.fct.2011.09.015.
[88] Lynch T, Price A. The effect of cytochrome P450 metabolism on drug response, interactions, and adverse effects. Am Fam Physician 2007;76:391–6.
[89] Zhou S, Gao Y, Jiang W, Huang M, Xu A, Paxton JW. Interactions of herbs with cytochrome P450. Drug Metab Rev 2003;35:35–98. https://doi.org/10.1081/dmr-120018248.
[90] Crespi CL, Penman BW. Use of cDNA-expressed human cytochrome P450 enzymes to study potential drug-drug interactions. Adv Pharmacol 1997;43:171–88. https://doi.org/10.1016/s1054-3589(08)60205-7.
[91] Tsukamoto S, Aburatani M, Ohta T. Isolation of CYP3A4 inhibitors from the black cohosh (Cimicifuga racemosa). Evid Based Complement Alternat Med 2005;2:223–6. https://doi.org/10.1093/ecam/neh086.

[92] Frank A, Unger M. Analysis of frankincense from various Boswellia species with inhibitory activity on human drug metabolising cytochrome P450 enzymes using liquid chromatography mass spectrometry after automated on-line extraction. J Chromatogr A 2006;1112:255–62. https://doi.org/10.1016/j.chroma.2005.11.116.

[93] Kang JJ, Wang HW, Liu TY, Chen YC, Ueng TH. Modulation of cytochrome P-450-dependent monooxygenases, glutathione and glutathione S-transferase in rat liver by geniposide from Gardenia jasminoides. Food Chem Toxicol 1997;35:957–65. https://doi.org/10.1016/s0278-6915(97)87265-1.

[94] Ganzera M, Schneider P, Stuppner H. Inhibitory effects of the essential oil of chamomile (Matricaria recutita L.) and its major constituents on human cytochrome P450 enzymes. Life Sci 2006;78:856–61. https://doi.org/10.1016/j.lfs.2005.05.095.

[95] Hansen TS, Nilsen OG. In vitro CYP3A4 metabolism: inhibition by Echinacea purpurea and choice of substrate for the evaluation of herbal inhibition. Basic Clin Pharmacol Toxicol 2008;103:445–9. https://doi.org/10.1111/j.1742-7843.2008.00307.x.

[96] Bent S, Goldberg H, Padula A, Avins AL. Spontaneous bleeding associated with ginkgo biloba: a case report and systematic review of the literature: a case report and systematic review of the literature. J Gen Intern Med 2005;20:657–61. https://doi.org/10.1111/j.1525-1497.2005.0121.x.

[97] Greenblatt DJ, von Moltke LL, Luo Y, Perloff ES, Horan KA, Bruce A, et al. Ginkgo biloba does not alter clearance of flurbiprofen, a cytochrome P450-2C9 substrate. J Clin Pharmacol 2006;46:214–21. https://doi.org/10.1177/0091270005283465.

[98] Anke J, Ramzan I. Pharmacokinetic and pharmacodynamic drug interactions with kava (Piper methysticum Forst. F.). J Ethnopharmacol 2004;93:153–60. https://doi.org/10.1016/j.jep.2004.04.009.

[99] Zou L, Harkey MR, Henderson GL. Effects of herbal components on cDNA-expressed cytochrome P450 enzyme catalytic activity. Life Sci 2002;71:1579–89. https://doi.org/10.1016/s0024-3205(02)01913-6.

[100] Guerra MC, Speroni E, Broccoli M, Cangini M, Pasini P, Minghett A, et al. Comparison between chinese medical herb Pueraria lobata crude extract and its main isoflavone puerarin antioxidant properties and effects on rat liver CYP-catalysed drug metabolism. Life Sci 2000;67:2997–3006. https://doi.org/10.1016/s0024-3205(00)00885-7.

[101] Hari Kumar KB, Kuttan R. Inhibition of drug metabolizing enzymes (cytochrome P450) in vitro as well as in vivo by Phyllanthus amarus SCHUM & THONN. Biol Pharm Bull 2006;29:1310–3. https://doi.org/10.1248/bpb.29.1310.

[102] Peng CC, Glassman PA, Trilli LE, Hayes-Hunter J, Good CB. Incidence and severity of potential drug-dietary supplement interactions in primary care patients: an exploratory study of 2 outpatient practices. Arch Intern Med 2004;164:630–6. https://doi.org/10.1001/archinte.164.6.630.

[103] Ponnusankar S, Pandit S, Babu R, Bandyopadhyay A, Mukherjee PK. Cytochrome P450 inhibitory potential of Triphala—a Rasayana from Ayurveda. J Ethnopharmacol 2011;133:120–5. https://doi.org/10.1016/j.jep.2010.09.022.

[104] Harwansh RK, Mukherjee K, Bhadra S, Kar A, Bahadur S, Mitra A, et al. Cytochrome P450 inhibitory potential and RP-HPLC standardization of trikatu—a Rasayana from Indian Ayurveda. J Ethnopharmacol 2014;153:674–81. https://doi.org/10.1016/j.jep.2014.03.023.

[105] Pandit S, Mukherjee PK, Mukherjee K, Gajbhiye R, Venkatesh M, Ponnusankar S, et al. Cytochrome P450 inhibitory potential of selected Indian spices—possible food drug interaction. Food Res Int 2012;45:69–74. https://doi.org/10.1016/j.foodres.2011.08.021.

[106] Pandit S, Ponnusankar S, Bandyopadhyay A, Ota S, Mukherjee PK. Exploring the possible metabolism mediated interaction of Glycyrrhiza glabra extract with CYP3A4 and CYP2D6. Phytother Res 2011;25:1429–34. https://doi.org/10.1002/ptr.3426.

[107] Kar A, Mukherjee PK, Saha S, Banerjee S, Goswami D, Matsabisa MG, et al. Metabolite profiling and evaluation of CYP450 interaction potential of 'Trimada'—an Ayurvedic formulation. J Ethnopharmacol 2021;266. https://doi.org/10.1016/j.jep.2020.113457, 113457.

[108] Bahadur S, Mukherjee PK, Pandit S, Ahmmed SKM, Kar A. Herb–drug interaction potential of Berberis aristata through cytochrome P450 inhibition assay. Synergy 2017;4:1–7. https://doi.org/10.1016/j.synres.2016.12.001.

[109] Bahadur S, Mukherjee K, Kar A, Ahmmed SM, Mukherjee PK, Al-Dhabi NA, et al. Evaluation of food-drug interaction potential of *Aegle marmelos* (L) Corrêa through metabolism mediated cytochrome P450 inhibition assay. vol. 14; 2015. p. 7.

[110] Kar A, Mukherjee PK, Saha S, Bahadur S, Ahmmed SM, Pandit S. Possible herb-drug interaction of *Morus alba* L- a potential antidiabetic plant from Indian Traditional medicine. vol. 14; 2015. p. 6.

[111] Ahmmed SM, Mukherjee P, Bahadur S, Kar A, Al-Dhabi N, Duraipandiyan V. Inhibition potential of Moringa oleifera lam. On drug metabolizing enzymes. Undefined; 2015.

[112] De Smet P. Health risks of herbal remedies: an update. Clin Pharmacol Ther 2004;76:1–17. https://doi.org/10.1016/j.clpt.2004.03.005.

[113] Colalto C. Herbal interactions on absorption of drugs: mechanisms of action and clinical risk assessment. Pharmacol Res 2010;62:207–27. https://doi.org/10.1016/j.phrs.2010.04.001.

[114] Anonymous. Directive 2004/24/EC of the European Parliament and of the Council of 31 March 2004, Amending, as regards traditional herbal medicinal products, directive 2001/83/EC on the community code relating to medicinal products for human use. Off J Eur Union 2009;11:85–90. https://doi.org/10.3233/PPL-2009-0236.

[115] Barnes J. A close look at synergy and polyvalent action in medicinal plants. Inpharma Wkly 1999;1185:3–4. https://doi.org/10.2165/00128413-199911850-00004.

[116] Wagner H. New targets in the phytopharmacology of plants. In: Herbal medicine, a concise overview for health care professionals. Butterworth-Heinemann; 1999. p. 34–42.

[117] Kitano H. Computational systems biology. Nature 2002;420:206–10. https://doi.org/10.1038/nature01254.

[118] Kell DB. Systems biology, metabolic modelling and metabolomics in drug discovery and development. Drug Discov Today 2006;11:1085–92. https://doi.org/10.1016/j.drudis.2006.10.004.

[119] Li P, Yang L-P. Application of systems biology method in the research of traditional Chinese medicine. Zhong Xi Yi Jie He Xue Bao 2008;6:454–7. https://doi.org/10.3736/jcim20080504.

[120] Li F, Gonzalez FJ, Ma X. LC–MS-based metabolomics in profiling of drug metabolism and bioactivation. Acta Pharm Sin B 2012;2:118–25. https://doi.org/10.1016/j.apsb.2012.02.010.

[121] Hood L, Perlmutter RM. The impact of systems approaches on biological problems in drug discovery. Nat Biotechnol 2004;22:1215–7. https://doi.org/10.1038/nbt1004-1215.

[122] Holmes E, Nicholls AW, Lindon JC, Connor SC, Connelly JC, Haselden JN, et al. Chemometric models for toxicity classification based on NMR spectra of biofluids. Chem Res Toxicol 2000;13:471–8. https://doi.org/10.1021/tx990210t.

[123] Choi YH, Choi H-K, Hazekamp A, Bermejo P, Schilder Y, Erkelens C, et al. Quantitative analysis of bilobalide and ginkgolides from Ginkgo biloba leaves and Ginkgo products using (1) H-NMR. Chem Pharm Bull (Tokyo) 2003;51:158–61. https://doi.org/10.1248/cpb.51.158.

[124] Choi YH, Kim HK, Hazekamp A, Erkelens C, Lefeber AWM, Verpoorte R. Metabolomic differentiation of Cannabis sativa cultivars using 1H NMR spectroscopy and principal component analysis. J Nat Prod 2004;67:953–7. https://doi.org/10.1021/np049919c.

[125] Mukherjee PK. Plant metabolomics and quality evaluation of herbal drugs. In: Mukherjee PK, editor. Quality control and evaluation of herbal drugs. Elsevier; 2019. p. 629–53 [chapter 17].

[126] Mukherjee P, Harwansh R, Bahadur S, Biswas S, Kuchibhatla LN, Tetali S, et al. Metabolomics of medicinal plants—a versatile tool for standardization of herbal products and quality evaluation of ayurvedic formulations; 2016. https://doi.org/10.18520/CS/V111/I10/1624-1630.

[127] Park H-W, In G, Kim J-H, Cho B-G, Han G-H, Chang I-M. Metabolomic approach for discrimination of processed ginseng genus (Panax ginseng and Panax quinquefolius) using UPLC-QTOF MS. J Ginseng Res 2014;38:59-65. https://doi.org/10.1016/j.jgr.2013.11.011.

[128] Mukherjee PK, Harwansh RK, Bahadur S, Banerjee S, Kar A, Chanda J, et al. Development of Ayurveda—tradition to trend. J Ethnopharmacol 2017;197:10–24. https://doi.org/10.1016/j.jep.2016.09.024.

[129] Mukherjee PK. Evidence based validation of traditional medicine—farm to pharma. Planta Med 2015;81:OA40. https://doi.org/10.1055/s-0035-1545122.

[130] Mukherjee PK. Safety related quality issues—approach for evaluation of herbal medicinal plants through marker analysis. Eur J Integr Med 2014;6:696. https://doi.org/10.1016/j.eujim.2014.09.026.

[131] Mukherjee PK, Banerjee S, Katiyar CK, Sharma S, Chattopadhyay N. Traditional medical system (TMS) for sustainable healthcare in India. In: Dikshit M, editor. Drug discovery and drug development: the Indian narrative. Singapore: Springer; 2021. p. 1–36. https://doi.org/10.1007/978-981-15-8002-4_1.

[132] Tu Y. The discovery of artemisinin (qinghaosu) and gifts from Chinese medicine. Nat Med 2011;17:1217–20. https://doi.org/10.1038/nm.2471.

[133] Panossian AG, Efferth T, Shikov AN, Pozharitskaya ON, Kuchta K, Mukherjee PK, et al. Evolution of the adaptogenic concept from traditional use to medical systems: pharmacology of stress- and aging-related diseases. Med Res Rev 2021;41:630–703. https://doi.org/10.1002/med.21743.

[134] Mukherjee PK, Banerjee S, Kar A. Molecular combination networks in medicinal plants: understanding synergy by network pharmacology in Indian traditional medicine. Phytochem Rev 2021. https://doi.org/10.1007/s11101-020-09730-4.

[135] Mukherjee PK, Banerjee S, Kar A. Globalisation of traditional medicine under the modern medicine portfolio. In: Natural Medicines. CRC Press; 2019.

[136] Katoch D, Sharma JS, Banerjee S, Biswas R, Das B, Goswami D, et al. Government policies and initiatives for development of Ayurveda. J Ethnopharmacol 2017;197:25–31. https://doi.org/10.1016/j.jep.2016.08.018.

[137] Jordan SA, Cunningham DG, Marles RJ. Assessment of herbal medicinal products: challenges, and opportunities to increase the knowledge base for safety assessment. Toxicol Appl Pharmacol 2010;243:198-216. https://doi.org/10.1016/j.taap.2009.12.005.

[138] Anon. Guideline on selection of test materials for genotoxicity testing for traditional herbal medicinal products/herbal medicinal products; 2009.

[139] Anon. Guidance on safety assessment of botanicals and botanical preparations intended for use as ingredients in food supplements. EFSA J 2009;7:1249. https://doi.org/10.2903/j.efsa.2009.1249.

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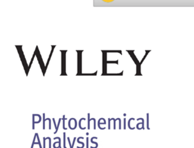
Phytochemical Analysis



RESEARCH ARTICLE

Metabolite Profiling and Integrated Network Pharmacology Based Mechanism of *Benincasa hispida* (Thunb.) Cogn. Fruit Against Non-insulin-Dependent Diabetes Mellitus

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Keywords: *Benincasa hispida* (Thunb.) Cogn | enzyme inhibition kinetics | microwave-assisted extraction | network pharmacology | NIDDM | UHPLC-QTOF-MS/MS

ABSTRACT

Introduction: *Benincasa hispida* (Thunb.) Cogn. (Cucurbitaceae) is an essential food plant in India possessing antihyperglycemic and antihyperlipidemic activities.

Objective: The objective included comparative estimation of α-glucosidase and α-amylase enzyme inhibition potential of *B. hispida* fractions prepared by microwave-assisted extraction and prediction of metabolite interaction against non-insulin-dependent diabetes mellitus by metabolite profiling based network pharmacology analysis.

Methods: A validated microwave-assisted extraction method was employed to obtain different fractions of *B. hispida* fruits. The in vitro enzyme assay was done with *p*-nitrophenyl-α-D-glucopyranoside and acarbose as standard to evaluate antidiabetic potential. The phytomolecules present in the active fraction were identified by UHPLC-QToF-MS/MS analysis. Network pharmacology analysis gave possible gene and disease association, combination synergy network, and predicted probable mechanism of action.

Results: The highest enzyme inhibition potential (IC_{50}) was shown by the ethyl acetate fraction (0.546 ± 0.17 mg/mL and 1.134 ± 0.42 mg/mL) compared to acarbose (0.298 ± 0.08 mg/mL and 0.532 ± 0.38 mg/mL), respectively, for α-glucosidase and α-amylase addressing the potential role in ameliorating non-insulin-dependent diabetes mellitus. Metabolite profiling resulted in the identification of 17 metabolites, and a synergy between the identified molecules suggested multimolecule action in the amelioration of non-insulin-dependent diabetes mellitus through insulin resistance pathway, AMPK signaling pathway, PPAR signaling pathway, and PI3K–Akt signaling pathway. Combination synergy of identified molecules was observed through a multitarget approach to manage non-insulin-dependent diabetes mellitus.

Abbreviations: AMPK, adenosine monophosphate-activated protein kinase; BHCF, *Benincasa hispida* (Thunb.) Cogn. fruit chloroform fraction; BHEF, *Benincasa hispida* (Thunb.) Cogn. fruit ethyl acetate fraction; BHEtF, *Benincasa hispida* (Thunb.) Cogn. fruit ethanolic fraction; BHPF, *Benincasa hispida* (Thunb.) Cogn. fruit pet ether fraction; DAVID, Database for Annotation, Visualization, and Integrated Discovery; DPPH, 2,2-diphenylpicrylhydrazyl; GAE, gallic acid equivalent; KEGG, Kyoto Encyclopedia of Genes and Genomes; NIDDM, non-insulin-dependent diabetes mellitus; PI3K-Akt, phosphoinositide-3-kinase-Akt; PPAR, peroxisome proliferator-activated receptors; PPI, protein–protein interaction; RE, rutin equivalent; STRING, Search Tool for the Retrieval of Interacting Genes/Proteins; TFC, total flavonoid content; TPC, total phenolic content; UHPLC-QTOF-MS/MS, ultra-high-performance liquid chromatography-quadrupole time-of-flight tandem mass spectrometry.

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Conclusion: Polyphenol-enriched fraction of *B. hispida* fruits and identified phytocompounds ameliorate non-insulin-dependent diabetes mellitus. Thus, enriched extract of *B. hispida* can be further investigated in order to develop high-quality, safe, and effective products for the management of non-insulin-dependent diabetes mellitus.

1 | Introduction

Non-insulin-dependent diabetes mellitus (NIDDM) is a mixed assembly of diseases characterized by varying levels of insulin resistance, improper insulin secretion, and increased glucose production from the liver, with postprandial hyperglycemia as a significant consequence. Natural products are significantly used as medicine in treating, mitigating and preventing different ailments, including NIDDM. Recent strategies for managing NIDDM target two major enzymes: α-glucosidase (EC 3.2.1.20) and α-amylase (EC 3.2.1.1). Both enzymes belong to the glycoside hydrolase superfamily of carbohydrate metabolizing enzymes that break down large oligosaccharides into glucose and enable absorption through the human gut [1, 2]. Inhibition of these enzymes resulted in the postponement of glucose absorption, leading to the reduction of postprandial hyperglycemia, ultimately ameliorating NIDDM. Many of the marketed formulations already used in the treatment of NIDDM are metformin, acarbose, voglibose, and miglitol. Nevertheless the frequent use of these available medications has produced flatulence, diarrhea, and abdominal disorders as severe side effects [3]. Several studies have reported the effect of metformin on gastrointestinal complications, including diarrhea, nausea, flatulence, indigestion, vomiting, and abdominal pain with lower hemoglobin and hematocrit levels [4]. Long-term metformin usage has been linked to vitamin B_{12} deficiency, leading to anemia and neuropathy [5, 6]. A population-based case–control study with Type 2 diabetic patients highlighted the risk factors associated with long-term use of sulfonylureas except for gliclazide, leading to colorectal cancer [7]. Thus, the need of the hour is to develop natural products based on alternative therapeutics with fewer side effects in short as well as long-term usage.

The Cucurbitaceae family has been a key part in the management of NIDDM and related disorders both in traditional and modern way of medicine [8]. *Benincasa hispida* (Thunb.) Cogn. (ash gourd) from the Cucurbitaceae family plays a major medicinal as well as culinary role in many Asian countries. Traditionally, it has been used for treating hyperglycemia, epilepsy, and asthma, as a diuretic and analgesic, and in liver diseases [9, 10]. The major phytochemical constituents present are flavonoids (naringenin), triterpenoids (Cucurbitacin), vitamins, phenolic compounds (catechin), and steroids (β-sitosterol) [11]. *B. hispida* has ethnopharmacological uses in anuria, dysuria, urinary disorder, renal stone, constipation, and psychological problem [8]. The fruit extract has several reported pharmacological activities like anticonvulsive, anxiolytic, anticompulsive, antidepressant, antiulcer, antioxidant, analgesic, anti-inflammatory, antiasthmatic, nephroprotective, hepatoprotective, and hypolipidemic in various experimental models [12]. Antihyperglycemic effect of *B. hispida* (Thunb.) Cogn. fruits are due to their high nutritive and low calorific value [8]. The methanolic stem extract has been reported to exhibit a dose-dependent decrease in blood glucose levels in alloxan-induced diabetic rats. Another study revealed the α-amylase inhibitory potential of methanolic, ethanolic, and aqueous extracts of the fruit peel. The ethanolic and ethyl ethanoate extract of the leaves also lowered blood glucose levels in diabetic mice [9]. Ethanolic fruit extract of *B. hispida* also showed antipyretic potential [12].

Ultra-high-performance liquid chromatography-quadrupole time-of-flight tandem mass spectrometry (UHPLC-QTOF-MS/MS) is useful in the quality control of natural products. It precisely identifies target compounds from databases by molecular formula as well as molecular feature. Metabolomics-based metabolite profiling is essential in the identification of large clusters of phytomolecules in medicinal plants and traditional formulations [13]. Metabolomics-based network pharmacology analysis has proven beneficial in understanding the mechanism of action, prediction, and validation of medicinal plants and their products. Network pharmacology analysis predicts synergistic mechanism of action of multiple compounds, thus leading to the discovery of novel therapeutic prospects. The presence of multiple compounds in plant extract provides a combinatory response due to synergy compared to the single isolated compounds [14].

In this study, we explored four fractions of *B. hispida* fruit hydroalcoholic extract and subjected to *in-vitro* α-glucosidase and α-amylase enzyme inhibition assay to identify the bioactive fraction. The total phenolic, flavonoid, and antioxidant properties of the four fractions were examined for their correlation with enzyme activity. The type of inhibition exhibited by the bioactive fraction was deciphered by enzyme inhibition assay in kinetics mode. Metabolite profiling coupled with network pharmacology analysis was incorporated to determine the possible mechanism of action and phytomolecule➔gene➔disease relationship concerning NIDDM. Thus, this study aims to understand and represent the effect of *B. hispida* fruits against NIDDM.

2 | Materials and Methods

2.1 | Instrument and Reagents

The α-glucosidase enzyme (*Saccharomyces cerevisiae*) Type I (≥10 units/mg protein), 4-nitrophenyl-α-D-glucopyrano side (p-NPG, purity ≥99%), α-amylase (from *Aspergillus oryzae*), powder 30 U/mg, starch from potato (soluble), 3,5-dinitrosalicylic acid (DNS) (98%), potassium sodium tartrate tetrahydrate ACS reagent (99%), sodium dihydrogen phosphate, and disodium hydrogen phosphate were procured from Sigma-Aldrich, USA. Acarbose extrapure, 95%, was procured from Sisco Research Laboratories Pvt. Ltd, India. Other chemicals and reagents were obtained from e-Merck

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(Mumbai, India). The sample was weighed in a digital weighing balance (Shimadzu Analytical (India) Pvt. Ltd., Mumbai; Model No. ATX124). Extraction and drying of the dried sample were done in a microwave extraction unit (Catalyst microwave synthesizer CATA R), rotary vacuum evaporator (Hahnshin, Republic of Korea; HS-2005V-N), and lyophilizer (Indian Instrumentation, Kolkata, West Bengal), respectively. Kinetics assay of the enzyme was done in a microplate reader (SpectraMax ID3, Molecular Devices LLC, USA), and metabolite profiling was carried out in UHPLC-QTOF-MS/MS (Santa Clara, CA, USA).

2.2 | Raw Material Collection and Microwave-Assisted Extraction

The fruits of *B. hispida* (ash gourd) were collected from the local market in Kolkata and authenticated (Voucher No. SNPS-JU/2019/1493). The fruits were cleaned and dried under shade and converted into coarse powder. The dried material (500 g) was extracted successively using a microwave-assisted extraction technique with petether, chloroform, ethyl acetate, and ethanol at 60°C and 595 W for 45 min (15 min/cycle). The rotary vacuum evaporator dried the fractions and were stored until further use [15]. The percentage (%) yield of each fraction was represented as % (w/w).

2.3 | Total Flavonoid and Phenolic Content Determination

The total flavonoid content (TFC) and total phenolic content (TPC) in *B. hispida* fruit fractions were determined according to the previously reported high-throughput assay method with slight modifications [3].

In TFC, 90 μL distilled water was added to 96-well plate with $NaNO_2$ (10 μL) and 25 μL of standard (rutin) and test sample solution in different rows. Solution of $AlCl_3$ (15μL) was added after 15 min, followed by 50 μL of NaOH solution. Absorbance was measured at 510 nm. In TPC, 70 μL distilled water was added to 96-well plate with 30 μL sample or standard (gallic acid) and 20 μL of Folin–Ciocalteu (FC) reagent. After 6 min, 100 μL of Na_2CO_3 was added to each well. The plate was left in the dark for 90 min, and absorbance was measured at 765 nm after shaking the plate for 20 s orbitally in the plate reader. All analyses were performed in triplicate. Standard and the test samples were taken in 0.025–0.5 mg/mL concentrations. The TPC and TFC were denoted as GAE mg/g and RE mg/g, respectively.

2.4 | DPPH Radical Scavenging Potential

The DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical scavenging assay (FRSA) method was done as per previously reported methods [16]. In each well, 100 μL of test sample solution or standard (ascorbic acid) was added in concentrations 0.025–0.5 mg/mL and 100 μL of 0.1 mM DPPH solution. The reaction was continued at room temperature in the dark before measuring absorbance at 517 nm.

The IC_{50} value (50% inhibitory concentration) was calculated by nonlinear regression using GraphPad Prism Version 8.0.2 (Boston, MA, USA). The concentration–response curve was obtained by plotting the percentage relative activity versus concentration. The FRSA activity was expressed in IC_{50} (mg/mL), and % inhibition was calculated.

2.5 | In Vitro Assay for Enzyme Inhibition

The α-glucosidase enzyme inhibition assay was done in 96-well plate of *B. hispida* fractions as per the previously reported method with minor modifications [17]. Briefly, 20 μL of α-glucosidase (0.5 U/mL) and a sample (10 μL) were taken in dilutions 0.8–0.1 mg/mL in a microtiter plate and incubated for 10 min (37°C). 5.0 mM, 20 μL 4-nitrophenyl-β-D-glucopyranoside (*p*-NPG) substrate was added and incubated for 5 min (37°C), and absorbance was measured at 405 nm (kinetic mode).

In vitro α-amylase inhibition assay was done as per previous methods with minor modifications [17]. The enzyme, substrate, and samples were prepared in 20 mM, pH 6.9 phosphate buffer. The reaction mixture consisted of 40 μL of α-amylase (1 U/mL) and 40 μL of sample solutions (0.8–0.1 mg/mL). The 96-well plate was preincubated for 10 min (37°C) before adding 40 μL of 1% w/v starch solution (substrate) and incubated for 30 min (37°C). The reaction mixture was terminated by 80 μL DNS colour reagent and heated for 10 min (100°C). Acarbose was used as the reference standard drug in both studies. The absorbance was measured at 540 nm, and percentage relative activity and IC_{50} were calculated as mentioned previously. All the experiments were performed in triplicate.

2.6 | In Vitro Enzyme Kinetic Assay

The inhibitory mechanism (reversible or irreversible) at different sample concentrations was determined by plotting reaction velocity (ΔOD/min) vs. concentrations of the enzyme [E] [18]. 1.5–6.0 mM substrate and 0.25–1 mg/mL inhibitor were used for the kinetic assay, and the inhibitory effect was calculated by taking absorbance at every one-minute interval (0–30 min). K_m and V_{max} were obtained from the Lineweaver–Burk double reciprocal plot using the equation, as mentioned previously. Sigma Plot 14.0 (CA, USA) was used to calculate the values.

2.7 | Metabolite Profiling Conditions

UHPLC-QTOF-MS/MS analysis with Agilent 1260 Infinity II LC System and Agilent 6530 LC/Q-TOF was executed to identify metabolites in BHEF. The chromatographic method and conditions were optimized as per the previously developed method and with slight modifications in the mobile phase [19]. The mobile phase consisted of acetonitrile (A) and water (B) both containing 0.1% formic acid in gradient elution of 5% A (0–1 min), 17% A (1–6 min), 100% A (6–35 min), and 5% A (from 35 to 45 min), operating at 0.5 mL/min. 100 μg/mL sample was injected, and phytocompounds were identified based

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on mass, score (0–100), and theoretical formula. The column temperature was set at 25°C with 5 µL injection volume. Data interpretation was done using MassHunter B.06.01 software (Agilent Technologies, CA, USA). The compound identification was performed by uploading a custom library, the phenolic compounds were obtained from the Phenol-Explorer database [20].

2.8 | Network Pharmacology Analysis

2.8.1 | Target Identification of Bioactive Phytoconstituents

The target list of each phytocompound was identified from Binding DB (https://www.bindingdb.org/) with a similarity ≥0.7. UniProt database was used to search the corresponding human gene targets (https://www.uniprot.org/) [21]. The target genes were filtered for status "Reviewed (Swiss-Prot)" and popular organism "Human." The gene names in bold were taken for further analysis.

2.8.2 | Construction of Gene Diseases Association Network

The target corresponding genes in specified format with double colon (::) were matched with the associated diseases to obtain the summary gene–disease association using DisGeNET (http://www.disgenet.org) [21] The results were filtered to understand the potential link of the targets with NIDDM and related diseases. The gene–disease association score was set to 0.1. The gene–disease association results were downloaded in an excel format containing the Gene, Gene_id, Disease, and Disease_id. The keywords include diabetes mellitus, non-insulin-dependent, and hyperglycemia, post-prandial to filter and sort the excel file.

2.8.3 | Protein–Protein Interaction, Target–Disease Network, and Pathway Analysis

Protein–protein interaction (PPI) was analyzed using STRING Version 12.0 [22]. The gene list was uploaded in "Multiple Proteins by Names/Identifiers" search, and the choice of organism was set to *Homo sapiens*. The string network was created with a minimum required interaction score set to 0.4. The high-resolution string network was downloaded in PNG format. Cytoscape was used to identify the Top 10 interactions based on degree centrality and shortest path length. The gene targets were mapped with respective diseases and created a target–disease network. DAVID 2021 was used to pinpoint the genes and integrated pathways in signal transduction [23].

2.8.4 | Network Analysis Based Combination Synergy

The combined synergy of the phytocompounds linked with NIDDM was analyzed based on a neighborhood approach to obtain a bioactive–target–disease network in Cytoscape (http://cytoscape.org/, Version 3.10.0) [21]. Separate files of plant–phytomolecule, phytomolecule–gene, and gene–disease were prepared in Excel format and uploaded separately in Cytoscape. The source node, target node, and target node attribute were set accordingly, and each network was created. The network were filtered for "self-loop" and "duplicate edge" and finally merged in union to create the bioactive–target–disease network.

2.9 | Statistical Analysis

All results ($n=3$) were statistically analyzed and expressed as mean value ± standard deviation (SD). Two-way ANOVA followed by Dunnett's multiple comparison test at $p<0.0001$ was done to analyze the significance differences. Pearson correlation test was employed to understand the correlation between the antioxidant, total phenolic, and total flavonoid with enzyme inhibitory activity. GraphPad Prism 8.0.2 software (Boston, USA) was used for this analysis.

3 | Results and Discussion

3.1 | TPC and TFC Analysis

The phenolic and flavonoid content was determined from the equation of gallic acid ($y=0.001x+0.0299$, $R^2=0.9913$) and rutin ($y=0.0008x+0.0367$, $R^2=0.9848$), respectively. The standard curve has been given in Figures S1 and S2. BHEF exhibited the highest TPC and TFC at 15.35 ± 1.01 mg gallic acid equivalent/g and 12.42 ± 0.97 mg rutin equivalent/g. The TPC and TFC values of the rest of the fractions have been given in Table S2.

3.2 | DPPH Inhibition Analysis

The DPPH free radical scavenging potential of the fractions of *B. hispida* fruits were determined based on DPPH free radical scavenging potential, and BHEF offered the highest antioxidant property (0.0618 ± 0.015 mg/mL), compared with ascorbic acid (0.0322 ± 0.064 mg/mL). The antioxidant potential of the rest of the fractions has been given in Table S3. The DPPH-FRSA curve has been given in Figure S3. Polyphenolic compounds and antioxidants in medicinal plants have a significant role in NIDDM via various mechanistic pathways related to α-amylase, α-glucosidase enzyme inhibition, glucose transporter and regulation of insulin secretion [24, 25].

3.3 | Analysis: Enzyme Inhibition Assay

The yield values of the fractions are given in Table S1. Both α-glucosidase and α-amylase enzymes have been reported to be key in the management of NIDDM [26]. In the α-glucosidase inhibition assay, half-maximal inhibitory concentration (IC_{50}) was found to be the highest in BHEF (0.546 ± 0.17 mg/mL) compared to acarbose (0.298 ± 0.08 mg/mL). The IC_{50} values of other fractions were found to be 1.062 ± 0.22 (BHPF), 1.384 ± 0.25 (BHCF), and 0.644 ± 0.3 (BHEtF) mg/mL,

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respectively (Figure 1). BHEF also showed maximum inhibition (IC_{50}) against α-amylase at 1.134 ± 0.42 mg/mL, comparable with acarbose (0.532 ± 0.38 mg/mL). The IC_{50} values of the other subfractions were 2.6 ± 0.44 (BHPF), 4.57 ± 0.57 (BHCF), and 1.9 ± 0.3 (BHEtF) mg/mL (Figure 1). Multiple comparisons of the IC_{50} values with two-way ANOVA between BHEF and acarbose for both enzymes, followed by Dunnett's test showed significant results ($p < 0.0001$), which are shown in Figure S8. The results highlight that *B. hispida* has inhibited both α-glucosidase and α-amylase in a strong manner comparable to standard inhibitor, which correlates with previous reports [27, 28]

3.4 | Analysis: Enzyme Inhibition Kinetics

Reaction velocity (v) vs. enzyme concentration (I = 0–1000 μg/mL) showed reversible inhibition of α-glucosidase and α-amylase by BHEF and inversely decreasing slope with increasing concentration of inhibitors (I). The α-glucosidase inhibition kinetics gave V_{max} and K_m as 24.6 mM/min and 3.4 mM, respectively, and α-amylase inhibition kinetics gave V_{max} and K_m as 23 mM/min and 3.7 mM, respectively (Figure 2). Both the values of apparent V_{max} (decrease) and K_m (increase) were changed with increasing concentration of BHEF. The secondary plot was used to calculate Ki and αKi as 208.2 and 17.4 μg/mL for α-glucosidase

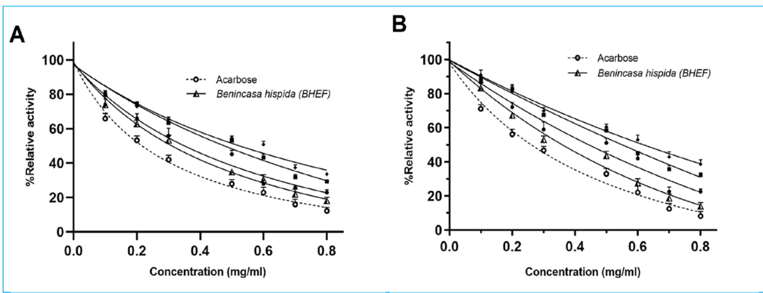

FIGURE 1 | Dose–response curve of fractions of *Benincasa hispida* (Thunb.) Cogn. and acarbose against (A) α-glucosidase and (B) α-amylase inhibition (—▲—) BHEF–ethyl acetate fraction; (- -O- -) acarbose; (—■—) chloroform; (—●—) ethanol; and (—◆—) pet ether fraction; data are presented as mean ± SD ($n = 3$).

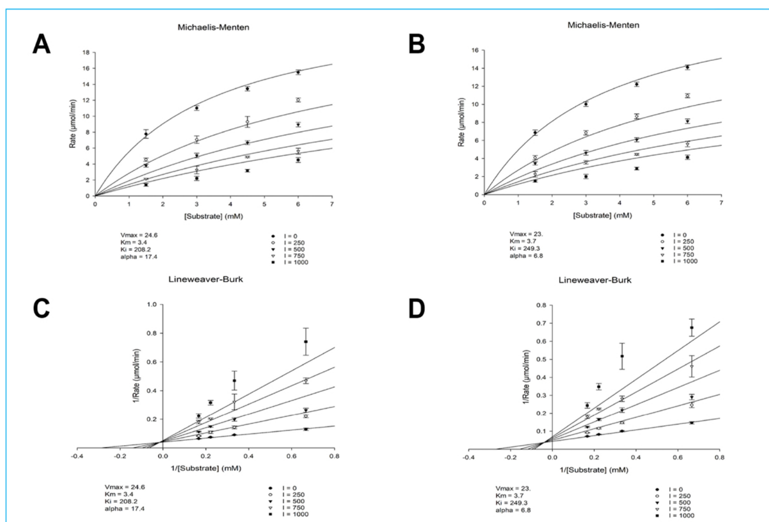

FIGURE 2 | Enzyme kinetics study of BHEF (● I = 0 μg/mL; ○ I = 250 μg/mL; ▼ I = 500 μg/mL; ▽ I = 750 μg/mL; ■ I = 1000 μg/mL). (A) Michaelis–Menten plot against α-glucosidase inhibitory activity. (B) Michaelis–Menten plot against α-amylase inhibitory activity. (C) Lineweaver–Burk plot against α-glucosidase inhibitory activity. (D) Lineweaver–Burk plot against α-amylase inhibitory activity.

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and 249.3 and 6.8 µg/mL for α-amylase, respectively (Figure 3). These observations confirm a probable mixed type of inhibition by BHEF against these enzymes.

3.5 | TPC and TFC With α-Glucosidase and α-Amylase Inhibitory Potential – Correlation Analysis

Pearson correlation analysis between α-glucosidase and α-amylase inhibitory potential with the TPC & TFC values were evaluated and shown in Table 1. It was observed that TPC and TFC of BHEF showed a positive correlation with α-amylase and a negative with α-glucosidase inhibitory potential, which was reflected in the Pearson r value at $p < 0.05$. The enzyme inhibitory activity of BHEF was found to be related to TPC and TFC, which indicates that phenolic and flavonoid compounds present in *B. hispida* fruits play a significant role in inhibitory activity.

3.6 | DPPH Free Radical Scavenging Potential With α-Glucosidase and α-Amylase Inhibitory Activity – Correlation Analysis

Pearson correlation analysis exhibited a significant correlation was observed between the α-glucosidase (0.99) and α-amylase (−0.99) inhibition potential and DPPH-FRSA in BHEF (Table 1). The correlation analysis exhibited that antioxidant potential was beneficial in exhibiting an effect against NIDDM.

3.7 | UHPLC-QTOF-MS/MS Analysis

The identified phytocompounds present in BHEF (Table 2) summarize 17 metabolites with the retention time, molecular formula, error (in ppm), major MS/MS fragment, and phytocompounds, including their class. The total ion chromatogram in positive ion mode is shown in Figure 4. Polyphenols,

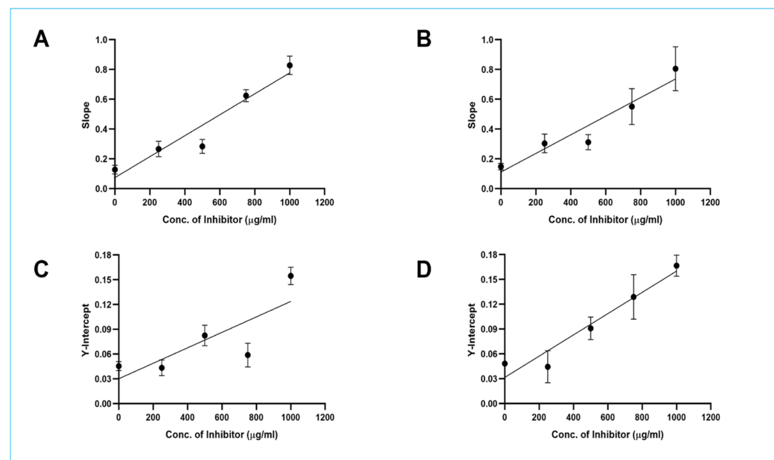

FIGURE 3 | Plot of slope (*K*m/Vmax) vs. BHEF concentration: (A) α-glucosidase and (C) α-amylase. Plot of *y*-intercept (1/*V*max) vs. BHEF concentration: (B) α-glucosidase and (D) α-amylase.

TABLE 1 | Correlation analysis between the TPC/TFC and antioxidant property (IC_{50} value) of the fractions of *Benincasa hispida* (Thunb.) Cogn. fruits and their α-glucosidase and α-amylase inhibition.

	Pearson correlation (*r*) (IC_{50} value of α-glucosidase inhibition)				Pearson correlation (*r*) (IC_{50} value of α-amylase inhibition)			
Parameters	**BHPF**	**BHCF**	**BHEF**	**BHEtF**	**BHPF**	**BHCF**	**BHEF**	**BHEtF**
Total phenol content (mg/g)	0.9^{ns}	0.97^{ns}	−0.99*	-0.25^{ns}	0.95^{ns}	-0.85^{ns}	0.99*	0.93^{ns}
Total flavonoid content (mg/g)	0.99^{ns}	0.99^{ns}	−0.99*	0.05^{ns}	0.96^{ns}	-0.89^{ns}	0.99*	0.99*
Antioxidant (DPPH free radical scavenging capacity) (IC_{50} mg/mL)	0.99^{ns}	0.84^{ns}	0.99*	0.049^{ns}	0.99*	-0.62^{ns}	−0.99*	0.99*

Note: ns for $p \geq 0.05$ (non-significant).
*$p < 0.05$ (significant).

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TABLE 2 | Compounds identified in ethyl acetate fraction of *Benincasa hispida* (Thunb.) Cogn. fruit by UHPLC-QTOF-MS/MS.

SI. no.	RT	m/z (Estimated)	m/z (Expected)	Chemical formula	Error PPM	Major MS-MS fragments	Name of compounds	Class
1	10.903	290.0790	290.0790	$C_{15}H_{14}O_6$	0.00	236, 202, 166, 124, 109	Catechin	Flavonoid
2	15.281	424.3702	424.3705	$C_{30}H_{48}O$	−0.70	300, 268, 245, 205	Friedoolean-8-en-3-one	Friedooleanane-type triterpenes
3	16.4	556.3030	556.3036	$C_{32}H_{44}O_8$	−1.07	518	Cucurbitacin E	Triterpenes
4	17.220	272.0687	272.0684	$C_{15}H_{12}O_5$	1.10	153, 147	Naringenin	Flavonoid
5	17.673	356.2929	356.2926	$C_{21}H_{40}O_4$	0.84	340, 254	Butanoic acid, 2,3-dihydroxypropyl ester	Glycerolipids
6	19.3	414. 3860	414.3861	$C_{29}H_{50}O$	0.84	379, 341, 287, 255, 213	β-Sitosterol	Stigmastanes and derivatives
	22.844	278.2250	278.2245	$C_{18}H_{30}O_2$	1.79	261, 243, 205, 149, 123	Linolenic acid	Polyunsaturated fatty acids
8.	23.174	280.2402	280.2402	$C_{18}H_{32}O_2$	0.00	202, 165, 124, 100	Linoleic acid	Polyunsaturated fatty acids
9	25.002	412.3705	412.3705	$C_{29}H_{48}O$	0.00	395, 315, 257, 199, 83	Stigmasterol	Stigmastanes and derivatives
10	25.222	256.2401	256.2402	$C_{16}H_{32}O_2$	−0.39	183, 149, 118, 75	Palmitic acid	Long-chain fatty acids
11	25.655	284.2717	284.2715	$C_{18}H_{36}O_2$	0.70	275, 257, 241, 153, 125	Stearic acid	Long-chain fatty acids
12	26.792	282.2555	282.2558	$C_{18}H_{34}O_2$	−1.06	265, 205, 135, 107, 83	Oleic acid	Monounsaturated omega-9 fatty acid
13.	26.803	280.2404	280.2402	C18H32O2	0.71	278, 254	9,12-Octadecadenoic acid	Fatty acyls
14.	27.181	178.0265	178.0266	$C_9H_6O_4$	−0.56	149, 133, 121, 105, 89	Esculetin	6,7-Dihydroxycoumarins
15.	28.946	310.2875	310.2871	$C_{20}H_{38}O_2$	1.28	290, 275, 227	Eicosenoic acid	Monounsaturated long-chain fatty acid
16.	29.292	594.1589	594.1584	$C_{27}H_{30}O_{15}$	0.84	422, 304, 288, 256	Kaempferol	Tetrahydroxyflavone
17.	29.723	431.0974	431.0978	$C_{21}H_{19}O_{10}$	−0.92	352, 288	Kaempferol 3-O-L-rhamnopyranoside	Flavonoid glycoside

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flavonoids, phenols, triterpenes, flavanones, stigmastanes, and amino acids were identified as significant compounds. The identified bioactive compounds like catechin, naringenin, kaempferol, kaempferol 3-O-L-rhamnopyranoside, cucurbitacin E, butanoic acid, linolenic acid, linoleic acid, palmitic acid, stearic acid, oleic acid, eicosenoic acid, β-sitosterol, stigmasterol, friedoolean-8-en-3-one, and esculetin were all previously reported to possess therapeutic potential against NIDDM [29–35].

3.8 | Target Screening of Identified Molecules

The identified targets were searched using BindingDB concerning the compounds found in the metabolomics study. The associated genes identified from the UniProt database were converted into a specified format. The bioactive–target network has been shown in Figure S4. The interacting targets and bioactive molecules were used to construct the bioactive–target network. Network analysis among the 13 compounds demonstrated kaempferol to show the highest degree of association of 7, whereas cucurbitacin E exhibited the highest closeness and betweenness centrality of 1.0.

3.9 | Target–Disease Association Network

Network-based target–disease association was achieved by uploading the target-associated genes in specified format to the DisGeNET database, and the results were filtered for diabetes mellitus, non-insulin-dependent, and postprandial hyperglycemia (Figure S5). The filtered genes were used to construct a target–disease association network with 141 nodes, 143 edges, 2.028 average number of neighbors, and a network density of 0.014. The gene–disease association network showed targets like INSR, NOS3, HSD11B1, PTGS2, PPARG, BCL2, EDNRA, NOS2, EDNRB, and PPARA to interact with diabetes mellitus, non-insulin-dependent, and post-prandial hyperglycemia.

3.10 | Target PPI and Pathway Analysis

The target genes involved with diabetes mellitus, non-insulin-dependent, and postprandial hyperglycemia were visualized in STRING, and the PPI was analyzed to get significantly higher interactions of PPI enrichment p-value < 1.0e−16. PPI analysis identified the signaling pathways and networks to understand the mode of action of compounds from *B. hispida* fruits. The PPI network was analyzed by degree and shortest path length using cytoHubba tool in Cytoscape to calculate the Top 10 hub genes (Figure 5). The pathway enrichment analysis by KEGG exhibited pancreatic secretions (0.0031), p53 signaling pathway (0.0071), and peroxisome proliferator-activated receptors (PPAR) signaling pathway (1.01E-08), which are connected to NIDDM (Figure 6). Other pathways involved are phosphoinositide-3-kinase-Akt (PI3K-Akt) signaling pathway (5.11E-11), AGE-RAGE signaling pathway in diabetic complications (1.30E-08), insulin resistance (1.68E-05), MAPK pathway (4.79E-05), adipocytokine signaling pathway (0.0026), and insulin signaling pathway (0.0069). Functional association clustering integrates functional genomics annotations to relate important targets, and pathway-associated NIDDM was analyzed. The enriched PPAR signaling pathway is involved through matching genes FABP4, FABP2, PPARG, FABP1, FABP5, PPARD, FABP3, PPARA, RXRA, and NR1H3. The pancreatic secretion pathway interacts through CFTR, CA2, PNLIP, PLA2G2A, and PLA2G10. The p53 signaling pathway interacts through SERPINE1, CDK6, CDK2, and BCL2. Genes are marked with red star and shown in Figure 6. The gene ontology (GO) enrichment analysis was visualized by ShinyGO 0.77 at FDR cutoff of 0.05 to find 50 enriched pathways based on pathway database KEGG, GO molecular function, and GO biological process and established the part of these proteins in NIDDM (Figure S7). GO analysis explored potential targets PI3K, PTP1B, MAPK, AKT, TNF, and NF-κB, which were involved in β-cell regulation and amelioration of diabetes through PI3K–Akt signaling pathway according to previous studies [36]. The AMPK signaling pathway shows marked

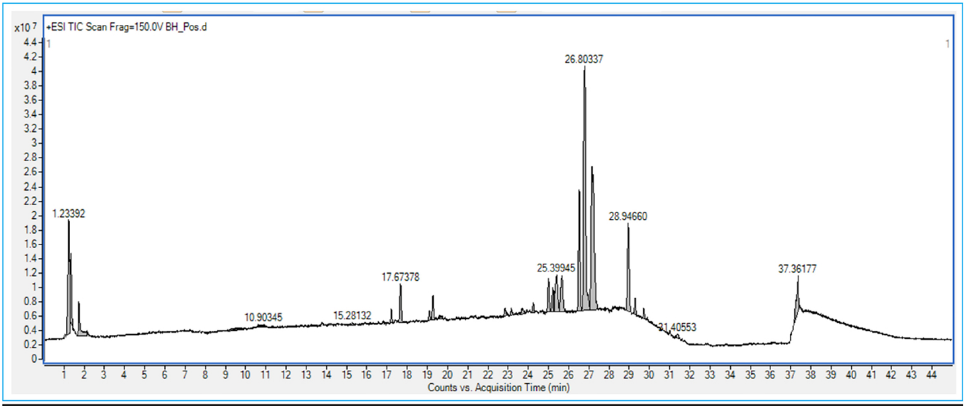

FIGURE 4 | Total ion chromatogram of BHEF in the positive ionization mode.

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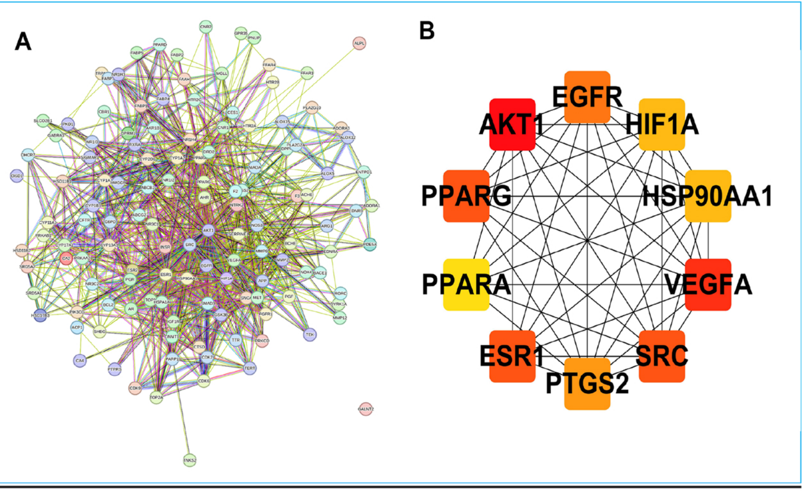

FIGURE 5 | PPI network of genes associated with NIDDM. (A) In STRING database network, multiple bonds between genes represent stronger interaction. (B) Top 10 gene interactions based on degree centrality and shortest path length; the more the red color, the more the interacting potential.

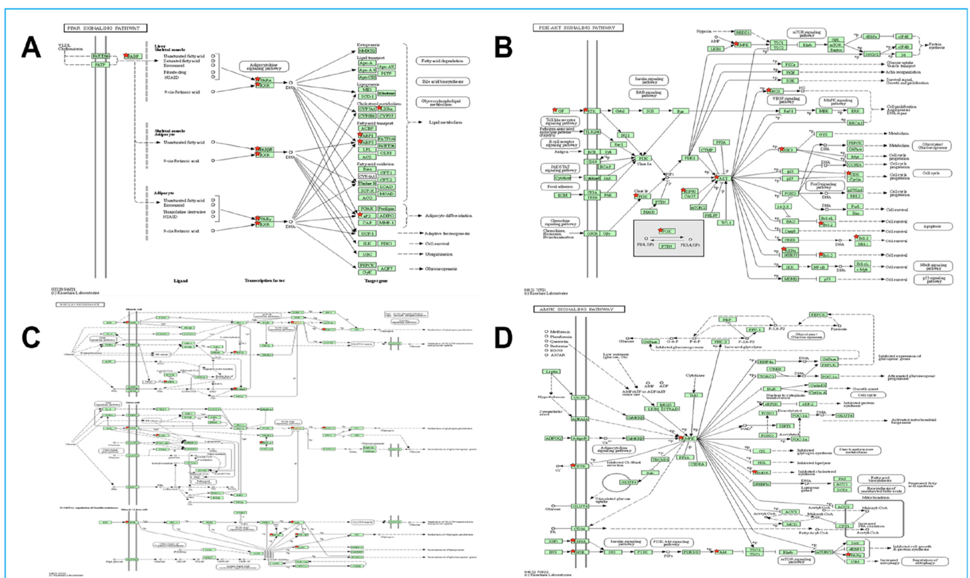

FIGURE 6 | KEGG enrichment pathway analysis of NIDDM. The targeted genes are marked with red star: (A) PPAR signaling pathway, (B) PI3K–Akt signaling pathway, (C) insulin resistance, and (D) AMPK signaling pathway.

genes acting through adipocytokine signaling pathway. Insulin resistance pathway shows involved genes INSR, AKT2, and GSK-3 in reduction of glycogen production, PI3K–Akt signaling pathway, and inhibition of GLUT4 translocation and glucose uptake. Recent studies have shown that PI3K–Akt signaling pathway activation exhibited antihyperglycemic effect.

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The target proteins in the PI3K–Akt signaling pathway CDK6, CDK2, IGF1R, EGFR, NTRK2, MET, GSK3B, HSP90AA1, PIK3CG, TEK, BCL2, FGFR1, RXRA, PGF, AKT1, and VEGFA show neighborhood connectivity with kaempferol, esculetin, catechin, naringenin, friedoolean-8-en-3-one, stigmasterol, and β-sitosterol and may act by regulating insulin homeostasis and promoting glucose transport, glycogen synthesis, and protein synthesis in skeletal muscles, liver, insulin secretion, and β-cell function in pancreas [37]. Upregulation of IRS, PI3K, AKT, and PPAR has been reported to reduce blood glucose levels [38]. Thus, the activation of PPAR–PI3K–Akt–IRS–AMPK cascade signaling pathway controls NIDDM [39].

3.11 | Combination Network Analysis

The combination synergy network was visualized in Cytoscape to understand the mode of action of the phytomolecule combinations to combat NIDDM and postprandial hyperglycemia. A neighborhood network connection analysis was developed to show the combination synergy networks related to the disease domains based on degree centrality. The combination network of NIDDM and postprandial hyperglycemia consisted of 148 nodes and 418 edges. The network has 5.649 average number of neighbors and a characteristic path length of 2.153 with a network density of 0.038 (Figure S6). The combination network was further filtered by degree centrality and shortest path length to obtain a hub network of 32 nodes selected as 2× median of degree centrality, shown in Figure 7. The modified network with hub genes based on betweenness and degree centrality found 13 phytomolecule interacting with 17 potential targets connected with NIDDM. Evidence shows that the role of oxidative stress in signaling pathways like the AMPK signaling pathway and insulin signaling pathway responses plays an important role in NIDDM and related disorders and may represent novel drug targets for alternative therapeutics [40]. Kaempferol was found to be the most interacting phytomolecule, and 9,12-octadecadenoic acid was found to be the least interacting one. Kaempferol, catechin, and naringenin act through PPAR signaling pathway by fatty acid–binding protein (FABP) regulating fatty acid metabolism, lipid storage, and fat oxidation through PPARα, PPARβ, and PPARγ. Experimental results have shown the activity of *B. hispida* fruit peels suppress PPAR receptor signaling and ameliorate NIDDM [41, 42]. The potential of kaempferol to improve insulin sensitivity and protect β-cell dysfunction by upregulation of glucose transport-4 (GLUT4) and AMP-dependent protein kinase (AMPK) expression in high-fat diet animal model [43].

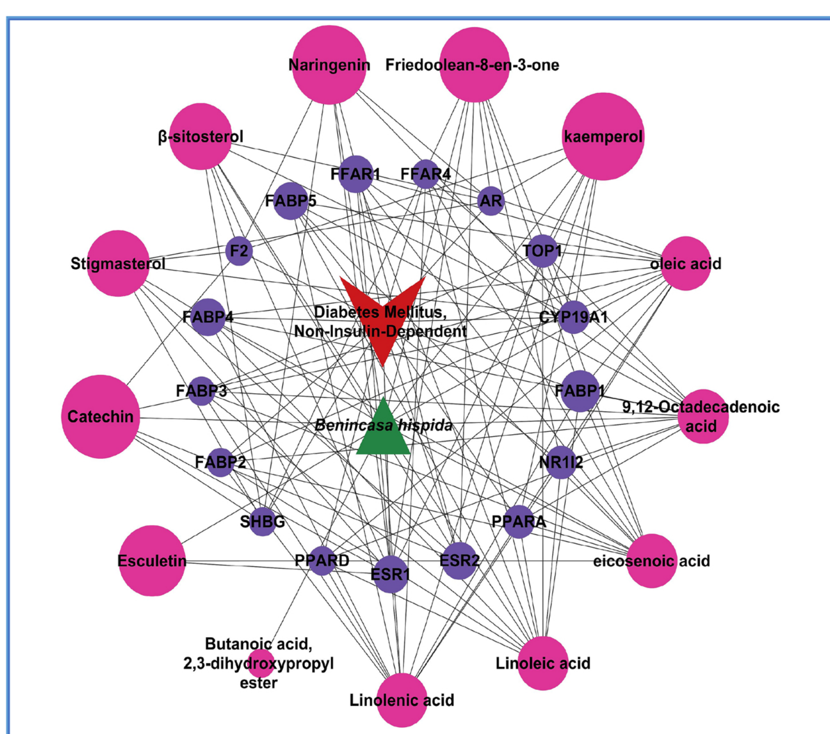

FIGURE 7 | Combination synergy network analysis based on degree centrality and shortest path length. Pink circles represent phytomolecules identified by metabolite profiling; violet circles represent human target genes; the bigger the size of the node, the more the interaction with other nodes.

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B. hispida is an important plant of the Cucurbitaceae family, widely used in culinary purposes in India and many parts of the world. *B. hispida* is already well established, but the evidence of mechanism and chemical validation is not properly explored. In this study, we are trying to elucidate probable mechanism of action based on its metabolite profiling through systems-based network pharmacology approach, which will be beneficial for development of natural healthcare alternatives.

The current study suggested the mixed inhibition of the polyphenolic-enriched fraction of *B. hispida* (Thunb.) Cogn. fruit against α-glucosidase and α-amylase enzymes. Metabolomics-based network pharmacology suggests the possible mechanism of action to combat NIDDM through PPAR signaling pathway, PI3K–Akt signaling pathway, insulin resistance, and AMPK signaling pathway. Combination synergy of identified phytomolecules was also observed. The results of this study provide evidence that polyphenolic-enriched fraction may be used to develop safe and effective functional food component for controlling the increased blood glucose levels in NIDDM. The protein targets obtained via network pharmacology approach can be validated by molecular docking analysis on α-amylase and α-glucosidase. These results can be correlated with proper in vivo studies for high-fat diet-induced diabetes including serum biochemical and histopathological studies. Further, studies should be performed in transcriptomic level to understand the upregulation and downregulation of key proteins in more details.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of the manuscript will be made available upon request to the corresponding author.

References

1. P. K. Mukherjee, "Therapeutic Evaluation of Herbs With Enzyme Inhibition Studies," in *Quality Control and Evaluation of Herbal Drugs*, ed. P. K. Mukherjee (Amsterdam: Elsevier, 2019), 539–571, https://doi.org/10.1016/B978-0-12-813374-3.00014-4.

2. A. Subramoniam, *Anti-Diabetes Mellitus Plants: Active Principles, Mechanisms of Action and Sustainable Utilization* (Florida: CRC Press, 2016), https://doi.org/10.1201/9781315368115.

3. J. Chanda, P. K. Mukherjee, A. Kar, et al., "LC–QTOF–MS-Based Metabolite Profiling and Evaluation of α-Glucosidase Inhibitory Kinetics of *Coccinia grandis* Fruit," *Biomedical Chromatography* 34, no. 12 (2020): e4950, https://doi.org/10.1002/bmc.4950.

4. A. M. Alatawi, A. F. Albogami, S. S. Aldharman, et al., "Therapeutic and Adverse Effects of Metformin in Diabetes: A Review Article: Life Sciences-Endocrinology," *International Journal of Life Science and Pharma Research* 13 (2023): 261–272, https://doi.org/10.22376/ijlpr.2023.13.1.L261-272.

5. T. J. Alharbi, A. M. Tourkmani, O. Abdelhay, et al., "The Association of Metformin Use With Vitamin B12 Deficiency and Peripheral Neuropathy in Saudi Individuals With Type 2 Diabetes Mellitus," *PLoS ONE* 13, no. 10 (2018): e0204420, https://doi.org/10.1371/journal.pone.0204420.

6. R. Yang, H. Yu, J. Wu, et al., "Metformin Treatment and Risk of Diabetic Peripheral Neuropathy in Patients With Type 2 Diabetes Mellitus in Beijing, China," *Frontiers in Endocrinology* 14 (2023): 1–8, https://doi.org/10.3389/fendo.2023.1082720.

7. C. M. Shin, N. Kim, K. Han, et al., "Anti-diabetic Medications and the Risk for Colorectal Cancer: A Population-Based Nested Case-Control Study," *Cancer Epidemiology* 64 (2020): 101658, https://doi.org/10.1016/j.canep.2019.101658.

8. P. K. Mukherjee, S. Singha, A. Kar, et al., "Therapeutic Importance of Cucurbitaceae: A Medicinally Important Family," *Journal of Ethnopharmacology* 282 (2022): 114599, https://doi.org/10.1016/j.jep.2021.114599.

9. M. T. Islam, C. Quispe, D. M. El-Kersh, et al., "A Literature-Based Update on *Benincasa hispida* (Thunb.) Cogn.: Traditional Uses, Nutraceutical, and Phytopharmacological Profiles," *Oxidative Medicine and Cellular Longevity* 2021 (2021): e6349041, https://doi.org/10.1155/2021/6349041.

10. M. S. Pagare, L. Patil, and V. J. Kadam, "*Benincasa hispida*: A Natural Medicine," *Research Journal of Pharmacy and Technology* 4 (2011): 1941–1944.

11. J. Chanda, S. Biswas, A. Kar, and P. K. Mukherjee, "Determination of Cucurbitacin E in Some Selected Herbs of Ayurvedic Importance Through RP-HPLC," *Journal of Ayurveda and Integrative Medicine* 11, no. 3 (2020): 287–293, https://doi.org/10.1016/j.jaim.2019.01.002.

12. S. Palamthodi and S. S. Lele, "Nutraceutical Applications of Gourd Family Vegetables: *Benincasa hispida*, *Lagenaria siceraria* and *Momordica charantia*," *Biomedicine & Preventive Nutrition* 4, no. 1 (2014): 15–21, https://doi.org/10.1016/j.bionut.2013.03.004.

13. A. Kar, P. K. Mukherjee, S. Saha, et al., "Metabolite Profiling and Evaluation of CYP450 Interaction Potential of 'Trimada'- An Ayurvedic Formulation," *Journal of Ethnopharmacology* 266 (2021): 113457, https://doi.org/10.1016/j.jep.2020.113457.

14. P. K. Mukherjee, S. Banerjee, A. Kar, et al., "Synergy and Network Pharmacology—Establishing the Efficacy of Herbal Medicine," in *Evidence-Based Validation of Herbal Medicine (Second Edition)*, ed. P. K. Mukherjee (Amsterdam: Elsevier, 2022), 501–510, https://doi.org/10.1016/B978-0-323-85542-6.00017-2.

15. P. K. Mukherjee, "Extraction and Other Downstream Procedures for Evaluation of Herbal Drugs," in *Quality Control and Evaluation of Herbal Drugs*, ed. P. K. Mukherjee (Amsterdam: Elsevier, 2019), 195–236, https://doi.org/10.1016/B978-0-12-813374-3.00006-5.

16. R. Banerjee, B. Das Gupta, A. Kar, et al., "Quality Evaluation of Different Black Rice Varieties of Northeastern Region of India," *Phytochemical Analysis* 34, no. 5 (2023): 507–517, https://doi.org/10.1002/pca.3230.

17. S. Singha, B. Das Gupta, A. Sarkar, et al., "Chemo-Profiling and Exploring Therapeutic Potential of *Momordica Dioica* Roxb. ex Willd. for Managing Metabolic Related Disorders: In-Vitro Studies, and Docking Based Approach," *Journal of Ethnopharmacology* 331 (2024): 118351, https://doi.org/10.1016/j.jep.2024.118351.

18. Y. Ji, D. Liu, Y. Jin, et al., "In Vitro and in Vivo Inhibitory Effect of Anthocyanin-Rich Bilberry Extract on α-Glucosidase and α-Amylase," *LWT* 145 (2021): 111484, https://doi.org/10.1016/j.lwt.2021.111484.

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19. B. D. Gupta, A. Kar, S. Narayan, C. P. Thakur, P. K. Mukherjee, and P. K. Haldar, "Ultra-Performance Liquid Chromatography-Quadrupole Time-Of-Flight Tandem Mass Spectrometry-Based Metabolite Profiling, Quality Evaluation, and Marker Analysis of *Trachyspermum ammi* (L.) Sprague by High-Performance Thin-Layer Chromatography," *Journal of Separation Science* 46 (2023): 2200872, https://doi.org/10.1002/jssc.202200872.

20. V. Neveu, J. Perez-Jiménez, F. Vos, et al., "Phenol-Explorer: An Online Comprehensive Database on Polyphenol Contents in Foods," *Database* 2010 (2010): bap024, https://doi.org/10.1093/database/bap024.

21. S. Sarkar, A. Kar, P. Shaw, et al., "Hydroalcoholic Root Extracts of *Houttuynia cordata* (Thunb.) Standardized by UPLC-Q-TOF-MS/MS Promotes Apoptosis in Human Hepatocarcinoma Cell HepG2 via GSK--3β/β-Catenin/PDL-1 Axis," *Fitoterapia* 171 (2023): 105684, https://doi.org/10.1016/j.fitote.2023.105684.

22. D. Szklarczyk, J. H. Morris, H. Cook, et al., "The STRING Database in 2017: Quality-Controlled Protein–Protein Association Networks, Made Broadly Accessible," *Nucleic Acids Research* 45, no. D1 (2017): D362–D368, https://doi.org/10.1093/nar/gkw937.

23. D. W. Huang, B. T. Sherman, and R. A. Lempicki, "Systematic and Integrative Analysis of Large Gene Lists Using DAVID Bioinformatics Resources," *Nature Protocols* 4, no. 1 (2009): 44–57, https://doi.org/10.1038/nprot.2008.211.

24. M. Guasch-Ferré, J. Merino, Q. Sun, M. Fitó, and J. Salas-Salvadó, "Dietary Polyphenols, Mediterranean Diet, Prediabetes, and Type 2 Diabetes: A Narrative Review of the Evidence," *Oxidative Medicine and Cellular Longevity* 2017 (2017): 6723931, https://doi.org/10.1155/2017/6723931.

25. Y. Kim, J. B. Keogh, and P. M. Clifton, "Polyphenols and Glycemic Control," *Nutrients* 8, no. 1 (2016): 17, https://doi.org/10.3390/nu8010017.

26. C. Ovatlarnporn, A. Basit, H. Paliwal, et al., "Untargeted Metabolomics, Optimization of Microwave-Assisted Extraction Using Box–Behnken Design and Evaluation of Antioxidant, and Antidiabetic Activities of Sugarcane Bagasse," *Phytochemical Analysis* 35 (2024): 1550–1564, https://doi.org/10.1002/pca.3395.

27. S. Bellur Nagarajaiah and J. Prakash, "Chemical Composition and Bioactive Potential of Dehydrated Peels of *Benincasa hispida*, *Luffa acutangula*, and *Sechium edule*," *Journal of Herbs Spices & Medicinal Plants* 21, no. 2 (2015): 193–202, https://doi.org/10.1080/10496475.2014.940437.

28. S. F. Sulaiman and K. L. Ooi, "Antioxidant and α-Glucosidase Inhibitory Activities of Cucurbit Fruit Vegetables and Identification of Active and Major Constituents From Phenolic-Rich Extracts of *Lagenaria siceraria* and *Sechium edule*," *Journal of Agricultural and Food Chemistry* 61, no. 42 (2013): 10080–10090, https://doi.org/10.1021/jf4031037.

29. H. Alkhalidy, W. Moore, Y. Wang, et al., "The Flavonoid Kaempferol Ameliorates Streptozotocin-Induced Diabetes by Suppressing Hepatic Glucose Production," *Molecules* 23, no. 9 (2018): 2338, https://doi.org/10.3390/molecules23092338.

30. T. Behl, K. Kumar, S. Singh, et al., "Unveiling the Role of Polyphenols in Diabetic Retinopathy," *Journal of Functional Foods* 85 (2021): 104608, https://doi.org/10.1016/j.jff.2021.104608.

31. T. Hussain, B. Tan, G. Murtaza, et al., "Flavonoids and Type 2 Diabetes: Evidence of Efficacy in Clinical and Animal Studies and Delivery Strategies to Enhance Their Therapeutic Efficacy," *Pharmacological Research* 152 (2020): 104629, https://doi.org/10.1016/j.phrs.2020.104629.

32. S. Maity, A. Acharyya, and C. A. Sankar, "Flavonoid-Based Polymeric Nanoparticles: A Promising Approach for Cancer and Diabetes Treatment," *European Polymer Journal* 177 (2022): 111455, https://doi.org/10.1016/j.eurpolymj.2022.111455.

33. M. Shahwan, F. Alhumaydhi, G. M. Ashraf, P. M. Z. Hasan, and A. Shamsi, "Role of Polyphenols in Combating Type 2 Diabetes and Insulin Resistance," *International Journal of Biological Macromolecules* 206 (2022): 567–579, https://doi.org/10.1016/j.ijbiomac.2022.03.004.

34. Y. Wang, T. Hu, J. Wei, X. Yin, Z. Gao, and H. Li, "Inhibitory Activities of Flavonoids From *Scutellaria baicalensis* Georgi on Amyloid Aggregation Related to Type 2 Diabetes and the Possible Structural Requirements for Polyphenol in Inhibiting the Nucleation Phase of hIAPP Aggregation," *International Journal of Biological Macromolecules* 215 (2022): 531–540, https://doi.org/10.1016/j.ijbiomac.2022.06.107.

35. Y. Yang, Z. Chen, X. Zhao, et al., "Mechanisms of Kaempferol in the Treatment of Diabetes: A Comprehensive and Latest Review," *Frontiers in Endocrinology* 13 (2022): 1–15, https://doi.org/10.3389/fendo.2022.990299.

36. C. Zhu, T. Cai, Y. Jin, et al., "Artificial Intelligence and Network Pharmacology Based Investigation of Pharmacological Mechanism and Substance Basis of Xiaokewan in Treating Diabetes," *Pharmacological Research* 159 (2020): 104935, https://doi.org/10.1016/j.phrs.2020.104935.

37. X. Huang, G. Liu, J. Guo, and Z. Su, "The PI3K/AKT Pathway in Obesity and Type 2 Diabetes," *International Journal of Biological Sciences* 14, no. 11 (2018): 1483–1496, https://doi.org/10.7150/ijbs.27173.

38. S. Bao, Y. L. Wu, X. Wang, et al., "*Agriophyllum* Oligosaccharides Ameliorate Hepatic Injury in Type 2 Diabetic db/db Mice Targeting INS-R/IRS-2/PI3K/AKT/PPAR-γ/Glut4 Signal Pathway," *Journal of Ethnopharmacology* 257 (2020): 112863, https://doi.org/10.1016/j.jep.2020.112863.

39. J. Zhu, M. Wu, H. Zhou, L. Cheng, X. Wei, and Y. Wang, "Liubao Brick Tea Activates the PI3K-Akt Signaling Pathway to Lower Blood Glucose, Metabolic Disorders and Insulin Resistance via Altering the Intestinal Flora," *Food Research International* 148 (2021): 110594, https://doi.org/10.1016/j.foodres.2021.110594.

40. H. Yaribeygi, T. Sathyapalan, S. L. Atkin, and A. Sahebkar, "Molecular Mechanisms Linking Oxidative Stress and Diabetes Mellitus," *Oxidative Medicine and Cellular Longevity* 2020 (2020): e8609213, https://doi.org/10.1155/2020/8609213.

41. E. Ebrahimi, S. Shirali, and R. Afrisham, "Effect and Mechanism of Herbal Ingredients in Improving Diabetes Mellitus Complications," *Jundishapur Journal of Natural Pharmaceutical Products* 12, no. 1 (2017): 1–8, https://doi.org/10.5812/jjnpp.31657.

42. A. Mahatma, M. D. S. Kumar, and A. Sonowal, "Evaluation of Antidiabetic Potential of Methanolic Extract of *Benincasa hispida* in Dexamethasone Induced Diabetic Rats," *IOSRJDMS* 13, no. 12 (2014): 118–125, https://doi.org/10.9790/0853-13125118125.

43. S. Banerjee, A. Tiwari, A. Kar, et al., "Combining LC-MS/MS Profiles With Network Pharmacology to Predict Molecular Mechanisms of the Hyperlipidemic Activity of *Lagenaria siceraria* Stand," *Journal of Ethnopharmacology* 300 (2023): 115633, https://doi.org/10.1016/j.jep.2022.115633.

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