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

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1. Title of the thesis:

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1. Background

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. The northeastern region of 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.

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 schematic diagram of the plan of work has been illustrated in Figure 1.

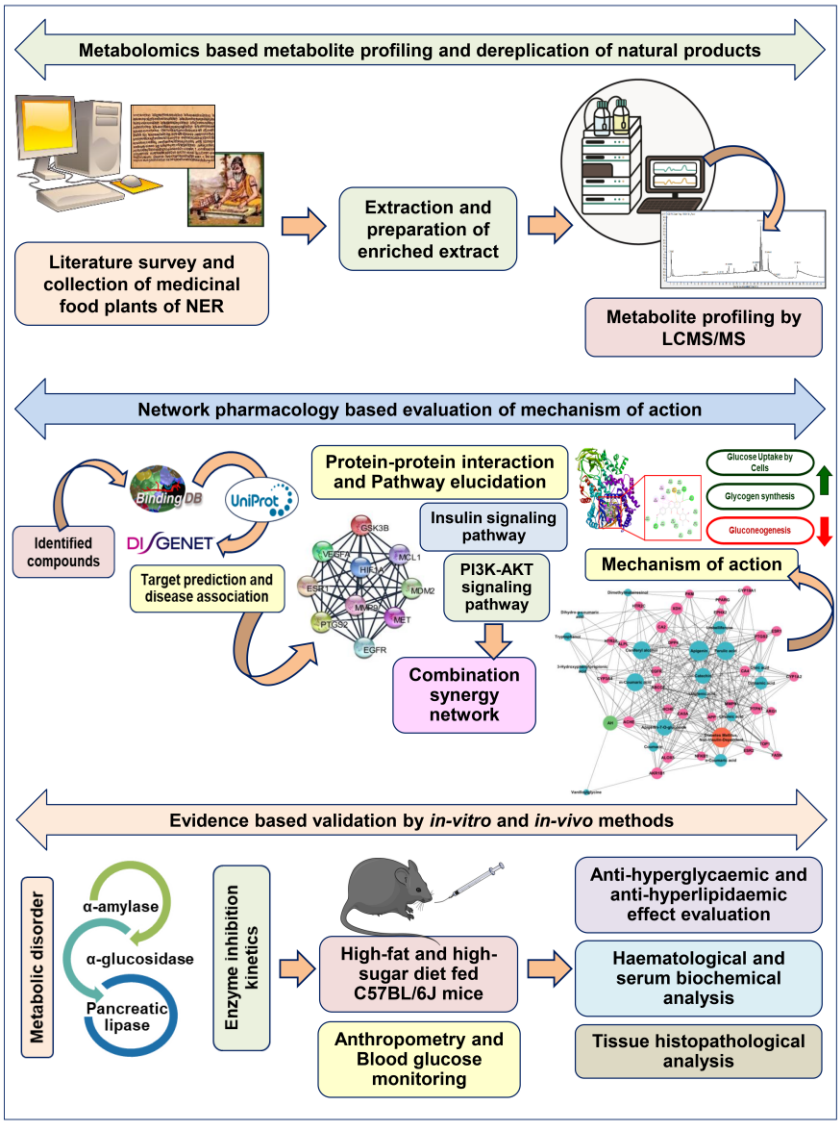

Figure 1. Schematic representation of the work plan

2. Work performed

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.

3. 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.

4. 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.

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