Therapeutic evaluation of medicinal plants as adaptogen from Cucurbitaceae family

Thesis Submitted By

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3. **Singha S**, Dasgupta B, Jana S, Debnath P, Kar A, Mukherjee P K. Metabolite analysis and *in-vitro* α-glucosidase enzyme inhibitory potential of *Trichosanthes dioica* Roxb. fruits. 8th Convention & National Seminar on "Ethnopharmacology for wellness: Tradition to Transition": CSIR-IICB, Kolkata, India, on December 10, 2021.

4. **Singha S**, Dasgupta B, Jana S, Gayen S, Chowdhury S, Kar A, Haldar P K, Mandal S C, Mukherjee P K. Assessment of In-Vivo Adaptogenic Activity of *Momordica* dioica Fruit Extract. 22nd International Congress of International Society for Ethnopharmacology (ISE) & 10th International Congress of the Society for Ethnopharmacology (SFE), India (ISE-SFEC, 2023): Imphal, Manipur, India during February 24- 26, 2023.

Statement of Originality

I, Seha Singha registered on 30.05.2018 to do hereby declare that this thesis entitled **"Therapeutic evaluation of medicinal plants as adaptogen from Cucurbitaceae family"** contains literature survey and original research work done by the undersigned candidates as part of Doctoral studies. All information in this thesis have been obtained and presented in accordance with existing academic rules 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 7%.

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Seha Singha

Declaration

I hereby declare that my research work embodied in this Ph.D (Pharmacy) thesis entitled **"Therapeutic evaluation of medicinal plants as adaptogen from Cucurbitaceae family"** have been carried out by me in the Department of Pharmaceutical Technology, Jadavpur University, Kolkata – 700032, India, under the direct supervision of **Prof. Pulok K Mukherjee**, Professor (on lien), Department of Pharmaceutical Technology, Jadavpur University, Kolkata – 700032, India & Director, Institute of Bioresources and Sustainable Development, Dept. of Biotechnology, Ministry of Science & Technology, Govt. of India, Takyelpat, Imphal-795001, Manipur, India. I also confirmed that this work is original and has not been submitted partly or in full for any other degree or diploma to this or other University or Institute.

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List of Abbreviations

Abbreviations	Full form	Abbreviations	Full form
DPPH	2,2-Diphenyl-1-Picrylhydrazyl	IC_{50}	50% inhibitory concentration
DNSA	3,5-Dinitro Salicylic Acid	IU/L	International Units Per Liter
4-NPA	4-Nitrophenyl Acetate	LOD	Limit of detection
p- NPG	4-Nitrophenyl-A-D-Glucopyranoside	LOQ	Limit of quantification
$AlCl_3$	Aluminium Chloride	LDL	Low-density lipoprotein
ANOVA	Analysis of Variance Test	*L. acutangula*	*Luffa acutangula* (L.) Roxb.
BuN	Blood urea nitrogen	LD_{50}	Median Lethal Dose
CO_2	Carbon dioxide	*M. dioica*	*Momordica dioica* Roxb. ex. Willd.
bCA	Carbonic anhydrase	NO	Nitric Oxide
bCA II	Carbonic anhydrase isozyme II from bovine erythrocytes	NBT	Nitro blue tetrazolium
CNS	Central nervous system	NADH	Nicotinamide adenine dinucleotide
CA	Chlorogenic acid	OD	Optical density
JNK	c-Jun N-terminal kinases	PL	Pancreatic lipases
µM	Micro Molar	PMS	Phenazine methosulfate
C. maxima	*Cucurbita maxima* Duchesne	p-NPC	p-nitrophenyl caprylate
Na_2HPO_4	Disodium hydrogen phosphate	RBC	Red Blood Cell
EDTA	Ethylenediaminetetraacetic acid	RSD	Relative standard deviation
EPM	Elevated plus maze	SALP	Serum alkaline phosphate
$FeCl_3$	Ferric chloride	SGOT	Serum Glutamic Oxaloacetic Transaminase
FC	Folin Ciocalteau reagent	SGPT	Serum Glutamic Pyruvic Transaminase
FST	Forced swimming endurance test	$NaHCO_3$	Sodium Bicarbonate
FTIR	Fourier transform infrared	NaH_2PO_4	Sodium dihydrogen phosphate
FFA	Free fatty acids	SAS	Sympatho adrenal system
GABA	Gamma-aminobutyric acid	TST	Tail Suspension test

H_2O_2	Hydrogen peroxide	TFC	Total Flavonoid Content
H_2SO_4	Sulfuric acid	TPC	Total Phenolic Content
Hsp70	Heat shock protein 70	*T. dioica*	*Trichosanthes dioica* Roxb.
HSF-1	Heat shock transcription factor 1	VLDL	Very low-density lipoprotein
RP-HPLC	Reverse Phase High Performance Liquid Chromatography	AA	α-amylase
HDL	High-density lipoprotein	AG	α-glucosidase
HPTLC	High-performance thin-layer chromatography	µg	micro gram
HPA	Hypothalamic–pituitary–adrenal	µL	Micro Litre

DEDICATED TO

MY BELOVED FAMILY

Chapter 1
Introduction

1.1 Adaptogen: Background and Perspective

Adaptogens are a group of herbal remedies frequently employed by herbalists across several traditional medicinal systems to help mitigate the detrimental effects of persistent stress on one's health (Amir et al., 2023). In 1958, the Soviet toxicologist Lazarev coined the name "adaptogen" to describe the synthetic stimulant 2-phenyl-imidazol. Lazarev hypothesized that adaptogens enhance the overall resilience of organisms when faced with stress, leading to improved endurance, stamina, and performance (Panossian et al., 2021). Figure 1.1 demonstrated the factors improved by Adaptogens.

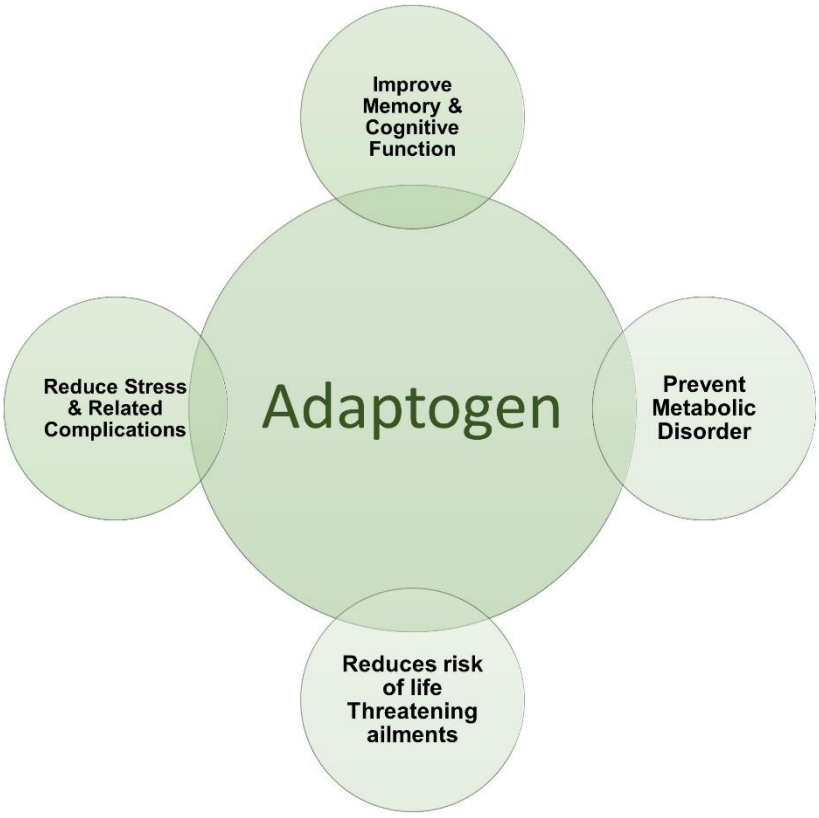


Figure 1.1. Factors Improved by Adaptogen

In 1969, Brekhman and Dardymov provided an additional definition of adaptogens derived from plants. Plant-originated adaptogens are defined by four criteria. Firstly, they must mitigate the adverse consequences of stressed states, for example exhaustion, infection, and depression. Secondly, they should be beneficial to stimulate the effects on the human body. Thirdly, unlike antidepressants, they should not induce

side effects like insomnia, reduced protein synthesis, or excessive energy consumption (Todorova et al., 2021). In the 1990s, a group of scientists conducted numerous investigations on adaptogens and put out the subsequent definition: Adaptogens are naturally occurring compounds that enhance the capacity to adjust to environmental variables and prevent the harm caused by such factors (Mendes, 2011). An adaptogen, as defined by the American FDA, is a specific metabolic modulator that has been scientifically proven to assist in adapting to the environment and safeguarding against external perils (Winslow and Kroll, 1998).

As general regulators, adaptogens can strengthen the body's defenses against a range of external stresses. Adaptogens primarily exert their effects via modulating the HPA axis in response to external stressors. Adaptogens have the ability to not only maintain or restore the balance of internal conditions in the body, but they can also enhance the process of rebuilding and repairing tissues (Panossian and Wikman, 2010). Another classification of adaptogens lacks the ability to directly impact the HPA axis. Nevertheless, these adaptogens possess the capability to regulate the immunological, neurological, and endocrine systems (Yance, 2013).

1.1.1 Functions of Adaptogen

1.1.1.1 Effect on adrenal fatigue

The adrenal glands activate a range of stress responses to many kinds of stress. Excessive stress can arise from either a single intense triggering event or the gradual build-up of chronic or recurring stress. Adrenal exhaustion impairs the adrenal gland's ability to maintain normal homeostasis. If the adrenal glands are able to effectively manage the situation affected by stress and maintain sufficiently high levels of cortisol to handle over time, symptoms of metabolic syndrome can be initiated (Panossian and Wikman, 2009). However, if the adrenal glands are unable to cope with the demands placed on them, adrenal fatigue may occur. This condition typically develops more rapidly than metabolic syndrome and can become so severe that it impairs body's functioning (Wilson, 2014). Adaptogens have the ability to boost the capacity for adrenal gland secretion, and this turn eliminates excessive stress hormone production (Bhatia et al., 2011).

In a study Gaffney and his researchers reported that *Panax ginseng* has the ability to inhibit 11-beta hydroxysteroid dehydrogenase and *E. senticosus* can inhibit catechol-O-methyl transferase, located near stress hormone receptors and are responsible for breaking down stress hormones into inactive forms (Gaffney et al., 2001). Adaptogens can expedite the closure of the adrenal gland when stress is not present. Moreover, adaptogens have the ability to enhance cellular energy production and protect against oxidative harm, thus promoting the proper functioning of the adrenal glands (Liao et al., 2018).

1.1.1.2 Effect on inflammatory disorder

Arthritis arises from tissue damage and joint disorders, generally accompanied by pain, swelling and inflammation. Osteoarthritis and rheumatoid arthritis are the predominant forms of arthritis. Fibromyalgia may coexist with arthritis, although it is not classified as a kind of arthritis due to its lack of inflammation or joint damage (Smolen et al., 2018). Adaptogens have the ability to significantly diminish inflammation and alleviate pain associated with arthritis (Liao et al., 2018).

1.1.1.3 Effect on Sleep and Cortisol

A significant number of individuals experience insomnia along with other sleep-related disorders. Ambient stressors disrupt regular release of circadian cortisol, a primary factor contributing to sleep-related issues. Cortisol secretion is regulated by the body's own biological clock and external circadian rhythms. Engaging in appropriate physical activity, adhering to a balanced and nutritious eating plan, and ensuring sufficient rest helps effectively regulate cortisol levels inside the human body (Potter et al., 2016). Various adaptogenic plants including *Eurycoma longifolia, Gynostemma Pentaphyllum, Lepidium peruvianum, Ocimum tenuiflorum* exhibited significant elevated cortisol level lowering at the morning time (Tóth-Mészáros et al., 2023).

1.1.1.4 Effect on neuroendocrine system

Adaptogens play a crucial role in stabilising the human body by influencing the neuroendocrine system (Panossian and Wagner, 2010). Plant-derived adaptogens contain compounds that improve the capacity to adjust to external conditions and

prevent harm (Carlini, 2003). Adaptogens can easily influence neuroendocrine system as well as cellular energy system and increase the efficiency of oxygen, nutrient, sugar and lipid utilisation (Liao et al., 2018).

1.1.1.5 Effect on Tumor growth

Adaptogens exhibited antiproliferative activity by suppressing neoplasm growth and possessing anticancer activity, maintaining physiological function, and aiding cell repair (Wiegant et al., 2009). The immunological processes are associated with the antiproliferative attributes of adaptogens. Adaptogens have the ability to stimulate various immune cells that include macrophages, T-lymphocytes, and NK cells, helps to prevent proliferation of tumours and promote cell-specific apoptosis (Yang et al., 2023).

1.1.2 Mechanism of action of Adaptogen

Adaptogens have the ability to trigger the modification of diverse responses in order to deal with different types of stress, influence the immune system (Panossian et al., 1999). The human stress response system comprises the CNS linked to corticotropin releasing hormone (CRH), arginine vasopressin (AVP), and the adrenalin nucleus, additionally the distal regions of the brainstem, the HPA axis, and the peripheral nervous system (Smith and Vale, 2006). The primary regulatory system for responding to external stressors includes CRH, AVP, catecholamine neurons, and other cellular tissues. The HPA axis and sympathetic nervous system (SNS) are the components that reflect the different branches of this system (Chrousos, 2009). CRH and catecholamine neurons engage in mutual interaction. The SNS and HPA system have interrelated functions and are closely related in terms of their anatomical structure. When reacting to the external environment, these systems can engage with each other at many stages. As an instance, catecholamine may trigger the HPA axis by emitting CRH, and the hormone generated by the HPA axis can affect the SNS (Panossian and Wagner, 2010). Recent research has indicated that the suppressive effects and prolonged increase in levels of naturally occurring glucocorticoids lead to stimulating effects that are regulated by the SNS during times of stress. The release of CRH and arginine vasopressin (AVP) is enhanced in response to external stress, leading to an increase in the secretion of cortisol and ACTH. SNS enables the human body to rapidly react to

external stressors. Besides catecholamine, the SNS and PSNS are capable of releasing several neuropeptides, ATP and NO (Herman et al., 2016). When the HPA axis is adjusted, an increase in energy circulation and regulation, a decrease in the perception of external pressure, an enhancement of resistance, and an improvement in mental concentration, is observed (Panossian et al., 2007). Adaptogens do not elevate the levels of cortisol and NO in the human system when exposed to acute physiological stressors (Darbinyan et al., 2000). Adaptogens have the ability to resist and withstand stress by stimulating the release of cortisol and NO in the bloodstream and saliva. This enables the body to adapt to higher levels of stress. Adaptogens can also enhance the production of stress-activating messenger molecules that involve NO and inhibit the release of cortisol (Liao et al., 2018). The proposed mechanism of action of adaptogen is illustrated in figure 1.2.

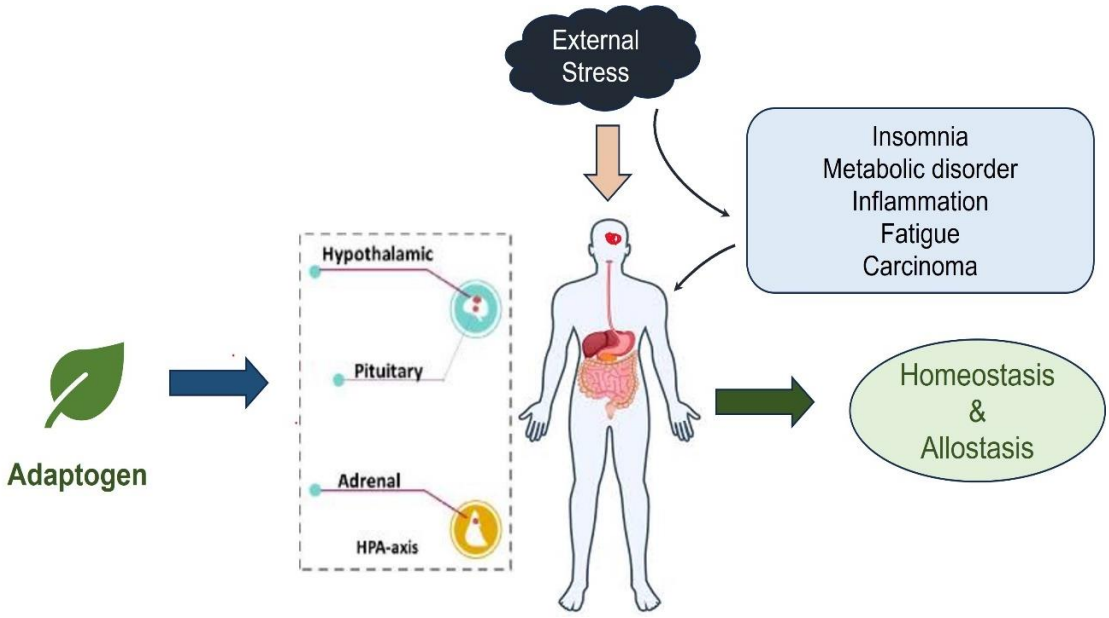


Figure 1.2. Mechanism of action of Adaptogen on Human Body

1.2 Stress and Physiological Response

Stress is a multifaceted and intricate phenomenon that involves different elements that initiate triggering events and elicit responses in the brain. The reactions might be emotional, biological, or physical, and they differ across individuals due to genetic and environmental influences. These responses frequently impact eating patterns. The stress response is a process that allows for adaptation. Typically, adaptation is physiologically suitable, but in certain instances, it can lead to harmful outcomes. When stress levels are minimal, the body typically achieves homeostasis, a state in which all bodily systems function harmoniously to maintain equilibrium. Stressors elicit a physiological response that puts the body in a state of crisis. Subsequently, the body tries to restore balance by employing an adaptive response to return to equilibrium. The phenomenon of returning to a state of equilibrium in reaction to a stressor is known as the general adaptation syndrome (GAS) (Yaribeygi et al., 2017). The GAS consists of three phases: alert, resistance, and weariness. This results in a multitude of physiological alterations within the body. Stress is commonly referred to as a condition that involves extended arousal, which triggers a series of detrimental health consequences that become more likely the longer stress persists. Almost, every bodily system can be vulnerable to attack, and the long-term consequences can be extremely destructive.

Hans Selye developed a model in which stress is conceptualised as a sequential process consisting of an initial triggering event, followed by a compensatory reaction, and ultimately resulting in the formation of a new state of homeostatic equilibrium (Tan and Yip, 2018). Figure 1.3 depicts a modified representation of Selye's stress curve. Stress can be defined as the physiological and psychological reaction of the body to conditions that present challenges, limitations, or possibilities. A stressor, on the other hand, refers to the specific stimulus that triggers a stress response. Stressors can be either tangible or perceived, either from within oneself or from external sources. The overall influence of a stressor will be contingent upon its specific attributes and the attributes of those individuals who have been impacted. Stress is frequently associated with ideas such as adaptability and anxiety (Tapas and Price, 2001).

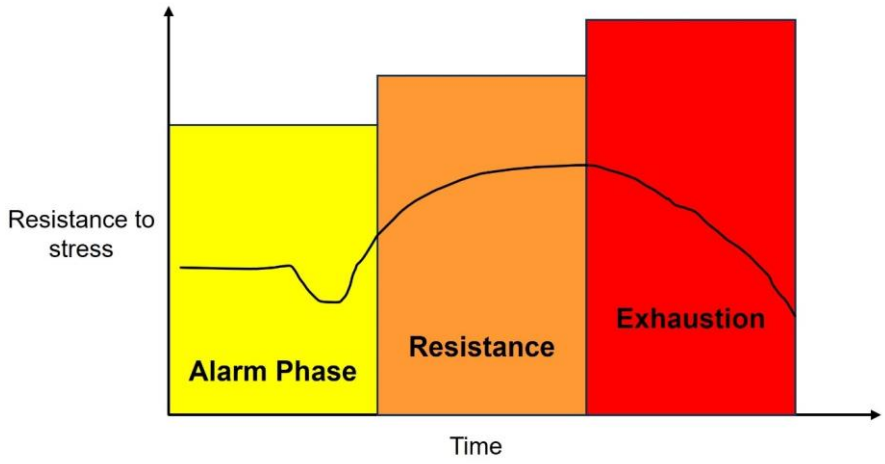


Figure 1.3. Selye's stress curve

Exposure to stressors triggers a sequence of synchronised reactions designed to increase the likelihood of survival. The coordinated reactions in the body, commonly known as stress responses, involve changes in conduct, autonomic functioning, and release of oxytocin, prolactin, corticosterone, catecholamine, ACTH, and renin (Smith and Vale, 2006). Physiological changes linked to the stress reaction encompass the process of gathering energy to sustain the functioning of the brain and muscles; increasing and concentrating attention on the apparent danger, elevating blood flow to the brain and enhancing the use of glucose in certain areas of the brain; Augmenting cardiac output and respiration, altering immunological function along with reducing food intake and diminished appetite (Bryan, 1990).

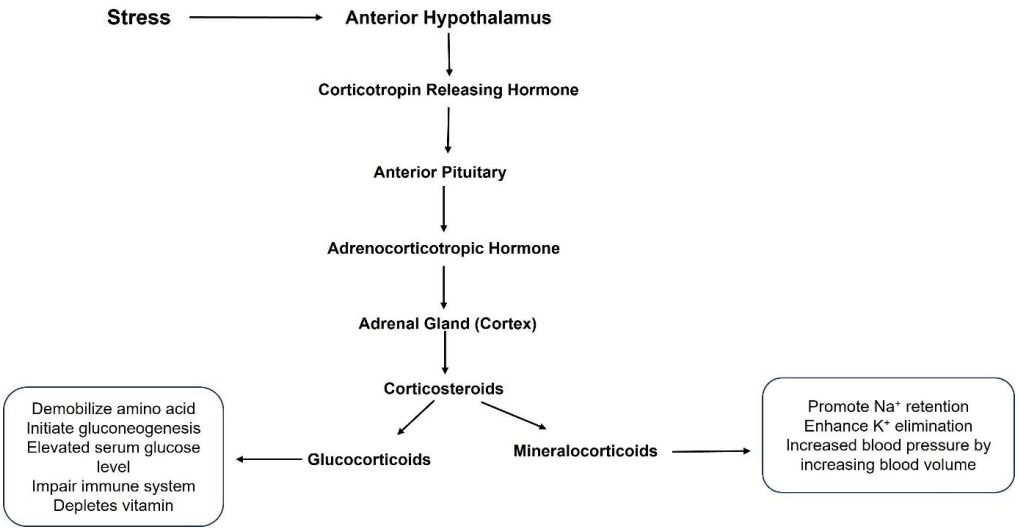


Figure 1.4. Physiological response to Stress

1.2.1 Stress and Metabolic Disorder

Chronic stress is associated with higher rates of illness and death. This is believed to happen because the body's stress response systems, known as the SAM and HPA responses, can be beneficial in the short term but can cause damage to organs and tissues over time, increasing the risk of disease (McEwen and Stellar, 1993). The frequent and persistent disruption of metabolic equilibrium, due to stress can susceptible to negative outcomes (Ryan et al., 2014). Long-term stress affects eating patterns and contributes to being obese. It is also considered as a significant factor to develop several metabolic disorders: cardiovascular disease (CVD), Type-2 diabetes mellitus, and polycystic ovarian syndrome (Fruh, 2017).

Glucocorticoids control the ingestion of food and the accumulation of fat. The signalling of the glucocorticoid receptor in the CNS leads to an increase in calorie intake and induces weight gain (Kuckuck et al., 2023). The presence of elevated glucocorticoid levels resulting from stress allows for the facilitation of lipolytic effects also caused by catecholamines, which directly promote the release of free fatty acids and glycerol into the bloodstream from fat deposits. This can occur due to increased blood flow through adipose tissue or through the stimulation of adipose-β2 adrenoceptors (Stimson et al., 2017). Adrenaline and cortisol prohibit lipoprotein lipase from working, which makes it harder for the body to get rid of triglycerides. This makes HDL levels drop and VLDL, IDL, and LDL levels rise in the blood (Maduka et al., 2015). Furthermore, stress prompts individuals to choose high-calorie, delicious foods instead of healthier, less satisfying options, which worsens its negative impact on metabolism (Yau and Potenza, 2013). Stress enhances the likelihood of both mice and humans to engage in work for enjoyable food. It also heightens the activity of neurons in the brain's motivation and reward circuitry when exposed to pleasurable foods. Significantly, the act of consuming enjoyable meals as a result of stress can happen even among individuals who reduce their overall calorie intake in reaction to stress (Morales and Berridge, 2020). Additionally, chronic stress store fat in abdomen due to glucocorticoids in the bloodstream. Visceral depots are believed to have a stronger response to adrenergic stimulation, leading to increased breakdown of fat. This suggests that chronic stress-induced fat redistribution may be a mechanism that allows the body to quickly release fatty acids in response to new stressful situations. Regrettably, the redistribution of fat

can lead to long-term pathophysiological effects. This is because an increase in visceral adiposity can contribute to metabolic disorders by causing fat to accumulate in the liver and vascular tissues (van der Valk, 2018).

Type-2 DM is a persistent metabolic condition caused by abnormalities in both the production and function of insulin. Psychological maltreatment from cohabitating partners greatly heightens the likelihood of developing Type-2 DM (Galicia-Garcia et al., 2020). In addition, brief exposure to psychological stress negatively affects the ability of diabetic individuals to remove glucose from their bloodstream after a meal. Chronic stress can contribute to insulin resistance by the direct effects of glucocorticoids, which cause cellular insulin resistance, accumulation of fat around internal organs including liver (Sharma et al., 2022). Hyperinsulinemia directly related to a spike in the release of catecholamines and allows cortisol and glucagon to have a greater impact to stimulate the activity of phosphatidate phosphohydrolase which affect the hepatic triglyceride production (Janssen, 2022). Metabolic diseases contribute to the heightened production of reactive oxygen species (ROS) in the body. Oxidative stress is a cellular event that happens at the molecular level when there is an imbalance between the production of ROS and the ability of the antioxidant defence systems to neutralise them (Pizzino et al., 2017). Figure 1.5. demonstrated the effect of stress in developing metabolic disorder.

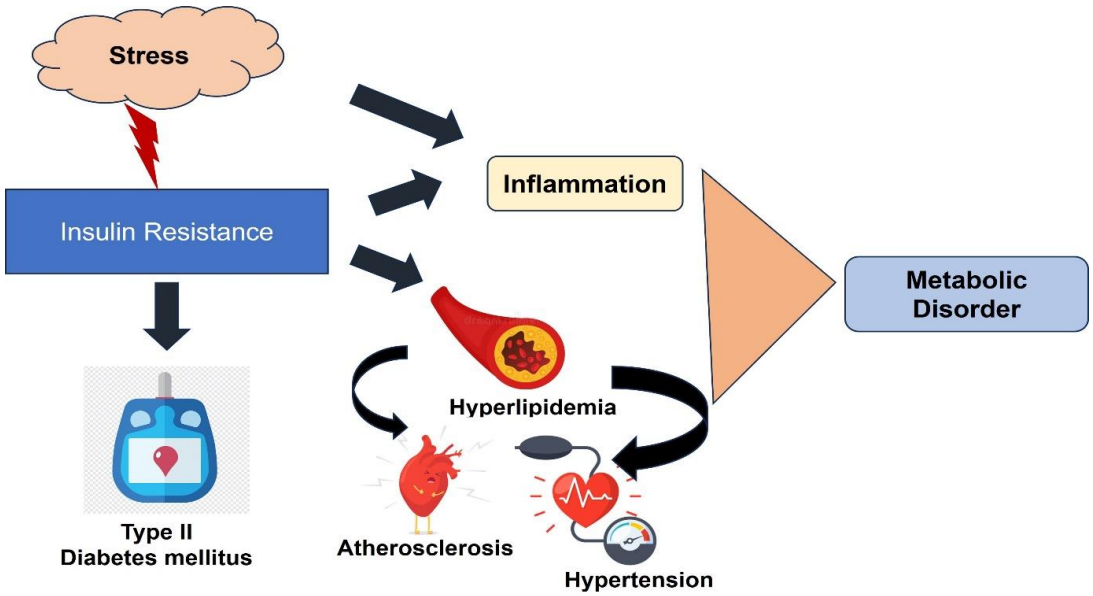


Figure 1.5. Effect of stress in developing metabolic disorder

1.3 Management of Stress using Adaptogen

Effectively managing stress can be a potent strategy for maintaining good health. Researchers have been examined the connections between stress and several medical conditions. Due to its association with various metabolic disorders, including obesity the impact of stress on individuals' eating behaviours, including the quantity and quality of food consumed should maintained (Kumar et al., 2022). Stress leads to increased physiological requirements including energy, oxygen, circulation, and consequently a greater amount of metabolic cofactors.

The role of particular nutrients in regulating food intake, maintaining homeostatic mechanisms, and influencing emotional processes is highly complex (Lopresti, 2020). 5-hydroxytryptamine is produced from the essential amino acid tryptophan found in diet. In addition, tyrosine serves as a precursor for the production of noradrenaline (NA). Psychosocial and physical stress lead to an elevated release of NA in both the nervous system, resulting in an increased need for protein, particularly tyrosine. Similarly, a range of other essential nutrients are necessary to decrease the concentrations of stress-inducing hormones that trigger the fight or flight response (Maffei, 2020). Plant adaptogen is very much acceptable now a days due to its direct effect on homeostasis and improve human well-being. Adaptogens not only reduces the stress but it also reduces the risk of developing metabolic disorder related ailments (Todovora et al., 2021). The list of plants with adaptogenic activity mentioned in Table 1.1.

Table 1.1. List of the plants possess adaptogenic activity

Plant	Part used	References
Aegle marmelos	Whole plant	Duraisami et al., 2010
Alstonia scholaris	Dried Bark	Kulkarni et al., 2008
Allium sativum	Bulb	Roshan et al., 2010
Annona muricata	Stem, Bark	Padma et al., 2001
Argyreia speciosa	Root	Habbu et al., 2010
Asparagus racemosus	Root milk	Garg and Gupta, 2010
Atragene sibirica	Aerial Part	Shilova et al., 2001
Azadirachta indica	Leaves	Yanpallewar et al., 2003

Bacopa moniera	Aerial Part	Gohil et al., 2010
Bergenia crassifolia	Fermented Leaves	Alexander et al., 2010
Boerhaavia diffusa	Root	Desai et al., 2011
Butea monosperma	Aerial Part	Pawar and Shivakumar, 2012
Caesalpinia bonduc	Seed	Kannur et al., 2006
Carum carvi	Fruit	Koppula et al., 2009
Centella asiatica	Leaves	Barbosa et al., 2008
Convolvulus pluricaulis	--	Agarwal et al., 2014
Chlorophytum Borivillianum L.	Leaves	Gopalkrishna et al., 2006
Chlorophytum borivilianum	Tuber	Deore and Khadabadi, 2009
Chrysactinia mexicana	Aerial Part	Cassani et al., 2015
Cicer arietinum	Fruit	Singh et al., 1983
Cnestis ferruginea	Root	Ishola and Ashorobi, 2007
Convolvulus pluricaulis	Root	Gupta and Fernandes, 2019
Curculigo orchioides	Aerial Part	Ramchandani et al., 2014
Curcuma longa	Rhizome	Mohanty et al., 2004
Dioscorea deltoidea	--	Volkova et al., 2013
Emblica officinalis	Fruit	Pareek et al., 2017
Eucommia ulmoides	Bark	Oshima et al., 1988
Evolvulus alsinoides	--	Siripurapu et al., 2005
Fagopyrum esculentum	Aerial Part	Kothiyal and Ratan, 2011
Hibiscus cannabinus	Leaves	Natraj et al., 2011
Hippophae rhamnoides	Leaves	Saggu et al., 2007
Hypericum perforatum	Aerial Part	Kumar et al., 2010
Labisia pumila	Leaves	Sarang et al., 2010
Lagenaria siceraria	Fruits	Lakshmi and Sudhakar, 2010
Mitragyna africanus	Stem bark	Aji et al., 2001
Momordica charantia	Fruit	Sumanth and Chaudhary, 2009
Morus alba	Root	Nade Vanda et al., 2009
Murraya koenigii	--	Saraf et al., 2011
Mussanenda frondosa	Root	Koul and Chaudhary, 2011

Nigella sativa	Seed	Khan et al., 2011
Oscimum santum	Leaves	Anju, 2012
Panax -ginseng	Root	Rai et al., 2003
Pandanus odorifer	Leaves	Adkar et al., 2014
Paullinia cupana	--	Espinola et al., 1997
Piper longum	Fruit	Yadav et al., 2015
Pueraria tuberosa	Tuber	Pramanik et al., 2010
Rhodiola crenulate	--	Kokoska and Janovska, 2009
Rubia cordifolia	Root	Patil et al., 2006
Salvia miltiorrhiza	Leaves	Tsai et al., 2012
Sida cordifolia	Root	Sumanth and Mustafa, 2009
Solanum torvum	Seed	Mohan et al., 2013
Terminalia chebula	Fruit	Belapurkar et al., 2014
Trichopus Zeylanicus	Whole plant	Singh et al., 2001
Trigonella foenom	Seed	Pawar et al., 2012
Tylophora indica	Root	Kulkarni et al., 2010
Vitis vinifera	Seeds	Satyanarayana et al., 2005
Withania somnifera	Root	Tiwari and Sahni, 2012
Zingiber officinale	Rhizome	Lakshmi and Sudhakar, 2010

1.4 Cucurbitaceae: Therapeutically Important Plant Family

The Cucurbitaceae plant family is an extensive family of food crops consisting of 115 genera and 960 species. The plants belonging to this family are primarily herbaceous annual vines or perennial lianas, often equipped with tendrils (Mukherjee et al., 2022). Ayurveda and other traditional Indian health systems acknowledge the therapeutic significance of this family plants, which could be explored as a promising resource for the creation of reliable and efficient treatments (Mukherjee, 2019). The plants can be either monoecious or dioecious and they possess hermaphroditic characteristics; predominantly found in tropical and subtropical regions. The Cucurbit are renowned for their bicollateral vascular bundles, which consist of phloem on both the outer and inner sides of the xylem. The several components of the plants belonging to the Cucurbitaceae family have been widely utilised in culinary uses from ages past. The

Cucurbitaceae family can be further divided into two main subfamilies, Cucurbitoideae and Zanonioideae, based on their morphological traits. The majority of edible varieties have their origins in the subfamily Cucurbitoideae, which can be further categorised into 15 tribes and allied genera. There are four specific tribes, namely Benincaseae, Cucurbiteae, Momordiceae, and Sicyoeae, that primarily cultivate edible cucurbitaceae species in the Indian subcontinent (Renner et al., 2013). The members of the Cucurbiteae tribe produce economically valuable fruits, called gourds, which include crops like squashes, luffas, and melons whereas the Benincaseae tribe contains a genus called Lagenaria. A details list of different tribes and genera of Cucurbitaceae family has been described in Table 1.2. A lot of medicinal benefits of the family cucurbitaceae have been noticed, among which the most widely cultivated are *Benincasa hispida, Benincasa fistulosa, Coccinia grandis, Luffa acutangula, Luffa Cylindrica, Momordica dioca, Momordica charantia, Cucumis sativus, Cucurbita maxima, Cucumis melo, Lagenaria siceraria, Citrulus lanatus, Trichosanthes cucumerina, Trichosanthes dioica, Scehium edule* etc. (Dhiman et al., 2012, Saboo et al., 2013). In figure 1.6. some selected food plants of the Cucurbitaceae family were represented.

1.4.1 Geographical Distribution

The majority of species in this family are found in Southeast Asia, Africa, Madagascar, Central and South America (Saboo et al., 2013; Avinash and Rai, 2017). The Cucurbitaceae family has its origins in India and other regions of southern Asia. India's tropical and subtropical regions, make it highly conducive for farming. The cultivation of Cucurbitaceous food plants is mostly concentrated in the states of West Bengal, Uttar Pradesh, Madhya Pradesh, Gujarat, Bihar, Tamil Nadu, Karnataka and Maharashtra (Renner et al., 2013). Furthermore, in the foothills of the Himalaya Mountains some other species of Cucurbitaceae can be found. These species cannot be cultivated as it is found in wild, bitter taste and full of thorns like characteristics present in the fruit. In China and the Middle East Asia some varieties of plant of Cucurbitaceae family can be found (Aryal et al., 2018).

Cucurbit plants thrive in warm, sandy soil filled with nutrients and moisture. Optimal plant growth of Cucurbitaceae seeds is achieved by faster germination in warmer temperatures ranging from 25 to 40ºC. Most cucurbitaceous plants require well-irrigated

and fertilised land with ample vertical and horizontal growing rooms for their development. Cucurbitaceae plants require extended periods of daylight, well drained lands, and ample moisture in order to grow. The development of certain plants in the cucurbitaceae family can be managed by employing fences, trellises, or other vertical structures that facilitate the climbing of the vine, thereby promoting their productive growth. It is advisable to prevent exposure to frost when cultivating cucurbitaceous crops, as this might have severe negative effects on the plants (McCreight, 2017). The geographical distribution of the plants belonging to the cucurbitaceae family has been documented in Table 1.2.

Table 1.2. Tribes of some edible fruits of Cucurbitaceae family (Renner and Pandey 2013)

Tribe	Binomial name	Common name	Geographical distribution in Indian State	Geographical distribution outside India
Benincaseae	*Benincasa hispida*	Wax Gourd	Tropical & Subtropical regions of India	Pakistan, Malayasia, Eastern Australia, Polynesia, China, Japan
	Benincasa fistulosa	Apple Gourd	Punjab, Rajasthan, Uttar Pradesh	Tropical Africa

	Citrullus colocynthis	Bitter Apple	Andhra Pradesh, Assam, Bihar, Jharkhand, Delhi, Goa, Gujarat, Karnataka, Kerala, Maharashtra, Odisha, Punjab, Rajasthan, Tamil Nadu, Uttar Pradesh	Afghanistan, Myanmar, Pakistan, Sri Lanka, west to the Sahara (Lybia) and Sahel region
	Citrullus lanatus	Watermelon	Andaman & Nicobar Islands, Assam, Bihar, Jahrkhand, Delhi, Gujarat, Karnataka, Madhya Pradesh, Maharashtra, Punjab, Rajasthan, Tamil Nadu, Tripura, Uttar Pradesh, Uttarakhand, West Bengal	Nepal, Pakistan, native to tropical Africa
	Coccinia grandis *Coccinia indica* *Coccinia palmate*	Ivy Gourd	Andhra Pradesh, Assam, Bihar, Kerala, M.P., Maharashtra, Manipur, Odisha, Rajasthan, Tamil Nadu, W.B., U.P., Gujrat, Goa	Africa, China, Japan, Malesia, Myanmar, Pakistan, Sri Lanka
	Cucumis hystrix		Arunachal Pradesh, Assam,	Myanmar, North and West Thailand, South

			Meghalaya, Mizoram	West China
	Cucumis indicus		Kerala, Maharashtra	---
	Cucumis melo	Muskmelon	Andhra Pradesh, Assam, Karnataka, Madhya Pradesh, Maharashtra, Manipur, Rajasthan, Tamil Nadu, Uttar Pradesh	Widely cultivated
	Cucumis sativus	Cucumber	Northern India (Ganges region)	Bhutan, China, Myanmar, Nepal, Thailand
	Cucumis javanicus		Assam	Java, China, and Thailand
	Cucumis ritchiei	Ritchie melon	Karnataka, Kerala, Maharashtra, Punjab, Tamil Nadu	---
	Lagenaria siceraria	Bottle gourd	Throughout India	Native of tropical Africa
Cucurbiteae	*Cucurbita ficifolia*	Figleaf gourd	Andhra Pradesh, Arunachal Pradesh, Assam, Bihar, Goa, Gujarat, Karnataka, Kerala Maharashtra, Rajasthan, Tamil Nadu, Tripura,	Native to Mesoamerica or northern South America, widely cultivated
	Cucurbita maxima	Pumpkin		Native to Central America
	Cucurbita	Crookneck		Native to Central

	moschata	pumpkin	Uttar Pradesh, Uttarakhand, West Bengal	or South America
	Cucurbita pepo	Field Pumpkin		
	Cucurbita argyrosperma	Cashew pumpkin		Native to Mesoamerica
Sicyoeae	*Luffa acutangula*	Ridge gourd	Native and cultivated throughout India	Cultivated globally
	Luffa cylindrica	Sponge Gourd		
	Luffa echinata	Bitter sponge gourd	Assam, Bihar, Gujarat, Himachal Pradesh, Madhya Pradesh, Maharashtra, Tamil Nadu, Uttar Pradesh, Uttarakhand, West Bengal	Wild from Egypt to Nigeria
	Luffa graveolens		Bihar, Maharashtra, Sikkim, Uttar Pradesh	Nepal
	Trichosanthes cordata		Andhra Pradesh, Arunachal Pradesh, Assam, Bihar, Chhattisgarh, Jharkhand, Madhya Pradesh, Manipur, Meghalaya, Mizoram, Nagaland, Rajasthan, Sikkim, Tamil	Bangladesh, Bhutan, China, Myanmar, Nepal

			Nadu, Tripura, Uttar Pradesh, Uttarakhand, West Bengal	
	Trichosanthes cucumerina	Snake gourd	Native and cultivated throughout India	Sri Lanka, tropical China through Malesia, West, North and North East Australia
	Trichosanthes dioica	Pointed gourd	Arunachal Pradesh, Assam, Bihar, Delhi, Himachal Pradesh, Jammu & Kashmir, Meghalaya, Punjab, Rajasthan, Uttar Pradesh, West Bengal	Bangladesh, Myanmar, Nepal, Pakistan, Sri Lanka
	Trichosanthes lobata	--	Andhra Pradesh, Karnataka, Kerala, Puducherry, Tamil Nadu, Uttar Pradesh, West Bengal	China
	Trichosanthes ovigera	--	Andaman & Nicobar Islands, Arunachal Pradesh, Assam, Meghalaya, Sikkim, Tripura, Uttar Pradesh, West Bengal	Australia, Bangladesh, China, Japan, Java, Myanmar, Nepal

	Trichosanthes tricuspidata	--	West Bengal	Myanmar, Thailand, Vietnam
	Trichosanthes truncata	--	Andhra Pradesh, Arunachal Pradesh, Assam, Meghalaya, Sikkim, West Bengal	Bangladesh, Bhutan, China, Thailand, Vietnam
Momordiceae	*Momordica balsamina*	Balsam apple	Gujarat, Haryana, Rajasthan	Native in the dry savannas of Southern Africa and northern margin of the tropical belt, tropical Asia, Americas, Pacific islands
	Momordica charantia	Bitter melon	Western and Eastern Ghats, all over Central and South India	Native in tropical and subtropical Africa, tropical Asia
	Momordica cochinchinensis	Gac	Andaman & Nicobar Islands, Arunachal Pradesh, Assam, Bihar, Karnataka, Manipur, Nagaland, Tripura, Uttar Pradesh, West Bengal	West to New Guinea, Southeast Australia
	Momordica cymbalaria		Andhra Pradesh, Karnataka, Madhya	North and East Africa

			Pradesh, Maharashtra, and Tamil Nadu	
	Momordica dioica	Spine gourd	Deccan plateau and Central India	Bangladesh, China, Myanmar, Nepal, Pakistan
--	*Sechium edule*	Winter Squash. chayote	Northeast India	Native to Mexico, cultivated throughout the tropics

1.4.2 Morphological Characteristics

Cucurbitaceae plants are predominantly herbaceous vines that can be either annual or perennial. They have weak stems that trail or lie flat on the ground, and they typically climb using tendrils. The leaves and stems of these plants contain a substantial amount of watery sap. The roots consist of a taproot that branches out and becomes thickened as a result of storing food and water. The stem of this plant is herbaceous and has the ability to ascend using tendrils or trail along the ground. It also has the ability to root at nodes and has an angular shape. Leaves are alternating leaves, typically uncomplicated but frequently deeply split or lobed and having veins that radiate from a central point, forming a network-like pattern. The petiole, or leaf stalk, is elongated and hollow. Tendrils can be either simple or branching and emerge from the axil or opposite to the leaf at the node (Dhiman et al., 2012). The species' floral characteristics display a diverse array of inflorescence, with flowers that are often solitary, large, and visually striking. These flowers can be found either individually or in racemes, cymes, or panicles. They may also be unisexual, with both male and female flowers present on the same plant (monoecious), as seen in species like Luffa and Cucumis, or on separate plants (dioecious), as seen in species like Trichosanthes. The blooms are regular, unisexual, occasionally bisexual, varying in size from tiny to large, and can be either white or yellow. They are epigynous in nature. The male blooms are often produced in far greater quantities and have a bell-shaped structure.

The calyx consists of five sepals that are united, pointed, petal-like, bell-shaped, and have an imbricate aestivation. The corolla is composed of five petals, which can either be united or separate (as seen in Luffa). The petals are generally deeply divided into five lobes and are arranged in a valvate, imbricate manner. They are placed on the calyx tube, and in the case of the free form of corolla, they can be either bell-shaped (campanulate) or wheel-shaped (rotate). The androecium is composed of either 5 or sometimes 3 stamens, which may be separate or fused together to create a central column that is attached to the calyx tube. The number of female flowers is lower than that of male blooms. The fruits are characterised by their soft, fleshy nature and are typically indehiscent, meaning they do not naturally split open. In certain cases, these fruits can reach huge sizes (Saboo et al., 2013). Figure 1.6 displays a collection of specific plants belonging to the Cucurbitaceae family.

A. *Lagenaria siceraria* (Bottle Gourd)

B. *Momordica charantia* (Bitter Gourd)

C. Momordica dioica (Spine Gourd)

D. Luffa acutangula (Ridge Gourd)

E. *Benincasa hispida* (Wax Gourd)

F. *Benincasa fistulosa* (Apple Gourd)

G. *Scheium edule* (Squash)

H. *Cucumis sativus* (Cucumber)

I. *Cucumis melo* (Melon)

J. *Citrullus lanatus* (Watermelon)

K. *Coccinia grandis* (Ivy Gourd)

L. *Trichosanthes dioica* (Pointed Gourd)

M. *Trichosanthes cucumerina* (Snake Gourd)

N. *Cucurbita Maxima* (Pumpkin)

Figure 1.6. (A-N) Some selected food crops of Cucurbitaceae family

1.4.3 Phytochemistry

The phytoconstituents found in plant species can be categorised primary and secondary metabolites. Primary metabolites possess nutritional value, while secondary metabolites are responsible for their therapeutic effects. Plants in the Cucurbitaceae family are highly valuable for their medicinal properties due to the presence of various beneficial compounds such as phenolics, flavonoids, tannins, cardiac glycosides, carbohydrates, resins, saponins, carotenoids, and particularly triterpenoid cucurbitacins. The subsequent sections have provided a comprehensive discussion on the characteristics and functions of primary and secondary metabolites. In figure 1.7. phytochemicals present in Cucurbitaceae family depicted.

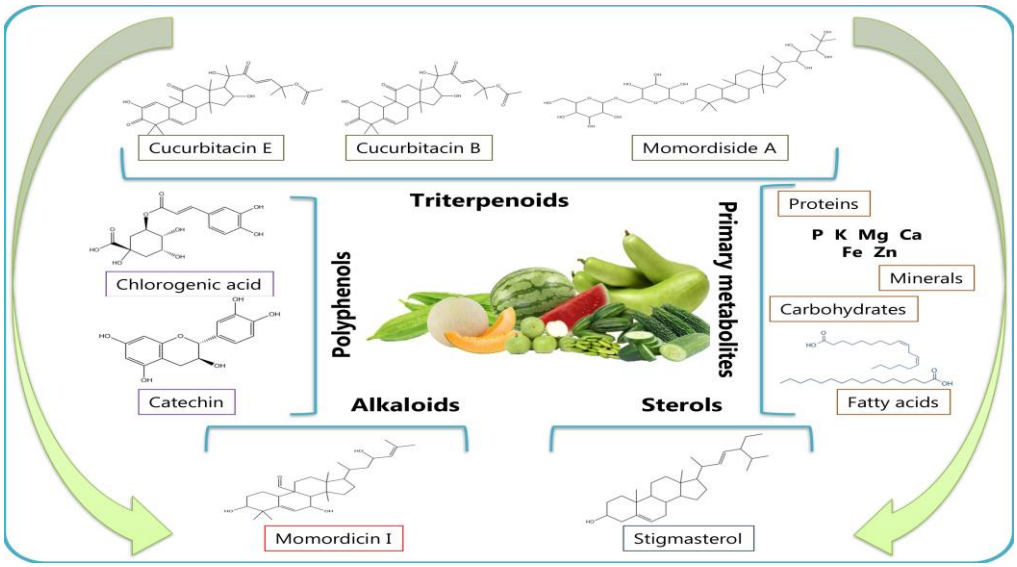


Figure 1.7. Phytochemicals Present in Cucurbitaceae family

1.4.3.1 Primary Metabolites

The plants belonging to the Cucurbitaceae family include a variety of amino acids and proteins that possess different pharmacological properties. The majority of plant-derived proteins exhibit significant efficacy against certain pathogens associated with fungal infections (Brederode et al., 1991). Protein derivatives, namely charantin and viciline, which were found in *Momordica charantia*, exhibited anti-diabetic and insulinomimetic properties. A novel ribosome inactivating peptide called Luffangulin was identified in *Luffa acutangula*. This peptide has been found to possess therapeutic effects (Ng and Prakah 2002; Wang et al. 2012). *Cucurbita maxima* is a rich source of vitamin A (Ragasa et al., 2014); on the contratry *Momordica dioica*, *Cucumis sativus*, and *Citrullus lanatus* contain relatively small levels of vitamin (Bawara et al., 2010). Furthermore, various kinds of Cucurbitaceae plants have been shown to contain vitamin C, thiamine, riboflavin, niacin, vitamin B-6, D, F, E and K (Shah and Seth, 2010; Avinash and Rai, 2017). *Tricosanthes cucumerina* and Coccinia indica have a certain quantity of vitamin C (Adebooye, 2008). The leaves of *Momordica charantia* are rich in calcium, magnesium, potassium, phosphorus, and iron, as stated by Kumar et al., in 2010. On the other hand, *Cucurbita pepo* includes phosphorus, potassium, magnesium, calcium, iron, zinc, and other minerals. According to Hashash et al. (2017), the seeds and fruit pulps of *Cucurbita pepo* have been found to contain significant amounts of potassium (K) and sodium (Na). The seeds of *Cucurbita pepo* and *Cucurbita maxima* have a

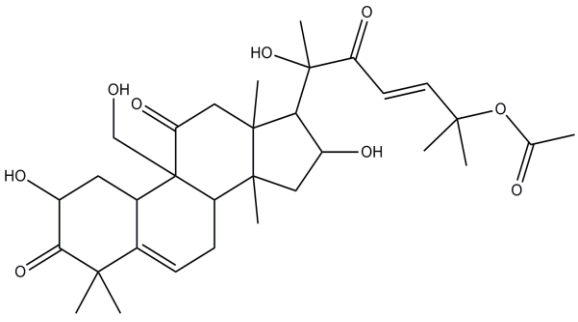
carbohydrate content (Hashash et al., 2017). *Citrullus lanatus* fruit consist adequate amount of carbohydrates (Adunola et al., 2015). The seeds of *Cucurbita pepo* contain 50% oil, primarily composed of linoleic and oleic acid. The seeds of *Momordica charantia* also has fixed oil and free acids (Kumar et al., 2010). The seed of *Citrullus colocynthis* contains a substantial quantity of palmitic, stearic, and linoleic acid (Dhakad et al., 2017). *Benincasa hispida* fruits contain a little amount of volatile oils (Al-Snafi, 2013).

1.4.3.2 Secondary Metabolites

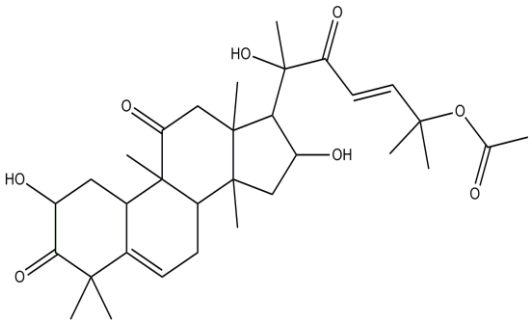
Cucurbitacin, a tetracyclic cucurbitane nucleus skeleton widely found in Cucurbits. The cucurbitacins exist in the form of non-glycosylated or glycosylated triterpenoids and are classified into twelve groups, including cucurbitacins A-T (Chen et al., 2005). The bitter taste of Cucurbitaceae plants is due to the high concentration of cucurbitacins, which are the main active components (Bartalis and Halaweish, 2005). Cucurbitacin E and its glycosides are the predominant chemical components found in food plants of the cucurbitaceae family (Dhiman et al., 2012). Cucurbitacins have been documented to have anti-inflammatory, anti-angiogenic, immunomodulatory, cytotoxic, cytostatic, and hepatoprotective attributes (Attard and Cuschieri, 2004; Shyam et al., 2010). Cucurbitacin A is exclusively present in *Cucumis* species, specifically in cucumbers (Chen et al., 2005). Cucurbitacin B exists in the form of dihydrocucurbitacin, dihydroisocucurbitacin, isocucurbitacin B; possess strong anticancer, anti-inflammatory and hepatoprotective effects (Zieniuk and Pawełkowicz et al., 2023; Miro, 1995). Cucurbitacin C is exclusively found in the species Cucumis sativus (Miro, 1995). Cucurbitacin E possess neuroprotective (Arel-Dubeau et al., 2014), anti-inflammatory, antipyretic, antitumor (Abdelkhalek et al., 2017), antiallergic (Yoshikawa et al., 2007), anthelmintic, and purgative activity (Miro, 1995). Cucurbitacin F derivatives, specifically 23, dihydro, and 15-oxo-cucurbitacin F, as well as 15-oxo-23,24-dihydrocucurbitacin F, have been found to exhibit anti-HIV activity, as reported by Konoshima et al. in 1994. The genus *Momordica* contains several distinct cucurbitacins and cucurbitane glycosides, including momordicosides A-E, momordicines, and neomorgoside; demonstrate promising antibacterial action (Miro, 1995). Few plants of the Cucurbitaceae family, include sweet cucurbitane glycosides, specifically mogrosides and oxomogroside (Chen et al., 2005).

Phenolic compounds, such as hydroquinone, phenolic acids, hydroxycinnamic acid derivatives, and flavonoids, are bioactive chemicals that are found in cucurbitaceous plants to a great extent (Oksana et al., 2012). It has been noted that chlorogenic, p-coumaric, gallic, p-hydroxybenzoic, vanillic, and ferulic acid are found in cucurbitaceae fruits. Flavonoid such as catechins, proanthocyanins, anthocyanidins, flavanones, isoflavones, flavonols, flavan-3-ols, and their glycosides are also present in the plants of this family. The majority of sterols and their derivatives are located in the seed portion of fruits from the cucurbitaceae family, specifically in *Benincasa cerifera, Cucumis sativus*, *Cucurbita maxima*. The primary sterols found in the seeds are 24-ethyl-Δ(7) and Δ(7,22)-sterols. Charantin, a steroidal glycoside, was extracted from M. charantia (Desai and Tatke, 2015). Furthermore, the seeds of Cucurbita pepo and Citrullus vulgaris contain a minimal quantity of cholesterol, as reported by (Badr et al., 2011; Hannah and Krishnakumari, 2015).

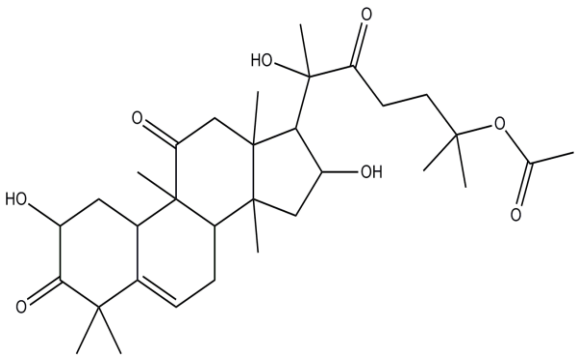
The *Momordica* genus consists a substantial presence of alkaloids. The compound momordicine, an alkaloid, was extracted from the fruits of *M. charantia* (Supraja et al., 2015). Vicine, a glycol alkaloid, was found in the seed extract of *M. charantia* (Haixia et al., 2004). 1-tert-butyl-5,6,7-trimethoxy isoquinolene, was extracted from the methanolic extract of *C. grandis* (Choudhury et al., 2013). *C. colocynthis* was found to contain colocynthin, a bitter compound consisting of a combination of alkaloid and crystalline alcohol. Structures of some important metabolites from Cucurbitaceae family have been illustrated in Figure 1.8 (1-24).



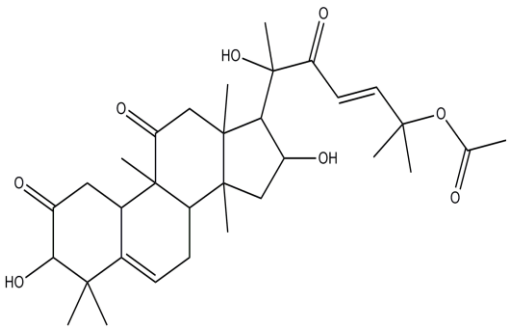
Cucurbitacin A (1)



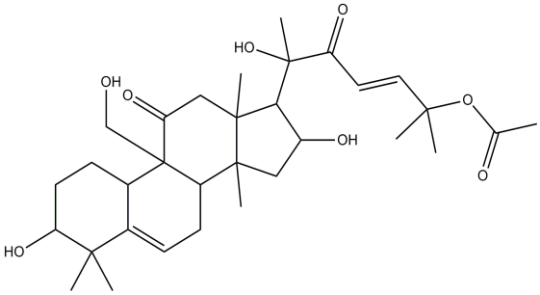
Cucurbitacin B (2)



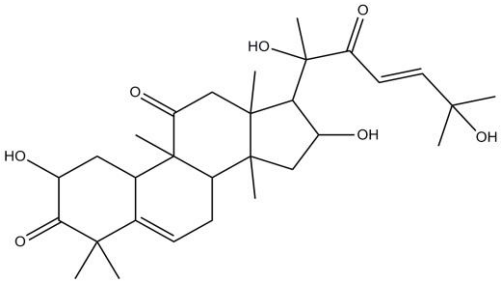
Dihydrocucurbitacin B (3)



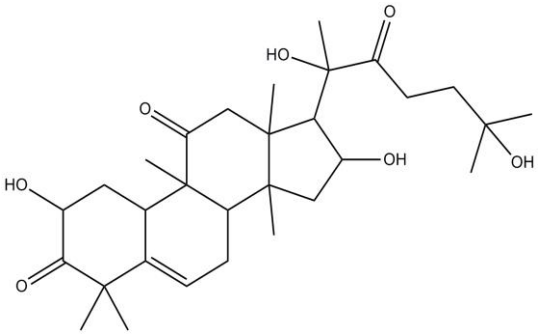
Isocucurbitacin B (4)



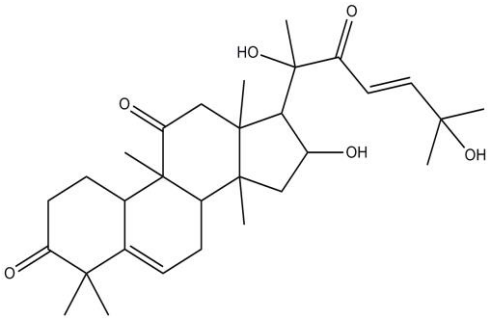
Cucurbitacin C (5)



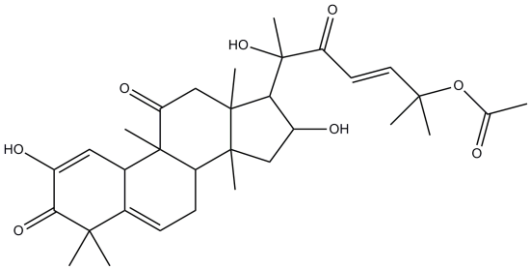
Cucurbitacin D (6)



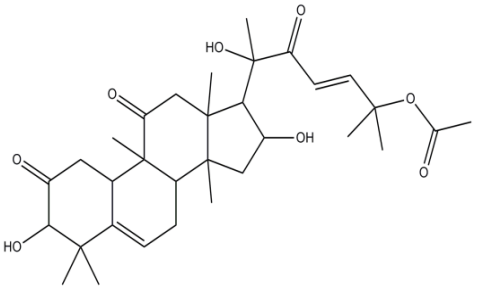
Dihydrocucurbitacin D (7)



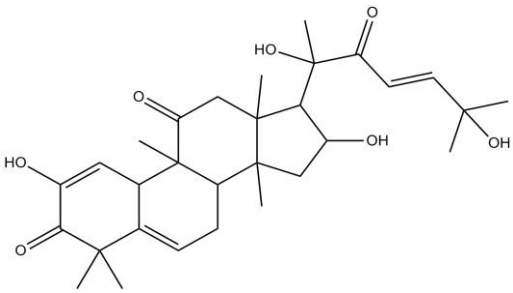
Deoxycucurbitacin D (8)



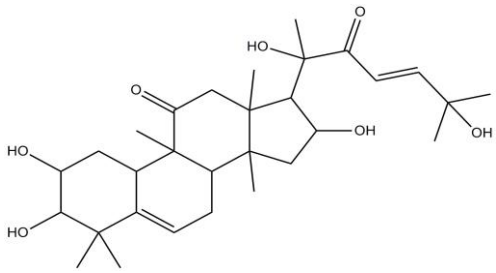
Cucurbitcin E (9)



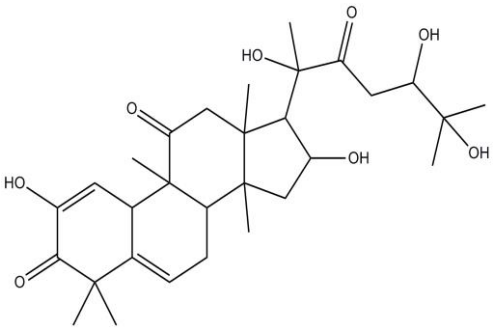
isocucurbitacin E (10)



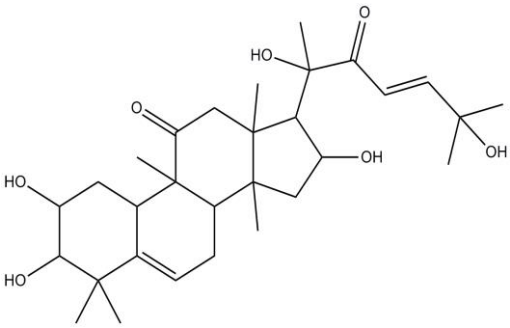
Cucurbitacin I (12)



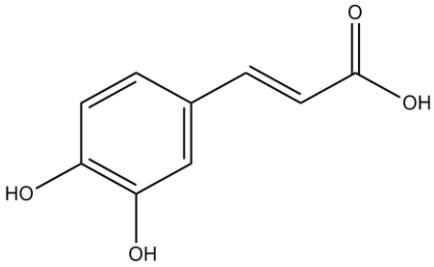
Cucurbitacin F (11)



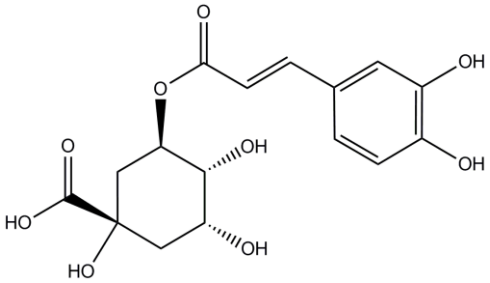
Cucurbitacin J (13)



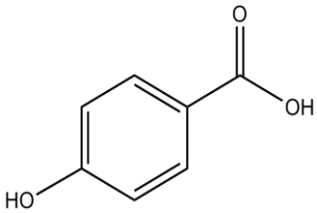
Cucurbitacin O (14)



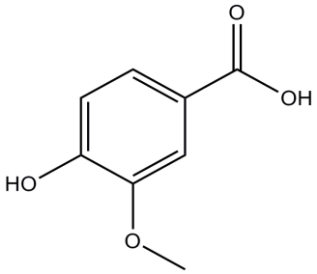
Caffeic acid (15)



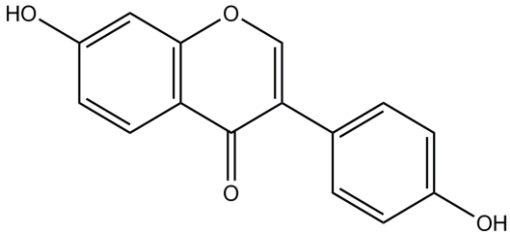
Chlorogenic acid (16)



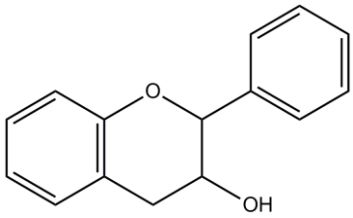
p-coumaric acid (17)



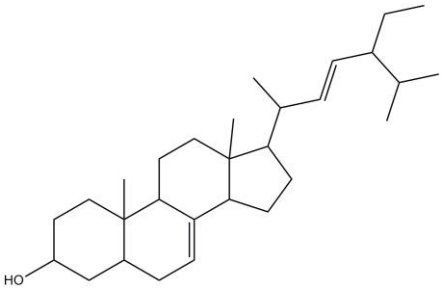
Vanillic acid (18)



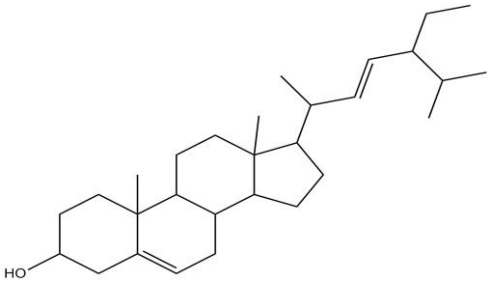
Isoflavone (19)



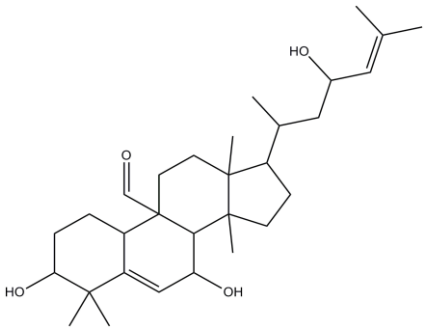
Flavan-3-ol (20)



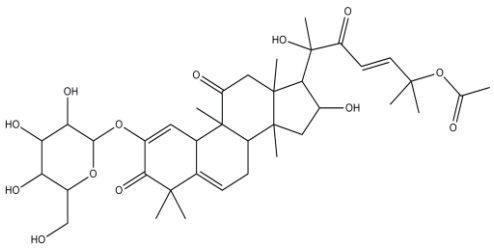
Spinasterol (21)



Stigmasterol (22)



Momordicin I (23)



Colocynthin (24)

Figure 1.8.(1-24) Chemical Structures of some important metabolites from Cucurbitaceae family

1.4.4 Ethnopharmacological Potential

In Ayurveda, *Citrullus colocynthis* used to treat worm infestation, jaundice, asthma, coughs, skin problems, tumour growth, and problems with the intestines. Whereas, *Luffa acutangula* widely used in skin disorder, anaemia, inflammation and indigestion. To treat various ailments including cough, asthma, any kind of poisoning, ulcer and pain fruits of *Lagenaria siceraria* is used (Anonymous, 2001). Dried fruits of *Benincasa hispida* is acceptable for the therapeutic properties against urinary disorder, metabolic disorder, dysuria, kidney stone, mental disorder, constipation (Anonymous, 2004). Ayurvedic formulations 'Hiraka Rasayana' Kakadani Taila', 'Kalagnirudra Rasa' and

'Visanasaka Yoga' consist of *Momordica dioica* dried root is used in the treatment of oro facial herpes, snake poisoning, dysuria, 'Sarpavisa' cold & cough, asthma, hiccough, debility, bleeding piles, diabetes, eye diseases, Neuro disorder, jaundice, and Kidney stones. Cucumber seeds are utilised as dried form to treat obstructive uropathy, bleeding disorder, general debility, burning sensation, 'Raktavikara' insomnia, headache, nausea & vomiting etc. *Trichosanthes bracteate* as ayurveduc herb used in food poisoning, metabolic disorder, abdominal swelling, skin related disorder, breast congestion in galactopoiesis, elephantiasis, and also in obstructed labour (Anonymous, 2006). The aerial parts of *Coccinia grandis* used to treat and symptomatic relief in Metabolic syndrome and Dysentery and rhizomes of *Corallocarpus epigaeus* also employed as antirheumatic, antidiarrheal antipyretic, chronic fever), antiasthmatic, anti-inflammatory agent (Anonymous, 2008).

Traditionally Fresh fruit juice of *M. dioica* employed in hypertension, unripe fruits in hyperglycemia and tender mature fruits used as e diuretic, laxative, hepatoprotective, anti-inflammatory, anti-pyretic (Talukdar and Hossain, 2014). Bitter gourd fruit used as an anthelmintic, stomachic, antibilious, laxative, antirheumatic and antidiabetic and leaf juice used in migraine. Root decoction of it possesses abortifacient effect. Leaf juice of loofah is used to treat conjunctivitis, fruits and roots are use as hepatoprotective and laxative (Khulakpam et al., 2015). Whole fruit of melon is ethnomedicinally used to treat chronic eczema and possesses laxative, galactagogue, diuretic and diaphoretic effect (Saboo et al., 2013). *Benincasa fistulosa* or apple gourd traditionally used as a pesticide and fodder (Gautam et al., 2011). Leaves and stems of pointed gourd are used in hypocholesterolemia, hyperglycemia; fruits are used as mouth freshener and antipyretic. Fruits of *C. grandis* are potent antidiabetic, anti-anaphylactic and antihistaminic and also used to treat leprosy, fever, asthma, bronchitis and jaundice. Watermelon acts as a natural diuretic and can be used in menstruation and pregnancy (Khulakpam et al., 2015). The seeds of *Telfairia occidentalis* are used traditionally to treat anaemia, convulsion, cardiovascular diseases (Teugwa et al., 2013). Pumpkin seeds are used to enhance the functioning of the urinary bladder and inhibit the growth of kidney stones (Smith, 1997). In India, Argentina and south American country, pumpkin seeds orally consumed in indigestion (Salehi et al., 2019). Black seed squash

is ethnomedicinally used for the treatment of haemorrhoids, fever and diabetes (Saboo et al., 2013).

In Thailand traditionally Bitter snake gourd is extensively used as laxative, anthelmintic and also helpful in the treatment of migraine (Dhiman et al., 2012). In China, India, Brazil, and Mexico *Cucurbita pepo* is used in the treatment of worm infestation. In Africa, *C. pepo* seeds are used to treat worm infestation, urinary bladder and kidney disorders. In Native America, bitter apple is used as a traditional medicine to heal urinary ailments and intestinal worms and also used to treat hypertension and prevent kidney stones. The leaves of this plant is used as cholagogue and roots are useful in breast inflammation, uterine and arthritic pain. The traditional healers in different stated of India prescribe Snake gourd for the treatment of headaches, abdominal tumors, fever, diarrhoea, and several allergies (Rolnik and Olas, 2020).

Table 1.3. Phytochemical profile and pharmacological aspects of some edible plants of Cucurbitaceae family

Scientific name	Major phytoconstituents	Pharmacological activity
Benincasa hispida	Bryonolic acid, lupeol, β-sitosterol, cucurbitin, avenasterol, multiflorenol, isomultiflorenyl acetate, stigmasterol 3-O-β-D-glucopyranoside, α-spinasterol 3-O-β-Dglucopyranoside, daucosterol, 2,5-dimethyl pyrazine (Han et al., 2013; Ghosh and Baghel 2011).	Antiulcer, anti-angiogenic, antioxidant, bronchodilator antipyretic (Qadrie et al., 2009), antidiraheal, antihyperlipidemic, antimicrobial (Natarajan et al., 2003) antihistaminic, hypoglycemic (Chakraborty et al., 2018)
Citrullus colocynthis	Flavone-C-glycosides, ursolic acid, Cucurbitacin I 2-O-β-D-glucopyranoside, cucurbitacin E 2-0-β-D-glucopyranoside,	Antidiabetic, antihyperlipidemic (Hussain et al., 2014), antimicrobial (Marzouk et al., 2009)

	colocynthoside A & B, khekadaengoside E, isosaponarin, isovitexin (Rajasree et al., 2016; Hussain et. al. 2014; Miao et al., 2012)	analgesic, anti-inflammatory (Pashmforosh et al., 2018), anticancer, cytotoxic (Rezai et al., 2017)
Citrullus lanatus	Vitamin A, ascorbic acid, lycopene, cucurbitacin E, flavonoids, thiamine, riboflavin, polyphenols, terpene, steroid, flavonoid, vicilin (Rajasree et al., 2016; Gupta et al., 2018)	Anti-urolithiatic, diuretic (Siddiqui et al., 2018), antimicrobial (Adunola et al., 2015), hepatoprotective, cardioprotective, analgesic, anti-inflammatory (Gupta et al., 2018)
Coccinia grandis	Polyprenol 1, saponin, flavonoids, glycosides, taraxerone, taraxerol, 24R-24-ethylcholest-5-en-3β-ol glucoside, Cephalandrins A and B, β-sitosterol, coccinoside, flavonoid glycoside, Lupeol, β-amyrin, β-sitosterol (Pekamwar et al., 2013)	Antidyslipidemic (Singh et. al., 2007), mast cell stabilizing, antianaphylactic, antihistaminic (Taur and Patil, 2011), analgesic (Hossain et. al., 2014b), antibacterial & cell proliferative (Sakharkar and Chauhan, 2017); antiproteolytic, leishmanicidal (Das et al., 2015), antidiabetic, insulinotrophic (Meenatchi et al., 2017)
Coccinia indica	Steroids, terpenoids, saponins, flavonoids alkaloids, tannins, glycosides, phenolic acid (Pushpa Rani et. al., 2016)	Antihyperglycemic, hypolipidemic (Balaraman et al., 2010), antifungal (Shaheen et al., 2018)

Cucumis melo	Phenolic glycosides, fatty acids, gallic acid, ellagic acid, catechin, quercetin, vanillin, eugenol, vanillic acid, luteolin-7-glucoside, naringenin, oleuropein, linoleic acid, tocopherols, oleic acid (Mallek-Ayadi et al., 2017, Mallek-Ayadi et al., 2018; Rajasree et al., 2016)	Promote skin hydration, antioxidant, anti-inflammatory (Dhiman et al., 2012), antiproliferative (Rolim et al., 2018)
Cucumis sativus	Glycoside, saponin, flavonoid, isovitexin, saponarin, acylated flavone-C-glycosides, cucumegastigmanes I and II, cucumerin A and B, vitexin, orientin, apigenin 7-O-(6″-O-p-coumaroylglucoside) (Mukherjee et al., 2013; Rajasree et al., 2016).	Diuretic, antihelmintic, hypolipidemic (Sudheesh and Vijayalakshmi, 1999), antiaging, (Nema et al., 2011), antimicrobial, antidiabetic (Dixit and Kar, 2010), hepatoprotective (Mukherjee et al., 2013), antiurolithiatic (Pethakar et al., 2017), antimicrobial (Sotiroudis et al., 2010)
Cucurbita maxima	Spinasterol, 24-ethyl-5α-cholesta-7, 22, 25-trien-3β-ol, polyphenolics, Cucurbitaxanthin, gibberellin, β-carotene, γ-amino butyric acid, 11E-octadecatrienoic acid, 13-hydroxy-9Z octadecatrienoic acid protocatechuic, caffeic, vanillic, p-coumaric, ferulic, oleic, linoleic, palmitic acids (Muchirah et al., 2018; Rezig et al., 2012)	Antidiabetic, antihyperlipidemic (Sharma et al., 2013), CNS stimulant (Doke et al., 2011), diuretic (Saravanan and Manokaran, 2012), immunosuppressive, antitumor, antihypertensive, anti-inflammatory, antibacterial, antihypercholestramia (Rajasree et al., 2016)

Cucurbita pepo	Linoleic acid, oleic acid, avenasterol, spinasterol, stigmasterol, triterpenoids, sesquiterpenoids, squalene, tocopherols, lutein, α and β-carotene, flavoxanthin, luteoxanthin, A and β-cryptoxanthin, cucurbitacin I, J, L, K, M (Gutierrez, 2016; Rajasree et al., 2016)	Diuretic, antiandrogenic, immunological, anti-inflammatory, hepatoprotective, anti-ulcer, antileprotic (Dhiman et al., 2012; Rajasree et. al., 2016), antibacterial, antioxidant, antitumor, antidiabetic, hypolipidemic (Adnan et al., 2017)
Lagenaria siceraria	Cucurbitacin B, D, G, H, saponins, flavone-C-glycoside, polyphenol, campesterol, fucosterol, sitosterol, Lagenin (Prajapati et al., 2010), D: C-Friedooleanane type triterpenoids (Chen et al., 2008).	Antimicrobial (Dash and Ghosh, 2018), lipase inhibitory activity, xanthine oxidase and alpha-amylase inhibitory activities (Maqsood et al., 2017), antitumor, cardioprotective, diuretic immunoprotective, antiproliferative, hepatoprotective, CNS depressant, anticancer, immunomodulatory (Rajasree et. al. 2016; Prajapati et al., 2010).
Luffa acutangula	Luffangulin, luffaculin, gallic acid, p-coumaric acid, ferulic acid, protocatechuic acid, acutoside C, acutoside D, carboxylic acids, fatty acids (Nagarajaiah and	Hepatoprotective (Jadhav et. al., 2010), antidiabetic, antimicrobial, cytotoxic, antibacterial, immunomodulatory

	Prakash, 2015; Suryanti et al., 2015)	(Manikandaselvi et al., 2016)
Luffa cylindrica	Lucyin A, lucyoside, maslinic acid, ginsenosides, luffin P1, luffin S, luffacylin, apigenin-7-O-D-glucuronidemethyl ester; luteolin-7- O--D-glucuronide, methyl ester, p-coumaric acid, chlorogenic acid, caffeic acid (Du et al., 2006; Lucy and Abidemi 2012)	Antiischemic , Antihyperlipidemic, immunomodulatory (Khajuria et al., 2007), antimicrobial (Indumathy et al., 2011), antiviral, antidiabetic (Partap et al., 2012)
Luffa echinata	Echinatin, saponin, cucurbitacin B and E, echinatol A and B, oleanolic acid (Dogar et al., 2018)	Antihepatotoxic, analgesic, anti-inflammatory (Dogar et al., 2018; Modi and Kumar, 2014), hepatoprotective (Ahmed et al., 2001)
Momordica charantia	Momordicin I, IV, kuguacin, charantoside, vicine, goyaglycoside, quercetin-, kaempferol- and isorhamnetin-*O*-glycosides (Jia et al., 2017; Grover and Yadav, 2004)	Antidiabetic, antihyperlipidemic, anticancer, hepatoprotective, antiviral, anti-inflammatory, analgesic (Jia et al., 2017; Grover et al., 2004)
Momordica cochinchinensis	Hydroxybenzoic acids, hydroxycinnamic, gallic, p-hydroxybenzoic acid, apigenin, oleic, palmitic, linoleic acids (Ishida et al., 2004), lycopene, momordin, (Chuyen et al., 2015;	Antioxidant, antimicrobial, antiproliferative (Yu et al., 2017), anticancer, provitamin A activity (Chuyen et al., 2015), trypsin inhibitor (Felizmenio-Quimio et al.,

	Müller-Maatsch et al., 2017)	2001)
Momordica dioica	Pleuchiol, momodicaursenol triterpenes of ursolic acid (Ali and Srivastava, 1998), hederagenin, oleanolic acid, α-spiranosterol, stearic acid, gypsogenin (Sadyojatha and Vaidya, 1996), steroidal tritrpenoids (Luo et al., 1998)	Hepatoprotective, antihepatotoxic, antidiabetic, antibacterial (Pingle et al., 2018), immunostimulant (Venkateshwarlu et al., 2017)
Trichosanthes cucumerina	Palmitic, stearic, arachidic, behenic, lignoceric acids, lutein, zeaxanthine, cucurbitacins, coumaric acid, p-coumaric acid, caffeic acid, chlorogenic acid (Adebooye et al., 2008)	Antidiabetic (Devi, 2017), antibacterial (Reddy et al., 2013), anticancer, (Kongtun et al., 2009), gastroprotective (Arawwala et al., 2010)
Trichosanthes dioica	Trichosanthin, lectin, euphol, α-amyrin, β-amyrin, butyrospermol, lupeol, taraxerol, betulin, and karounidiol, cucurbitacin B and E, sterols, steroidal saponin, flavonoids (Khandaker et al., 2018)	Antihyperglycemic (Rai et al., 2008), antihyperlipidemic (Sharmila et al., 2007), antitumor (Bhattacharya et al., 2011), antiinflammatory (Bhattacharya and Haldar, 2013), immunomodulatory (Bhadoriyal and Mandoriya, 2012), wound healing (Shivhare et al., 2010)
Trichosanthes tricuspidata	Cucurbitane glycosides (cucurbitacin K 2-O-beta glucopyranoside), palmitic,	Anthelmintic (Duvey et al., 2012), antioxidant, antibacterial (Xavier and

	bryonolic acids, cucurbitacin B, isocucurbitacin B, 23,24-dihydrocucurbitacin, hexanorcucurbitane octanorcucurbitane glycosides (Duvey et al., 2012; Kanchanapoom et al., 2002)	Dhanasekaran, 2018), antihyperglycemic (Kulandaivel et al., 2013)
Sechium edule	Cucurbitacin B, D and I, luteolin 7-O-rutinoside, naringenin, phloretin, apigenin, chlorogenic, vanillic, p- hydroxybenzoic, 3-octadecenoic, α-linoleic acids (Ragasa et al., 2014; Aguiñiga-Sánchez et al., 2017	Antioxidant, α-glucosidase inhibitory, antidiabetic (Sulaiman et.al., 2013), cardioprotective (Neeraja et al., 2015), antiatherosclerotic (Ragasa et al., 2014), hepatoprotective (Firdous et.al., 2012a), antibacterial (Ordonez et al., 2009), antiepileptic, CNS depressant (Firdous et.al., 2012b), antiproliferative (Aguiniga-Sanchez et al., 2017)

1.4.5 Pharmacological Potential

Cucurbitaceae plants offer a rich supply of bioactive functional components that have diverse medicinal significance. The metabolites derived from plants belonging to the Cucurbitaceae family exhibit a wide range of biological activities, including as antidiabetic, anti-inflammatory, cytotoxic, hepatoprotective, and antibacterial properties. This section explores the diverse pharmacological and therapeutic capabilities of plants from the Cucurbitaceae family. Table 1.3 listed phytochemical and pharmacological

aspects of some edible plants of Cucurbitaceae family. Pharmacological and therapeutic activities of Cucurbitaceae family plant presented in figure 1.9.

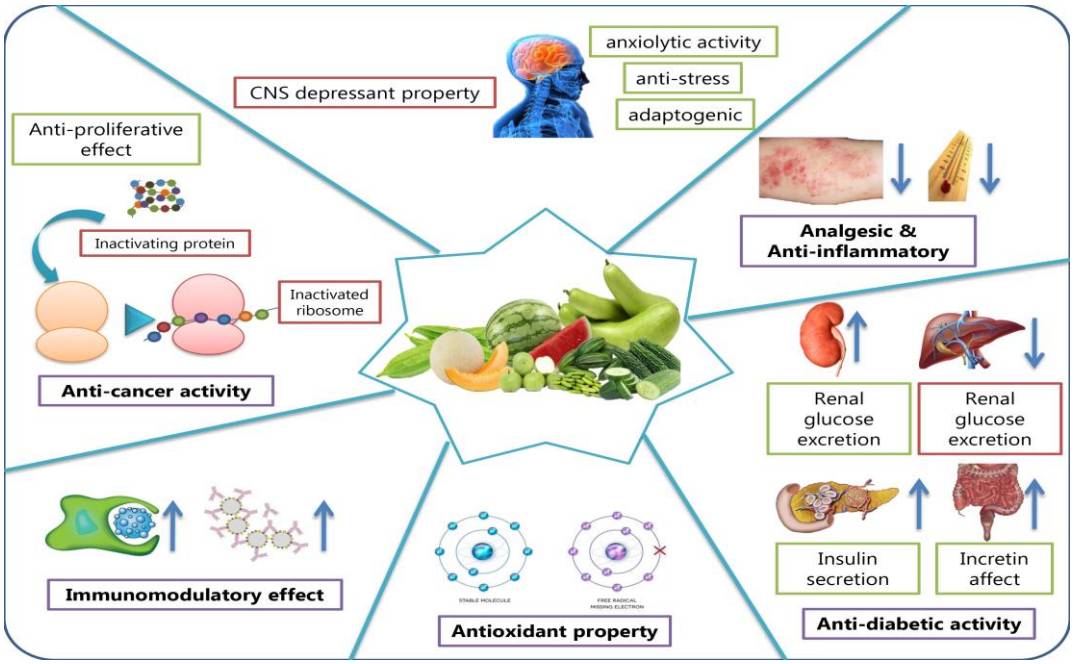


Figure 1.9. Pharmacological and Therapeutic activities of Cucurbitaceae family plants

1.4.5.1 Neuroprotective activity

The anxiolytic effect of alcoholic extract of *B. hispida* was observed on various behavioural models in in-vivo manner (Nimbal et al., 2011). *Lagenaria siceraria* fruit exhibited anti-stress, adaptogenic and CNS depressant activity (Pawar et. al., 2009). In another study, *Sechium edule* and *Cucumis sativus* were also reported to possess antiepileptic and CNS depressant activity in a dose-dependent manner (Firdous et al., 2012b; Nasrin et al., 2013).

1.4.5.2 Antidiabetic, Antihyperglycemic and antihyperlipidemic activity

Globulins isolated from *C. lanatus* and *C. moschata* seeds was found effective as antihyperglycemic agent (Teugwa et al., 2013). Polysaccharides found in some cucurbitaceae plants were reported to have antidiabetic activity (Dabaghian et al., 2012). *M. charantia* exhibits beneficial effects in diabetes management due to the presence of some peptides, sterol glucosides, saponins (Raman and Lau, 1996).

Coccinia indica exerts hypoglycemic effects by altering the effect of glucose-6-phosphatase enzyme. ß-sitosterol, and pectin were found as major antihyperglycemic bioactive components (Hossain et al., 1992). Additionally, the fruits of *Lagenaria siceraria, Sechium edule* and *Benincasa hispida* have potential antidiabetic effect by inhibit α-glucosidase and α- amylase enzyme (Sulaiman et al., 2013; Saeed et al., 2007). *Momordica charantia* have a prominent effect on lipid metabolism shows antihyperlipidemic activity (Nerurkar et al., 2010). Fruit extract of *M. charantia* also possess hypolipidemic activity by inhibit pancreatic lipase enzyme in a dose dependent in-vitro model (Chanda et al., 2019). The hypolipidemic activity of *Coccinia indica* was reported in the streptozotocin-induced diabetic rat model (Balaraman et al., 2010) and *Citrullus colocynthis* possess lipid-lowering activity (Hussain et al., 2013). Alcoholic and aqueous extract of *Lagenaria siceraria* showed potential activity in lowering total cholesterol, triglyceride, VLDL and LDL and as well as increasing HDL level (Ghule et. al., 2006b). *Cucurbita maxima*, *Luffa cylindrica* and also having an antihyperlipidemic effect (Rajasree et al., 2016).

1.4.5.3 Analgesic and Anti-inflammatory activity

Citrullus colocynthis (Hussain et al., 2014), *Cucumis melo* (Dhiman et al., 2012), *Trichosanthes dioica* (Kumar et. al., 2012), *Cucurbita maxima* and *Citrullus lanatus* (Rajasree et al., 2016) have been reported for their analgesic and anti-inflammatory properties. The aqueous and methanolic fruits extract of *Lagenaria siceraria* reported to possess analgesic and anti-inflammatory properties (Shah and Seth, 2010; Ghule et. al., 2006 (a). The anti-inflammatory activity of *Benincasa hispida* was observed in in-vivo model (Rachchh et. al., 2011) in a dose-dependent manner.

1.4.5.4 Anticancer and antiproliferive activity

A large number of cucurbitacin and cucurbitane-type triterpene glycosideswere found effective as cytotoxic and antiproliferative phytocompounds. α-momorcharin, trichosanthin, cyclic bisdesmosides, gypenosides, trichosanthin etc. have cytotoxic and tumour growth inhibitory activity. The antitumor activity of trichosanthin isolated from *Trichosanthes kirilowii* and Luffins a and b, Luffaculin isolated from *Luffa cylindrica* exerts inhibitory effects on different carcinoma cell lines by reducing the proliferation

(Méndez-Cuesta et al., 2018). *Cucumis sativus*, *Benincasa hispida*, *Coccinia indica, Cucurbita maxima*, and *Luffa acutangula* were studied on HeLa Cell Line and possess anticancer activity (Varalakshmi and Rao, 2012). The seed extract of *Momordica cochinchinensis* showed anti-proliferative effect on human lung cancer (Yu et al., 2017); whereas, aqueous extract of *Momordica charantia* was reported to mitigate skin carcinoma and inhibit proliferation (Ganguly and Das, 2000; Kusamran et al., 1998). Root extract and fruit juice of *Trichosanthes cucumerina* and *Citrullus colocynthis* having potential cytotoxic activity (Kongtun et al., 2009; Hussain et al., 2014).

1.4.5.5 Antioxidant activity

Fresh and dried fruits of *Lagenaria siceraria* was evaluated as antioxidant (Katare et al., 2014). *Momordica cochinchinensis* is a reach source of antioxidant (Chuyen et. al., 2015); due to the existence of polyphenol and flavonoid components. Seeds of eighteen varieties of edible Cucurbitaceae plants exhibit antioxidant activity (Sabo et al., 2014). *Cucurbita ficifolia* also possess antioxidant activity due to the presence of phenolic acid (Moreno-Quiroga et al., 2023).

1.4.5.6 Antimicrobial and Antifungal activity

Antimicrobial activity of *Momordica charantia* extract was evaluated against *S. aureus*, *B. cereus* and *E. coli* (Costa et al., 2011). Additionally, α- and ß- momorcharin has also been shows anti-HIV activity (Au et al., 2000). *Citrullus colocynthis* was reported to exhibit potential antimycobacterial activity against some drug-sensitive and drug-resistant strain of *M. tuberculosis* (Mehta et al., 2013). *Momordica cochinchinensis* (Chuyen et al., 2015), *Trichosanthes cucumerina* (Reddy and Jose 2013), *Trichosanthes dioica* (Bhattacharya and Halder, 2010) and *Sechium edule* (Ordonez et al., 2009) were studied for antimicrobial activity. Phytocompounds isolated from aerial parts of *Cucumis sativus* exert antifungal activity (Das et al., 2012). Cucurmoschin, hispin, luffacylin, vicillin have been reported as potential biofungicide protein (Yadav et al., 2013). In a study, synergistic antifungal activity of metronidazole and *M. charantia* extract was observed (Santos et al., 2012). Aerial parts of *Solena amplexicaulis* was observed against nine fungal strains (Moorthy et al., 2013).

1.4.5.7 Cardioprotective effect

Trichosanthes cucumerina fruit possess cardioprotective effect in doxorubicin-induced cardiotoxicity in the rat model (Shah et al., 2012). Fruits of *Lagenaria siceraria* reported to reduce the doxorubicin-induced cardiotoxicity (Fard et. al., 2008). The cardiotonic activity of *Lagenaria siceraria* fruit juice was also reported (Dhiman et al., 2012). *Citrullus lanatus* and *Cucurbita pepo* have also been found to be therapeutically active against cardiovascular disease (Rajasree et al., 2016).

1.4.5.8 Immunomodulatory effect

Lagenaria siceraria fruit epicarp extract exhibited immunoprotective, immunomodulatory along with immunostimulative effect (Rajasree et al., 2016; Deshpande et al., 2008). Saponins isolated from *Lagenaria siceraria* fruits are also showed immunomodulatory activity (Gangwal et al., 2008a). Crude extract of *Luffa acutangula* fruit possesses immunomodulatory activity in a dose-dependent manner (Shendge and Belemkar, 2018). Gallic acid, p-hydroxybenzoic acid, present in *L. acutangula* var. amara showed potential immunomodulatory activity (Kalaskar and Surana, 2014). The exploration of immunomodulatory activity of *Cucurbita pepo* and *Momordica dioica* was reported in various literature earlier (Rajasree et. al., 2016).

1.4.5.9 Hepatoprotective activity

Sechium edule fraction of ethyl acetate and n-butanol possess hepatoprotective activity by inhibiting carbon tetrachloride-induced hepatic injury in rats (Firdous et al., 2012a). *Momordica charantia*, *Luffa echinata* and *Momordica dioica* act as hepatoprotective and antihepatotoxic agents (Ahmed et al., 2001; Talukdar & Hossain, 2014). *Trichosanthes dioica* prevents gastric ulcers and liver necrosis, observed in rat model (Kulkarni & Raghavan, 2013). *Luffa acutangula* exhibited hepatoprotective activity against CCl_4 and rifampicin-induced liver toxicity (Jadhav et al., 2010).

1.4.6 Toxicity

Cucurbitaceae family are well known for their therapeutic benefits along with culinary uses. Occasionally, it may be necessary to limit the usage of certain substances due to

the possible adverse reactions or toxicity (Jia et al., 2017). Alcoholic extract of *M. charantia* was observed in normal and alloxan induced diabetic rat toxic. In histology, congestion of internal organ and alteration of blood color was observed (Batran et al., 2006). In another research, *Citrullus colocynthis* extract exhibited hepato toxicity by inhibiting the Fe^{3+} stimulated liver peroxide in a dose-dependent manner (Barth et al., 2002). *Lagenaria siceraria* juice also found toxic are due presence of cucurbitacin. Dehydration, gastrointestinal injury and renal toxicity was observed (Puri et al., 2011). Leaf extract of *Coccinia grandis* showed severe toxicity in alloxan-induced diabetic Wister rats (Attanayake et al., 2013). Animals often experience toxicity when given the therapeutic dose of cucurbitacin. It is important to mention that making changes to the structure of functional groups can potentially decrease the toxic effects of cucurbitacin and its related compounds. These modified compounds have been discovered to be promising candidates for drug development research.

1.4.7 Nutritional and Economical importance

Different components of Cucurbitaceae plants are consumed in everyday diets due to their numerous nutritional advantages. Certain cucurbitaceous plants have used in the production of drinking vessels, cooking pots, kitchenware, bath sponges, industrial filters, etc. Outer layer of *B. hispida* composed of a white, powdery wax like substance acts as a barrier against the growth of bacteria. The pulp of the fruit is utilised to create the broth. *Citrullus colocynthis* contributes to achieving a well-rounded diet, while only the seeds are edible (Badifu and Ogunsua 1991). Aerial parts of *Coccinia cordifolia*, *Lagenaria siceraria* and *Luffa acutangula* are commonly utilised in culinary applications and can be incorporated into cake icing. The flesh of *Cucurbita ficifolia* can be combined with sugar to prepare sweets or produce beer by fermentation. Raw fruits of *Sechium edule* are utilised for making salads and can also be consumed in cooked form due to excellent source of vitamin C. *Cucumis sativus* has a high-water content and a low fibre level and have vitamins A, K, and C, potassium. The fruit of *Cucumis melo* is utilised in the preparation of desserts.

Cucurbita pepo and *maxima*'s aerial parts including fruits are eaten as vegetables, as they contain vitamin A, C, E, thiamin, niacin, copper, iron, magnesium and dietary fibre (Ajuru & Nmom, 2017). Pumpkin seeds are good source of protein, and minerals.

Lagenaria siceraria fruits are utilised for crafting ladles, boxes, water jugs, planters, flutes, and various other musical instruments. The desiccated peels of bottle gourd serve as receptacles for palm wine, and are utilised by fisherman as buoyancy aids for fishnets and rafts (Jimoh et al., 2013). Cucumber fruit flesh is used to massage over the skin to promote smoothness due to cooling, and soothing impact and is also used in the preparation of body soap. The fully developed fruits of *Luffa aegyptiaca*, use as sponges (Vouldoukis et al., 2004). The tuber of *Cucumis ficifolius* is utilised for the management of blackleg, colic, and emaciation in ethnoveterinary field (Tamiru et. al., 2013). *Cucurbita pepo* leaves are utilised for treating trypanosomosis in animals. *Lagenaria siceraria* is utilised for the treatment of rabies and trypanosomosis; *Momordica foetida* is beneficial in the treatment of fracture, rabies, trypanosomosis, myiasis, lice, sedative and some ectoparasite infestations (Sori et al., 2004).

Chapter 2

Scope, objective and plan of work

2.1 Scope and rationale of the study

2.2 Objective of the study

2.3 Plan of work

2.1 Scope and rationale of the study

Medicinal plants have been utilised since ages for their therapeutic properties as well as for food purposes. Plants have been exploited in the treatment of diseases since ancient times and have consistently expanded as a kind of supplemental medicine. This is due to their accessibility and cost-effectiveness as alternative healthcare options (Chaachouay and Zidane, 2024). Currently, there is a rising fascination with harnessing crude extracts and dry powder samples from medicinal and aromatic plants for the purpose of creating alternative traditional medicine and food additives. Pharmaceutical substances produced from traditional herbs may potentially be used to alleviate anxiety. A study has been carried out to explore natural substances that can reduce anxiety, as an alternate option (Sofowora et al., 2013).

According to the World Health Organization's report, an estimated 1 in 8 individuals are affected by mental or behavioural illnesses, such as stress. This represents 12.3% of the worldwide burden of disease and is projected to increase to 15% by 2030 (Kayiteshonga et al., 2022). Stress is an inherent aspect of human existence and refers to any state that disrupts the body's equilibrium. Under conditions of severe stress, the homeostatic processes of the body become impaired, posing a threat to the organism's existence (Yaribeygi et al., 2017). Stress has been hypothesised to play an integral part in the development of various diseases, such as arthritis, hypertension, peptic ulcer, diabetes, hyperlipidemia, reproductive dysfunctions, cancer, immunological dysfunctions, depression, and neurodegenerative disorders. The traditional deployment of stimulants and anabolic steroids for stress management proves to be inefficient in addressing the various impacts of stressors and is moreover linked to significant negative consequences. Unlike conventional medications, adaptogens are chemicals derived from plants that do not have any bad effects (Dhama et al., 2019). Non-communicable disorders (NCDs) are chronic conditions that are influenced by various factors such as genetics, physiology, environment, work habits, diets, and particularly stress. Common NCDs include cardiovascular disease, hypertension, stroke, diabetes, dyslipidemia, and obesity (Tabish, 2017). The NCDs are typically influenced by multiple metabolic pathways that involve numerous enzymes. The hypothesis suggests that the catalytic efficiency of enzymes is mostly associated with the metabolic processes of the

human body. Several enzymes play a significant role in the key biochemical processes of disease development. These include α–amylase, α–glucosidase, angiotensin-converting enzyme (ACE), pancreatic lipase, HMG - CoA reductase, carbonic anhydrase, and aldose reductase, among others. Inhibiting these enzymes is a significant strategy for combating these diseases by targeting the transportation of proteins, carbohydrates, or lipids (Kumar et al., 2022).

Adaptogens have multiple ways of working and can provide resistance against stressful conditions by regulating the different components of the stress response system in a non-specific manner. Adaptogens are commonly referred to as "medicines for healthy individuals" and are thought to enhance energy and resilience while dealing with stress. They are known to improve both physical and mental performance, strengthen the body's defence mechanisms, and promote longevity (Panossian and Wikman, 2010). Adaptogenic plants have been utilised in traditional medicine in many countries including India, China, Korea, and Japan for several centuries to enhance resistance to stress by normalising effects and promoting adaptation, resilience, and survival, irrespective of the specific stressor (Todorova, 2021). Over the past years, numerous molecular targets, networks, and signalling pathways that are modulated by adaptogens have been established. Adaptogens are now being used and have the potential to be used in the treatment of exhaustion caused by stress, cognitive dysfunction, mental illnesses, and disorders connected to behaviour and ageing (Panossian and Efferth, 2022). During the last decade, many molecular targets, networks, and signalling pathways that are regulated by adaptogens have been identified. The current and potential uses of adaptogens include treatment of stress-induced fatigue, impaired cognitive function, mental illness, and behavioral- and aging-related disorders.

In Hippocratic medicine, it was considered that dietary intervention plays a significant effect in human wellness. The therapeutic significance of natural products has been recognised due to their broad range of pharmacological abilities, greater lesser adverse reaction, and lower costs compared to synthetic medications. Multiple findings suggest that consuming a large amount of fruits and vegetables can lower the risk of non-communicable diseases (NCDS) due to the presence of beneficial secondary and

primary metabolites, and the ability to maintain homeostasis (Kleisiaris et al., 2014). The prevalence of natural phenolic molecules in the human diet, mostly found in fruits, vegetables, and beverages, has been noted to significantly contribute to the mitigation of metabolic syndrome and its associated stress-related consequences (Rahman et al., 2022). Plant polyphenols, functioning as potent dietary antioxidants, may provide a degree of defence against oxidative damage. Furthermore, these plants contain distinct bioactive chemicals that are responsible for maintaining their bioactivities (Pandey and Rizvi, 2009). Standardisation of plant materials is crucial in this context. This can be achieved by utilising several analytical techniques such as high-performance liquid chromatography (HPLC), high-performance thin layer chromatography (HPTLC), and LC-MS/MS. These techniques are necessary for the accurate profiling of bioactive metabolites. Standardisation is beneficial for chemical fingerprinting and dereplication of plant extracts to define benchmarks for quality, safety, and efficacy.

The Cucurbitaceae family is widely recognised and comprises 125 genera and 960 species of edible vegetable and fruit crops. The food plants belonging to this family are regularly consumed on a wide scale (Renner and Pandey, 2013). The significance of these food plants in Indian traditional medicine, such as Ayurveda and other medical systems, is extensively documented. The Cucurbitaceae family is a significant family of plants that holds both therapeutic along with economic value. Phytomolecules have been proven to be very beneficial in treating metabolic illnesses triggered by stress and helping to preserve the equilibrium of the body. By virtue of their abundant presence of phenolic glycosides, flavonoids, terpenoids, minerals, water-soluble polysaccharides, dietary fibres, and other phytocompounds, plants in the Cucurbitaceae family have been widely acknowledged for their ability to effectively control NCDs and to function as adaptogens (Mukherjee et al., 2022). It has been established that the majority of metabolic ailments arise from the irregularity of certain enzyme activity. α- amylase and α-glucosidase, has a significant impact on our body. When the both the enzyme is inhibited, it slows down the digestion and absorption of carbohydrates, time leading to a longer overall digestion and a decrease glucose level in blood stream and prevents hyperglycemia. Carbonic anhydrase is associated with conditions which include hypertension, oedema, obesity, and cardiac hypertrophy. On the other hand, pancreatic

lipase is correlated with hyperlipidemia, diabetes, obesity, and related illnesses. The pancreatic lipase inhibitor impedes the breakdown of triglycerides, hence preventing the absorption of fat and preventing obesity. The therapeutic assessment of Cucurbitaceae plants as adaptogens was conducted by assessing their *in-vitro* enzyme inhibition and *in-vivo* adaptogenic activities. Therefore, the scientific verification of the extensive medicinal applications of these plants was established in order to evaluate their quality, effectiveness, and safety. The study aimed to evaluate the therapeutic activity of medicinal plants as adaptogen from Cucurbitaceae family and quality evaluation of the plant extracts. Figure 2.1 depicts the schematic diagram of the workflow.

2.2 Objective of the study

- Collection authentication and extraction of selected Cucurbitaceae food plants.
- Marker Profiling of selected Cucurbitaceae food plants.
- Standardization and quality evaluation by HPTLC and RP-HPLC.
- *In-vitro* α-amylase, α-glucosidase, carbonic anhydrase and pancreatic lipase enzyme inhibition study.
- *In-vivo* adaptogenic activity potential of selected fruit extract.

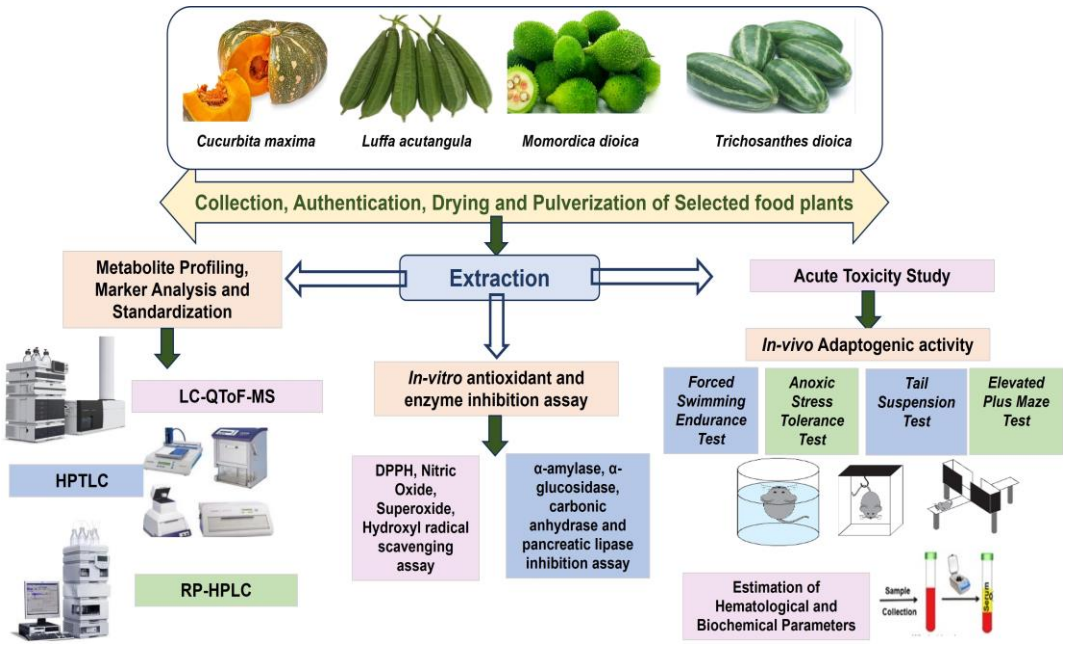


Figure 2.1. Schematic diagram of workplan of the study

2.3 Plan of work

In this study, the specific plan of work was as follows:

- Plant profile, extraction and Quantitative analysis of the selected food plants
  - ❖ Selection of the food plants on the basis of traditional uses
  - ❖ Collection and authentication of the plants and plants parts
  - ❖ Extraction of the selected fruits with suitable solvent
  - ❖ Quantitative analysis of the extracts by Total phenolic and total flavonoid content.
- Metabolite profiling, marker analysis and standardization of the selected food plant extracts
  - ❖ Liquid Chromatography-Quadrupole Time of Fly-Mass Spectrometry (LC-QToF-MS)
  - ❖ High Performance Thin Layer Chromatography (HPTLC)
  - ❖ Reverse Phase High Performance Liquid Chromatography (RP-HPLC)
- *In-vitro* antioxidant and enzyme inhibition assay of the selected fruit extracts
  - ❖ *In-vitro* antioxidant activity of the fruit extract by employing DPPH, Nitric Oxide, Superoxide and Hydroxyl free radical scavenging activity
  - ❖ *In-vitro* enzyme inhibition assay including α-amylase, α- glucosidase, carbonic anhydrase and pancreatic lipase
- Evaluation of *in-vivo* adaptogenic potential of selected fruit extract
  - ❖ *In-vivo* adaptogenic activity of selected fruit extract through forced swimming endurance, anoxic stress tolerance, tail suspension and elevated plus maze test; along with estimation of haematological as well as biochemical parameters

Chapter 3

Plant profile, extraction and Quantitative analysis of the selected food plants

3.1 Rationale for selection of plants

The Indian subcontinent serves as a central location for a diverse array of plants with therapeutic properties. The Indian traditional system of medicine employs medicinal plants due to their broad availability and diverse range of therapeutic properties. Over 7500 species of medicinally active plants have been utilised to treat a wide range of health conditions such as hyperglycemia, hyperlipidemia, gastrointestinal illnesses, and to improve various cognitive functions, among others (Pandey et al., 2013). Plants from the Cucurbitaceae family are commonly and extensively utilised for culinary purposes. There are subfamilies, namely Cucurbiteae, Momordiceae, and Sicyoeae, that mostly cultivate edible food plants on the Indian subcontinent. The plants are cultivated extensively in most states, with a particular focus on northern, eastern and central India (Renner and Pandey, 2013).

A survey has been done in India and Bangladesh to study the ethnomedicinal practices of plants belonging to the Cucurbitaceae family. The survey was conducted using data provided by indigenous healers from different locations of both countries. The study primarily focuses on the ethnomedicinal significance of cultivated plants from the plant family, which are commonly consumed as vegetables. *Citrullus lanatus*, *Coccinia grandis*, *Cucurbita maxima*, *Cucumis melo*, *Momordica charantia*, *Momordica cochinchinensis*, *Trichosanthes kirilowii*, and other plants from this family are commonly recommended for the treatment of diabetes and oedema. *Benincasa hispida*, *Citrullus lanatus, Coccinia grandis* have been documented as being used to treat cardiac diseases (Rahmatullah et al., 2012). Additionally, ethnopharmacological use of Cucurbitaceae plants, specifically *Luffa cylindrica, Momordica charantia, Momordica cochinchinensis, Trichosanthes dioica*, and *Trichosanthes kirilowii* have been seen in the treatment of metastasis (Shrivastava and Roy, 2013).

Based on the literature reports and ethnopharmacological relevances, the present study aims to validate the traditional claim of the selected plants viz. *Cucurbita maxima, Luffa acutangula*, *Momordica dioica* and *Trichosanthes dioica* based on their phytochemical composition and therapeutic evaluation. This chapter provides a comprehensive discussion on the morphological, phytochemical and pharmacological features of the selected food plants.

3.2 Profiles of the plants under study

3.2.1 *Cucurbita maxima* Duchesne

3.2.1.1 Botanical Taxonomy

Scientific classification			Vernacular names		
Kingdom	:	Plantae	English	:	Giant Pumpkin, great pumpkin
Division	:	Spermatophyta	Sanskrit	:	kusmandakah
Class	:	Dicotylednae	Hindi	:	Kaddu
Order	:	Cucurbitales	Bengali	:	Kumro
Family	:	Cucurbitaceae	Tamil	:	Carkkarai-p-pucani
Genus	:	*Cucurbita*	Assamese	:	Ranga
Species	:	*Cucurbita maxima*	Marathi	:	Lal bhopala

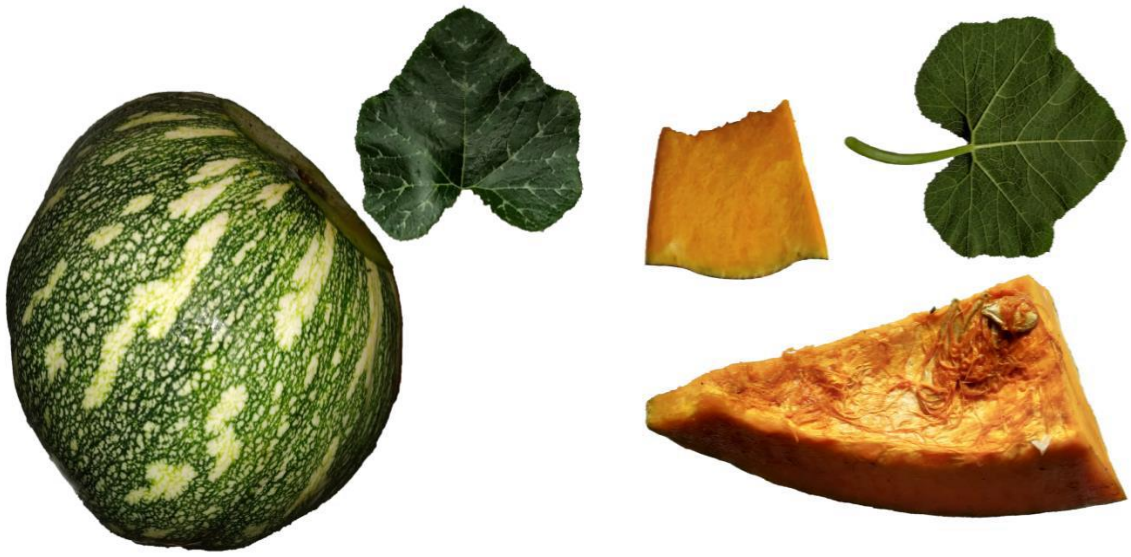


Figure 3.1. *Cucurbita maxima* Plant and Plant Parts

3.2.1.2 Description of plant and plant parts

Cucurbita maxima is a rapidly growing annual vine that can reach heights of up to 5 metres. The blooms exhibit monoecism, meaning that each individual flower is either male or female, but both genders can be present on the same plant. These flowers rely on insects for pollination. The plant is capable of self-fertilization. The stems are cylindrical, elongated, velvety and adorned with fine, short hairs. Roots frequently emerge from the nodes. The leaves possess a velvety texture and are covered in fine hairs, arranged in an alternating pattern. The length of the petiole measures between 10 - 20 cm. The leaf blades are kidney-shaped and have a profoundly heart-shaped base, measuring 25cm in diameter. The flower has five lemon-yellow to orange-yellow petals. The sepals are independent, needle-shaped to elongated and can reach a length of up to 2cm. The male blooms are elongated and possess three stamens. The female flowers have a smaller height compared to the male flowers and possess a unilocular ovary. There are typically three to five stigmas, which are frequently divided into two lobes. The fruits are of considerable size, having a round form and a weight of up to 50 kg. There is a vast variety of colours available, however the colour green is commonly seen in India. The interior is composed of soft tissue and has a vibrant orange hue. Seeds are little and have an elliptical form. The margin is conspicuous, ranging in tint from white to pale brown. Seeds are tiny and have an oval shape. The

hypocotyls measure 3-3.5 cm in length, while the cotyledons are elliptical and can reach up to 4 cm in length (Flowers of India). The pictorial description of the *C. maxima* plant and plant parts has been depicted as Figure 3.1.

3.2.1.3 Geographical distribution

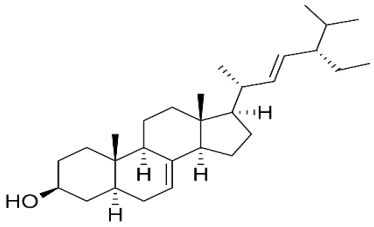
In India, Andhra Pradesh, Arunachal Pradesh, Assam, Bihar, Goa, Gujarat, Himachal Pradesh, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Rajasthan, Tamil Nadu, Tripura, Uttar Pradesh *C. maxima* widely cultivated. The plant globally native to Central America (Renner and Pandey 2013; Mukherjee et al., 2022).

3.2.1.4 Ethnopharmacological Relevance

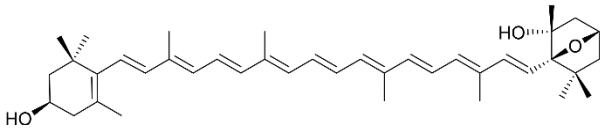
Cucurbita maxima is utilised to optimise urinary bladder function and prevent the creation of kidney stones. Traditionally, in Indian and Chinese medicine, it is utilised as an anti-inflammatory, antiviral, antidiabetic, and antioxidant. Additionally, the seeds have been employed as a vermifuge (Mukherjee et al., 2022). Oral administration of a mixture of *C. maxima* fruits, leaves, and seeds is used to treat neurological disorders and inhibit tumour growth. Fruits also have a cooling impact on the body. (Rahmatullah et al., 2012).

3.2.1.5 Phytochemical Profile

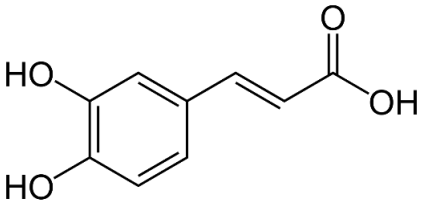
C. maxima is rich in a wide range of phytocomponents, which include both primary and secondary metabolites. According to Ragasa and Lim (2005), it is a valuable source of Vitamin A. This plant also contains a little amount of Vitamin E and K (Avinash and Rai, 2017). The seeds and fruits of *C. maxima* have a carbohydrate content ranging from 6 to 10%. The plant components of *C. maxima* contain secondary metabolites such as α-Spinasterol, flavonoids, polyphenolics, Cucurbitaxanthin A, gibberellin, 11 Eoctadecatrienoic acid, 13-hydroxy-9Z, protocatechuic, caffeic, syringic, vanillic, *p*-coumaric, oleic, linoleic, and palmitic acids (Mukherjee et al., 2022).



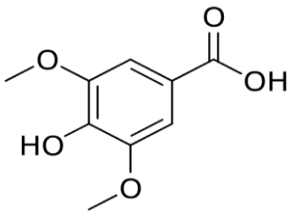
Spinasterol (1)



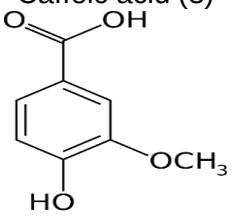
Cucurbitaxanthin A (2)



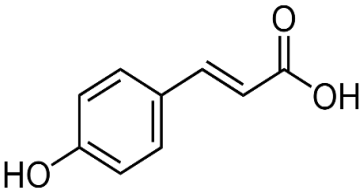
Caffeic acid (3)



Syringic acid (4)



Vanillic acid (5)



p-coumaric acid (6)

Figure 3.2. (1-6) Some structures of the chemical constituents present in *Cucurbita maxima* fruits

3.2.1.6 Pharmacological activity

The pharmacological activities of *Cucurbita maxima* are listed in Table 3.1.

Table 3.1. Pharmacological activities of *C. maxima*

Parts used	Pharmacological activity	References
Seeds	Antidiabetic, Antihyperlipidemic activity	Rajasree et al., 2016
Fruits	Antihypertensive	Sharma et al., 2013
Seeds	CNS Stimulant	Doke et al., 2011
Seeds	Immunosuppressive	Sharma et al., 2013
Seed	Antiinflammatory activity	Rajasree et al., 2016
Fruits	α-Glucosidase Inhibitory	Sulaiman et al., 2013
Fruits	Anti-proliferative and anticancer activity	Varalakshmi and Rao, 2012

Aerial parts	Anticancer and Antitumor properties	Saha et al., 2011a
Seeds	Hepatoprotective activity	Saha et al., 2011b

3.2.2 *Luffa acutangula* (L.) Roxb.

3.2.2.1 Botanical taxonomy

Scientific classification

Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Order	:	Cucurbitales
Family	:	Cucurbitaceae
Genus	:	Luffa
Species	:	*Luffa acutangula*

Vernacular names

English	:	Ridge gourd
Sanskrit	:	Kosataki
Hindi	:	Jhimani
Bengali	:	Jhinga
Tamil	:	Itukari
Telugu	:	Adavibeera
Marathi	:	Divali

Figure 3.3. *Luffa acutangula* Plant and Plant Parts

3.2.2.2 Description of plant and plant parts

Luffa acutangula is a tropical, creeping vine, characterised by its circular leaves and yellow blossoms. The plant roots have a yellowish-brown coloration and possess a nearly cylindrical shape, measuring approximately 8-12 cm in length and 0.7 cm in thickness. Their rough texture is due to the presence of longitudinal wrinkles, and they also exhibited the emergence of new adventitious roots. The stems have a glabrous stem with five angles and a three-fid tendril. The leaves have a round shape with a length of 15-20 cm. They are divided into 5-7 angles or lobes and have a rough texture. The veins and veinlets are highly visible. The fruits have an obovate shape and are pale yellowish brown in colour. They are 4-10 cm in length and 2-4 cm in width. The outer surface of the fruits is covered with 8-10 conspicuous longitudinal ribs. The object narrows towards the bottom and has longitudinal ridges. The fruits are partitioned into three compartments. The inner component exhibits a fibrous composition and can be readily separated in its entirety from the outer component. The fruits possess a bitter flavour. The cross-section of the fruit, namely through a rib, reveals a papillose epidermis that is coated with a thick striated cuticle. This is then followed by 4-6 layers of parenchymatous cells. The seeds are black in colour, possess a bitter taste, and have an ovoid-oblong form (Flowers of India; Pingale et al., 2018). Figure 3.3. displays a visual representation of the fruits and leaves of *L. acutangula*.

3.2.2.3 Geographical distribution

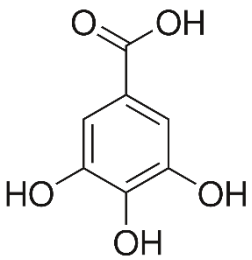
L. acutangula is native and cultivated throughout India and it is also cultivated globally (Renner and Pandey, 2013; Mukherjee et al., 2022).

3.2.2.4 Ethnopharmacological Relevance

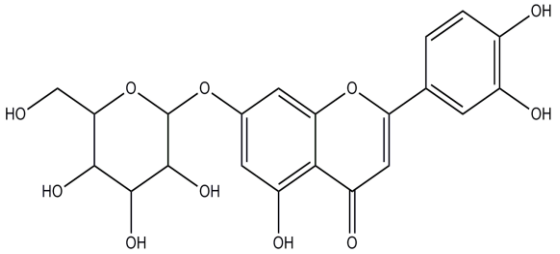
In Ayurveda, *L. acutangula* used to treat skin related disorders, anaemia, splenomegaly, inflammation, tumor growth, indigestion, neurological disorder, poisoning (Anonymous, 2001). The oral ingestion of seed powder is widely employed for the management of urinary bladder stones. The leave juice is used to cure granular conjunctivitis in children (Panicker, 2020).

3.2.2.5 Phytochemical Profile

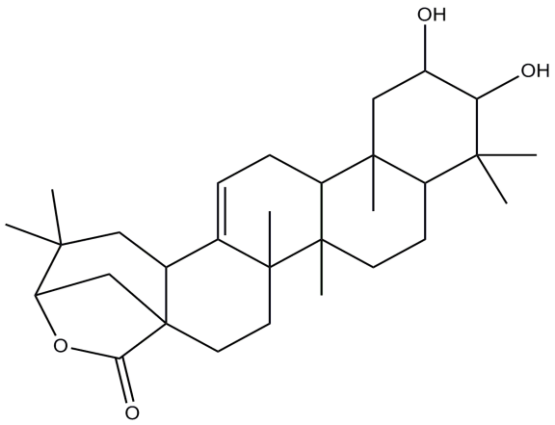
The primary constituents found in *L. acutangula* are protein, fat, amino acids, carbs, carotene, and saponins. The plant is rich in polyphenols, particularly phenolic acids and flavonoids such as ferulic acid, gallic acid, p-coumaric acid, Luteolin-7-glucoside, apigenin-7-glucoside, quercetin, and anthocyanins. These compounds are notably abundant in the fruits of the plant (Swetha and Muthukumar 2016). Caffeine is found in fruits as a bitter compound. Lectin, a type of oligosaccharide molecule, has been also identified in *L. acutangula*. The seeds contain a high concentration of fixed oil. Luffangulin, a ribosome inactivating peptide, was found in the seeds of *L. acutangula* (Shendge and Belemkar, 2018). The seeds of *L. acutangula* were found to contain sapogenin, oleanolic acid, and a bitter principle known as Cucurbitacin B and E (Anitha and Mrithula, 2014). A study by Wang and Ng (2002) identified a protein called Luffaculin 2 in the seeds of *L. acutangula*.



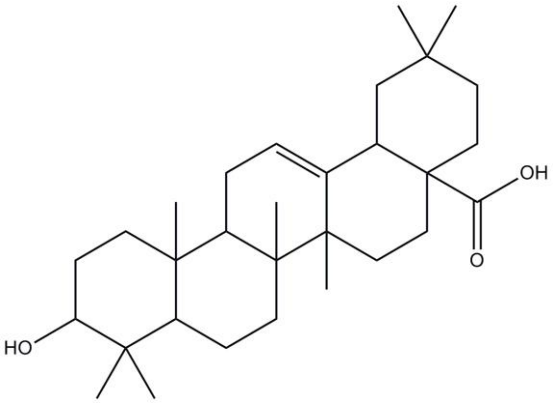
Gallic acid (1)



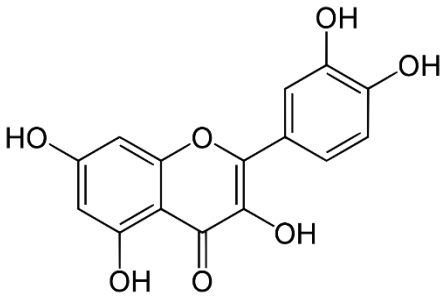
Luteolin-7-glucoside (2)



Sapogenin (3)



Oleanolic acid (4)



Quercetin (5)

Figure 3.4. (1-5) Some structures of the chemical constituents present in *Luffa acutangula* fruits

3.2.2.6 Pharmacological activity

The pharmacological properties of *Luffa acutangula* are outlined in Table 3.2.

Table 3.2. Pharmacological activities of *L. acutangula*

Parts used	Pharmacological activity	References
Fruits	Antimicrobial effects	Shendge and Belemkar, 2018
Fruits	Antidiabetic Activity	Mohan Raj et al., 2012
Fruits	Hepatoprotective activity	Ibrahim et al., 2014
Fruits	Antioxidant property	Pimple et al., 2012
Fruits	CNS depressant and neuroprotective activity	Panicker, 2020.
Fruits	Antibacterial Activity	Moideen and Prabha, 2014

Seeds and leaves	Analgesic and Anti-inflammatory activity	Iyyamperumal et al., 2013; Gill et al., 2011
Fruits	Antitumor and Anticancer Activity	Dashora and Chauhan, 2005

3.2.3 *Momordica dioica* Roxb. ex Willd.

3.2.3.1 Botanical taxonomy

Scientific classification			Vernacular names		
Kingdom	:	Plantae	English	:	Spine Gourd
Division	:	Angiosperms	Sanskrit	:	Vahisi
Class	:	Eudicots	Hindi	:	Ban karela, Kakora, Parora, Golbandra
Order	:	Cucurbitales	Bengali	:	Kartoli, kakrol
Family	:	Cucurbitaceae	Tamil	:	Aegaravalli, Thloopavai,
Genus	:	Momordica	Assamese	:	Bhaat karela
Species	:	*Momordica dioica*	Marathi	:	Kartoli

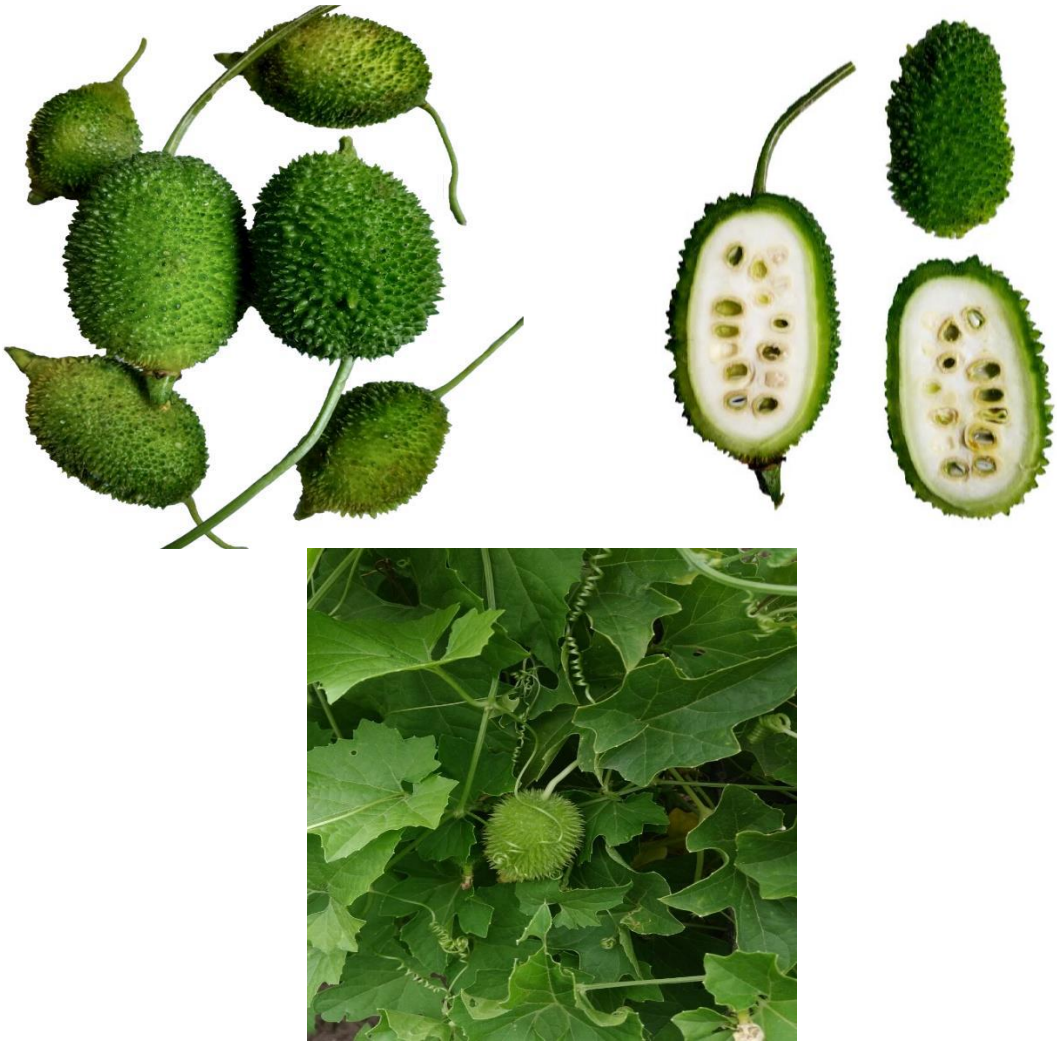


Figure 3.5. *Momordica dioica* Plant and Plant Parts

3.2.3.2 Description of plant and plant parts

M. dioica is a large vine that can reach heights of 3-5 m. The leaves are oval in shape and grouped in an alternating pattern, divided into 3-5 lobes. Stems are the main structural parts of plants that support leaves, flowers, and fruits. The climber is characterised by its solid structure, which consists of five distinct angular ridges. It has a twisting growth pattern and its tendril is not branched. Leaves are foliage and organised in a spiral pattern and have toothed edges. They are heart-shaped, deeply divided, and have a network of veins. The leaves are 9-10 cm in length and 10 cm in width. Flowers can be either monoecious or dioecious. The petals are whitish yellow in colour, about 4-6

cm across, and arranged in an imbricate manner. The male plant produces a single flower at each node, with a longer and thin peduncle of 11.9cm, and a very short pedicel. The female plant exhibits a regular pattern of flower production, with 2-3 consecutive nodes. The peduncle of the flowers is short, measuring 3.8cm, while the pedicel is longer. A fruit is a type of pepo that is heavily covered with soft tuberculate spines. Two types are available, elongated round-end and short round-end. Fruits are a type of edible plant product that often have a sweet or sour taste. The shape of the fruit is generally rectangular, with five rounded edges and a glossy surface. The size of the fruit varies depending on the type (Flowers of India; Talukdar and Hossain, 2014). Leaves and fruits of *M. dioica* presents in figure 3.5.

3.2.3.3 Geographical distribution

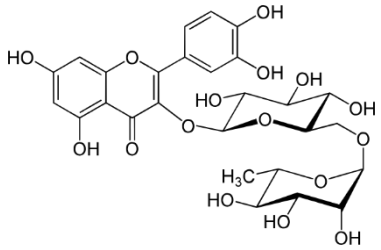
Momordica dioica plant mostly in Deccan plateau and Central part of the India cultivated. In Bangladesh, China, Myanmar, Nepal and Pakistan it is widely found (Renner and Pandey 2013; Mukherjee et al., 2022).

3.2.3.4 Ethnopharmacological Relevance

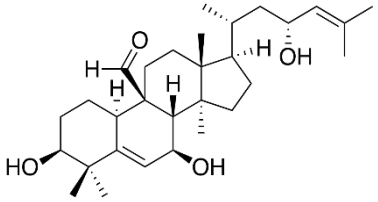
In Ayurvedic traditional system various parts of *M. dioica* widely used to formulate 'Hiraka Rasayana', 'Visanasaka Yoga' (Ayurvedic Prakasa), '*Kakadani Taila', 'Kalagnirudra Rasa', 'Sannipata Vidhvamsa', 'Candrarudra Rasa'.* It is a remedial of several ailments including oro facial herpes, snake poisoning, dysuria, snake poison, fever, cough, bleeding piles, diabetes, Neuro disorder, jaundice etc (Anonymous, 2006). Traditionally, fresh fruit juice, unripe fruits and tender fruits are utilised in hypertension, diabetes, skin disorder accordingly. *M. dioica* also exert diuretic, laxative, hepatoprotective, anti-inflammatory, anti-asthmatic effect. (Talukdar and Hossain, 2014).

3.2.3.5 Phytochemical Profile

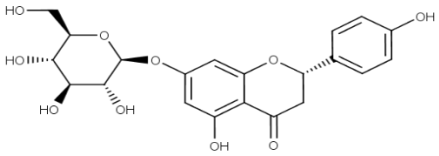
Momordica dioica fruit is rich in protein, fibre, minerals, and a moderate amount of carbohydrates. It also includes minor amounts of vital vitamins such as vitamin A, Vitamin B, Vitamin E (Singh et al., 2009). Various parts of this plant also contain α-spinasterol-3-O-β-D-glucopyranoside, Oleanolic acid, Momordicin, Naringenin-7-O-glucoside, Quercetin, Gallic acid, Rutin, Ferulic acid, Ellagic acid, Rosmarinic acid, Caffeic acid, Hesperetin (Talukdar and Hossain, 2014; Luo and Li, 1997).



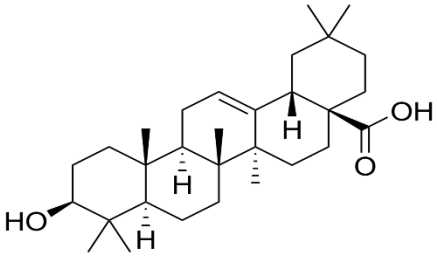
Rutin (1)



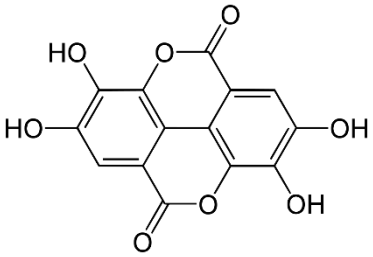
Momordicin (2)



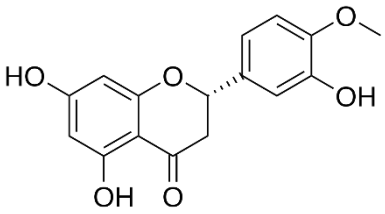
Naringenin-7-O-glucoside (3)



Oleanolic acid (4)



Ellagic acid (5)



Hesperetin (6)

Figure 3.6. (1-6) Some structures of the chemical constituents present in *Momordica dioica* fruits

3.2.3.6 Pharmacological activity

The pharmacological properties of *Momordica dioica* plants are presented in Table 3.3.

Table 3.3. Pharmacological activities of *M. dioica*

Parts used	Pharmacological activity	References
Roots	Antioxidant activity	Shreedhara and Vaidya, 2006
Fruits	Analgesic activity	Ilango et al., 2003
Fruits	Nephroprotective activity	Jain and Singhai, 2010

Fruits	Neuroprotective activity	Rakh and Chaudhary, 2010
Fruits	Antiulcer activity	Fernandopulle et al., 1996
Roots	Anticancer activity	Luo et al., 1998
Leaves	Antimicrobial activity	Arekar et al., 2013
Fruits	Antidiabetic activity	Singh et al., 2011
Roots	Hepatoprotective and antihepatotoxic activity	Chaudhary et al., 2010

3.2.4 *Trichosnathes dioica* Roxb.

3.2.4.1 Botanical taxonomy

Scientific classification			Vernacular names		
Kingdom	:	Plantae	English	:	Pointed Gourd
Division	:	Angiosperms	Sanskrit	:	Patola
Class	:	Eudicots	Hindi	:	Parval
Order	:	Cucurbitales	Bengali	:	Potol
Family	:	Cucurbitaceae	Tamil	:	Kambupudalai
Genus	:	Trichosanthes	Assamese	:	Potol
Species	:	*Trichosanthes dioica*	Telegu	:	Kommu Potla

Figure 3.7. *Trichosanthes dioica* Plant and Plant Parts

3.2.4.2 Description of plant and plant parts

T. dioica is a perennial plant that grows in a vine-like manner and has separate male and female individuals. The vines possess oblong-shaped leaves that are not lobed. These leaves are dark green and have a cordate and ovate shape, resembling leaves. The roots of the plant have a tuberous form and a lengthy taproot. The blooms are cylindrical and white in colour. The time from the beginning of flower development to full bloom is 16-19 days for female flowers and 10-14 days for male flowers. The stigma remains viable for around 14 hours, and 40-70% of blooms successfully produce fruit. Fruits are categorised into four distinct categories according to their shape, size, and striation. The leaves are long and dark green with white stripes, measuring 10-13 cm in length. They are thick and have extremely pale green stripes, measuring 10-16 cm in length. The leaves are roundish, dark green with white stripes, measuring 5-8 cm in length (Kumar et al., 2012).

3.2.4.3 Geographical distribution

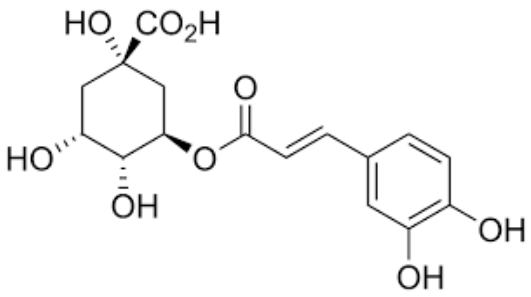
In Arunachal Pradesh, Assam, Bihar, Delhi, Himachal Pradesh, Jammu & Kashmir, Meghalaya, Punjab, Rajasthan, Uttar Pradesh, West Bengal states of India the plant of *T. dioica* widely found and cultivated. In other countries including Bangladesh, Myanmar, Nepal, Pakistan, Sri Lanka the plant was widely found (Renner and Pandey, 2013; Mukherjee et al., 2022).

3.2.4.4 Ethnopharmacological Relevance

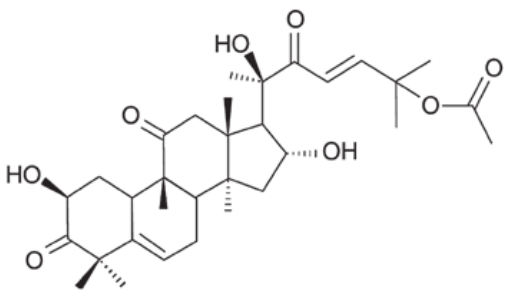
The leaves and stems of *T. dioica* have traditionally been found to have properties that lower cholesterol levels, reduce blood sugar levels, decrease phospholipid levels, and protect the nervous system. Fruits serve as a means to freshen the breath and are also utilised in the management of fever, wounds, and boils (Khulakpam et al., 2015). The plant is utilised for its stomachic, anticancer properties (Saxena and Dave 1995). In Ayurveda, the leaves and fruits of *T. dioica* are utilised for the treatment of drunkenness and jaundice. Fruit is also utilised to enhance appetite and facilitate digestion (Kumar et al., 2012).

3.2.4.5 Phytochemical Profile

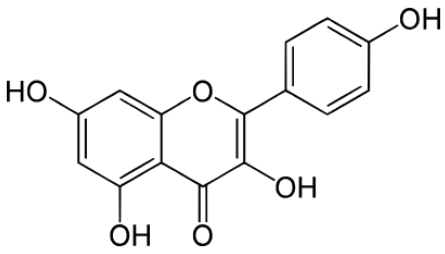
Pointed gourd is abundant in vitamins and encompasses many minerals. *T. dioica* seeds are rich in peptides. The seed peptides possess a distinctive characteristic of being impervious to the effects of silver nitrate, a delicate reagent frequently employed for protein staining. *T. dioica* has a range of chemical components, including vitamin A, vitamin C, tannins, and saponin. The seed of *T. dioica* includes a compound called 7-oxidihydrokarounidol-3-benzoate, as well as two primary phytosterols known as 24α-ethylcholest-7-enol and 24β-ethylcholest-7-enol (Kumar et al., 2012). The major phytochemical groups identified in the sample included alkaloids, glycosides, flavonoids, carbohydrates, fixed oils, steroids, tannins, and phenols. Notable compounds within these groups included Lutein, Zeaxanthin, Kaempferol, Luteolin, Quercetin, Vanillic acid, Chlorogenic acid, Caffeic acid, Cucurbitacin B, Cucurbitacin E, and others (Mukherjee et al., 2022).



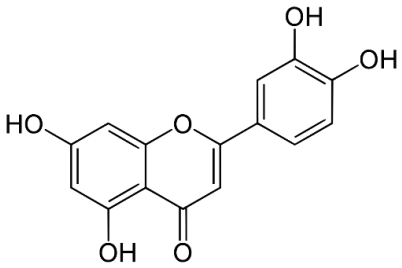
Chlorogenic acid (1)



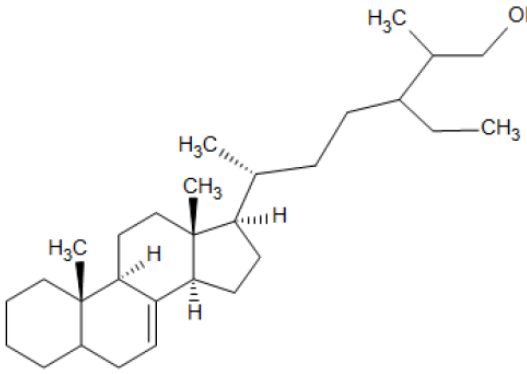
Cucurbitacin B (2)



Kaempferol (3)



Luteolin (4)



24β-ethylcholest-7-enol (5)

Figure 3.8. (1-5) Some structures of the chemical constituents present in *Trichosanthes dioica* fruits

3.2.4.6 Pharmacological activity

The pharmacological and therapeutic activities of *Trichosanthes dioica* plants are presented in Table 3.4.

Table 3.4. Pharmacological activities of *T. dioica*

Parts used	Pharmacological activity	References
Fruits	Antidiabetic activity	Shahana and Nikalje, 2019
Fruits	Antihyperlipidemic activity	Rai et al., 2013
Roots	Antitumor activity	Bhattacharya and Haldar, 2012b
Roots	Anti-inflammatory activity	Bhattacharya and Haldar, 2012a
Roots	Antinociceptive activity	Bhattacharya et al., 2012

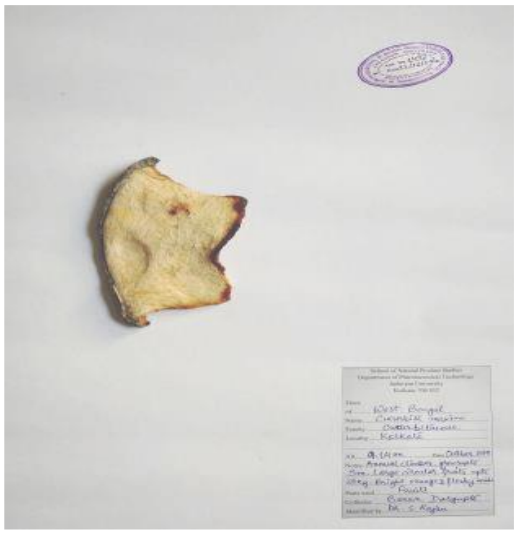
Leaves	Hepatoprotective activity	Khandaker et al., 2018
Whole Plant	Immunomodulatory activity	Bhadoriyal and Mandoriya, 2012
Fruits	Wound healing activity	Shivhare et al., 2010

3.3 Collection and Identification of the selected fruits

The different plants from Cucurbitaceae family were collected from various local markets situated in Kolkata, West Bengal, India. All the plants were authenticated by field botanist and the voucher specimens were prepared (Figure 3.9). Further the prepared voucher specimens were kept in School of Natural Product Studies (SNPS), Department of Pharmaceutical Technology, Jadavpur University, Kolkata for future references. The details of collection and authentication of plants has been described in Table 3.5.

Table 3.5. Authentication details of selected plants of Cucurbitaceae family

Plant Name	Common Name	Tribes	Voucher specimen no.
Cucurbita maxima Duchesne	Pumpkin	Cucurbiteae	SNPS-JU/2019/1492
Luffa acutangula (L.) Roxb.	Ridge Gourd	Sicyoaea	SNPS-JU/2019/1488
Momordica dioica Roxb. ex Willd.	Spine Gourd	Momordiceae	SNPS-JU/2019/1490
Trichosanthes dioica Roxb.	Pointed Gourd	Sicyoaea	SNPS-JU/2019/1489



Cucurbita maxima Duchesne

Luffa acutangula (L.) Roxb.

Momordica dioica Roxb. ex Willd.

Trichosanthes dioica Roxb.

Figure 3.9. Authentication details of selected plants of Cucurbitaceae family

3.4 Extraction of the selected fruits

To achieve the most effective separation of the phytomolecules, a method of serial extraction using solvents of varying polarity was found to be efficient, allows for the isolation and characterization of the active components. Cold maceration is a simple form of extraction where powdered plant material is placed in a sealed container and soaked with a solvent for a specific amount of time until the soluble parts dissolve in the

solvent. This approach is commonly utilised in the field of medicinal plant research. The goal of the technique is to extract the therapeutically useful component and remove the inert material by treating it with a selective solvent called the menstruum. Maceration consists of three primary stages. Initially, plant materials undergo a process of pulverisation to transform them into a fine powder. With an increase in surface area, there is enhanced contact between the solvent and substance, facilitating effective mixing with the solvent. Following the process of grinding, a selected solvent is introduced into a sealed container. Next, the liquid is separated by straining, while the solid residue from this extraction procedure is compressed to retrieve a significant quantity of enclosed solutions. During maceration, intermittent shaking enhances the extraction yield by promoting diffusion and eliminating the concentrated solution from the surface of the sample. The effectiveness of the maceration process is influenced by two primary factors: solubility and efficient diffusion. Solvent polarity is another crucial component in the cold maceration process. For instance, when extracting non-polar compounds such as fats, oils, and lipids, non-polar solvents are employed. Conversely, highly polar compounds like glycosides, phenolics, sugars, amino acids, proteins, and polysaccharides are primarily extracted using polar solvents like ethanol and water (Abubakar and Haque, 2020; Zhang et al., 2018).

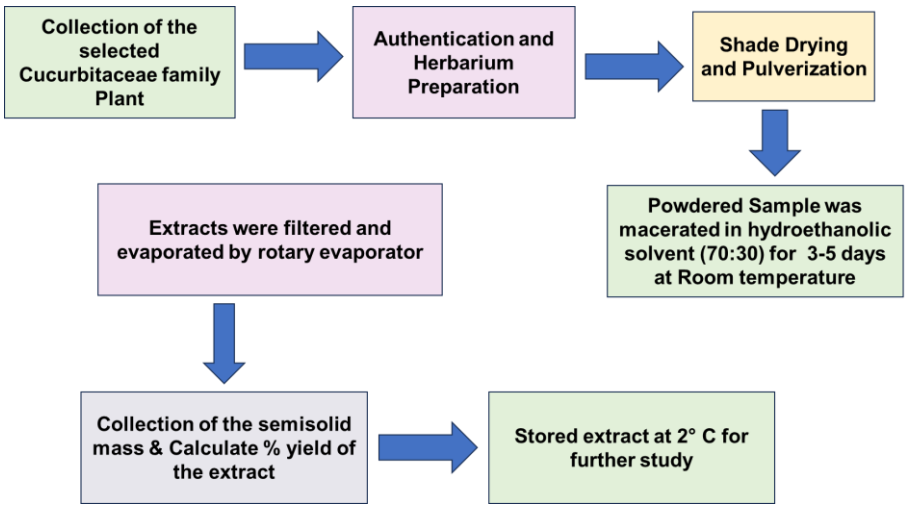


Figure 3.10. Schematic diagram of extraction process

All the selected plants of Cucurbitaceae family were shade dried and pulverized into coarse powder. Then the plant materials were macerated in hydroalcohol solvent (70:30 ratio) for cold maceration extraction procedure for 3-5 days at room temp. with occasional shaking. After that the filtrates were collected and evaporated by rotary evaporator (Instrumentation, India) at 40–45 °C. After complete evaporation the extracts were further lyophilised and stored in air tight container at 2-8ºC in a refrigerator for further use (Singha et al., 2020; Chibuye et al., 2023) In figure 3.10. the schematic diagram of extraction process was presented. The % (w/w) yields for the selected plants were presented in Table 3.6.

Table 3.6. Percentage yields of the Cucurbitaceae family plants under study

Name of the Plant	% Yield (w/w)
Cucurbita maxima Duchesne	15.02
Luffa acutangula (L.) Roxb.	10.64
Momordica dioica Roxb. ex Willd.	12.70
Trichosanthes dioica Roxb.	18.90

3.5 Quantitative Analysis

3.5.1 Determination of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

Phenolic substance are plant compounds that have the characteristic of having an aromatic ring with one or more hydroxyl groups. Approximately 8000 plant phenolics occur naturally, with flavonoids accounting for over half of this total. Phenolics have a diverse range of biochemical properties, including antioxidant, antimutagenic, anticarcinogenic, and the potential to alter gene expression. Phenolics are the

predominant category of phytochemicals responsible for the majority of antioxidant activity found in plants or plant-derived products (Tungmunnithum et al., 2018).

Flavonoids are the most extensive category of phenolic chemicals that are found naturally in various portions of plants, existing either in their free form or as glycosides. These compounds have been discovered to possess numerous biological actions, such as antibacterial, mitochondrial adhesion inhibition, antiulcer, antiarthritic, antiangiogenic, anticancer, and protein kinase inhibition. Flavonoids consist of two benzene rings that are separated by a propane unit. The flavones and flavonols have the broadest distribution among all phenolics. Flavonoids have notable advantages, as they function as antioxidants and provide defence against cardiovascular disease, specific types of cancer, and the deterioration of cell components associated with ageing. Due to their polyphenolic nature, they have the ability to eliminate harmful free radicals including superoxide and hydroxyl radicals. Experimental animal models have shown that various dietary plant flavonoids can effectively suppress the formation of tumors. Biflavonoids have pharmacological properties such as the ability to suppress the production of histamines, prevent the adhesion of blood platelets, and limit the action of lens aldose reductase. They also have anti-inflammatory effects against hepatotoxins and can function as a heart stimulant (Baker, 2022; Kumar and Pandey, 2013).

3.5.1.1 Materials and methods

3.5.1.1(i) Chemicals and Reagents

Gallic acid and Rutin standard compound obtained from Sigma Aldrich, India. Folin Ciocalteau reagent (FC), Sodium Bicarbonate ($NaHCO_3$), methanol, Sodium Hydroxide (NaOH), Sodium Nitrite ($NaNO_2$), Aluminium Chloride ($AlCl_3$) were procured from Merck, Mumbai, India.

3.5.1.1(ii) Methodology of TPC

Standard compound Gallic acid solution was prepared (1 mg/mL) using methanol and further diluted into 1000, 500, 250, 125, 62.5, 31.25 µg/ml by serial dilution. Each selected plant extract of Cucurbitaceae family were also dissolved in methanol (1 mg/mL) and were filtered through 0.45µm filter (Millipore). 18 µL aliquots of each plant

extract and gallic acid was mixed with 90 µL of 10% FC and 90 µL of 7.5% $NaHCO_3$. Further the reaction mixture incubated at 45°C for 45 minutes and measured the absorbance at 765 nm by using Spectramax ID3 (Molecular Devices LLC, United States) (Singha et al., 2020). The TPC has been stated in Gallic acid equivalent (GAE), mg/g. Figure 3.11. depicted the schematic representation of the workflow of TPC.

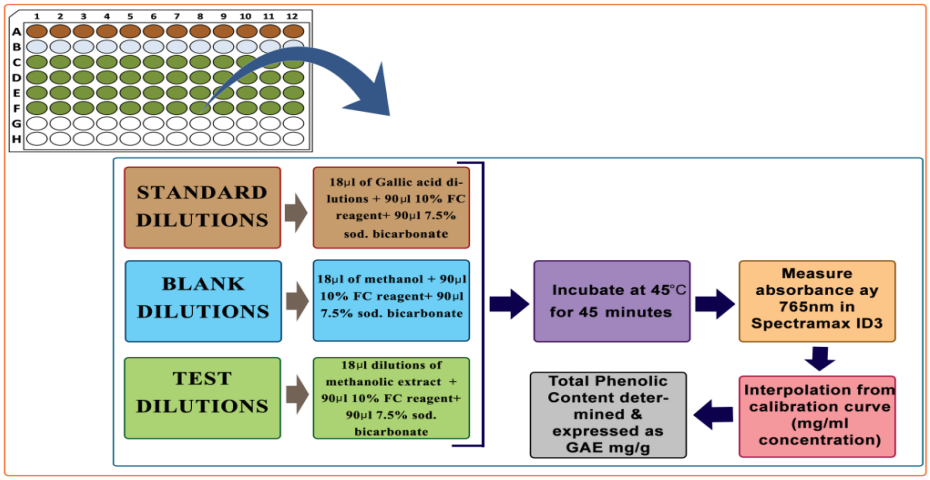


Figure 3.11. Schematic diagram of the workflow of TPC

3.5.1.1(iii) Methodology of TFC

All the selected fruit extracts (1 mg/mL) and Rutin (1 mg/mL) were dissolved in methanol. 100 µL standard or sample or blank mixed with 50µL of 4% NaOH, 10 µL of 5% $NaNO_2$ and 10 µL of 10% $AlCl_3$. The absorbance of the admixture was measured by using Spectramax ID3 (Molecular Devices LLC, United States) at 518 nm (Singha et al., 2020; Fattahi et al., 2014) and expressed as Rutin Equivalent (RE), mg/g.

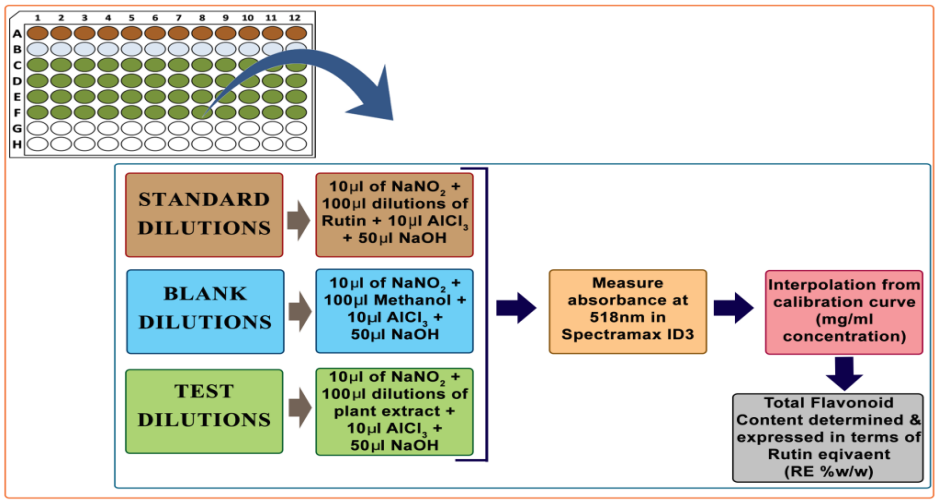


Figure 3.12. Schematic diagram of the workflow of TFC

3.6 Results and Discussions

The % yield of the selected fruit extract were *C. maxima*: 15.02% w/w, *L. acutangula*:10.64% w/w, *M. dioica*: 12.70 % w/w and *T. dioica*:18.90% w/w. The regression equation for Gallic acid and Rutin was found out y= 0.008x + 0.005 and y= 0.002x-0.005 respectively; the R^2 value of gallic acid was 0.924 whereas the R^2 value of Rutin was 0.979. It suggests a strong regression analysis that is statistically significant. The experimental values of TPC and TFC represented in Table 3.7. Both the calibration curve of Gallic acid and Rutin has been shown in Figure 3.13 (i-ii).

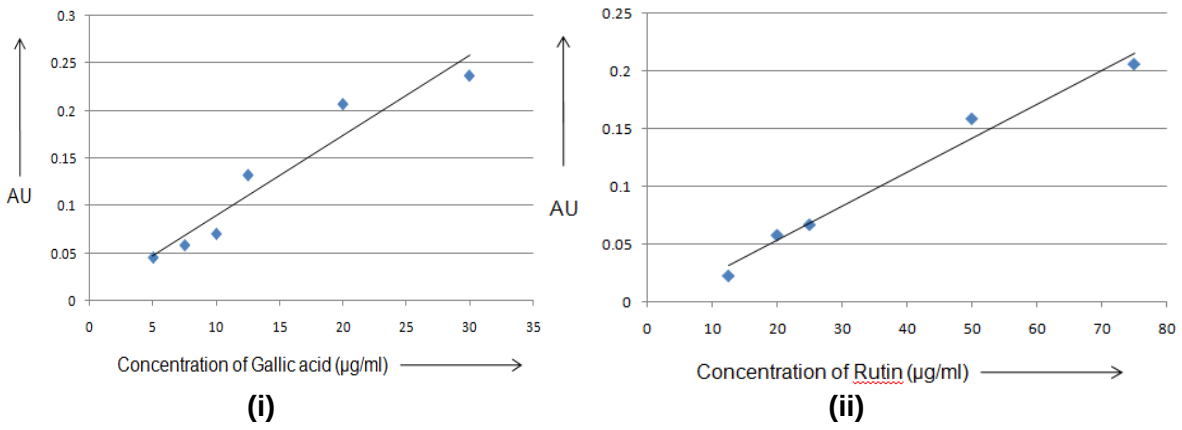


Figure 3.13. Calibration curve of Gallic acid (i) and Rutin (ii)

Table 3.7. Estimation of TPC and TFC of the selected food plant of Cucurbitaceae family

Plant Name	Total phenolic content (GAE, mg/g)	Total flavonoid content (RE, mg/g)
Cucurbita maxima Duchesne	22.32 ± 0.368	4.09 ± 0.029
Luffa acutangula (L.) Roxb.	8.33 ± 0.422	0.856 ± 0.041
Momordica dioica Roxb. ex Willd.	25.59 ± 0.329	4.90 ± 0.03
Trichosanthes dioica Roxb.	9.73 ± 0.39	3.12 ± 0.043

3.7 Conclusion

The cold maceration extraction method was used to extract all of the selected plant extract. The quantitative preliminary phytochemical studies in the forms of TPC and TFC studies have given promising findings. The plants with the greatest TPC (25.59 ± 0.329 mg/g and 22.32 ± 0.368 mg/g, respectively, expressed as GAE) and TFC (4.90 ± 0.03 mg/g and 4.09 ± 0.029 mg/g, respectively, expressed as RE) were *Momordica dioica* and *Cucurbita maxima*. Therefore, it is evident from the initial phytochemical investigations that the plants are of the highest calibre. To further establish their qualities, they will undergo metabolite profiling through LC-QTOF-MS, HPTLC, and HPLC assessments. Additionally, the concentrated extracts will be investigated for their *in-vivo* adaptogenic activity as well as their *in-vitro* antioxidant, α-amylase, α-glucosidase, carbonic anhydrase, and pancreatic lipase enzyme inhibition assays.

Chapter 4

Metabolite profiling, marker analysis and standardization of *Cucurbita maxima, Luffa acutangula, Momordica dioica* and *Trichosanthes dioica* fruits

4.1 Marker profiling and standardization in medicinal plant analysis

The composition of medicinal plants encompasses a diverse array of secondary metabolites, such as alkaloids, terpenoids, saponins, flavonoids, phenolic acids which possess promising therapeutic characteristics. The utilisation of LC-MS technology offers a comprehensive equipment for the profiling of plant metabolites, analysis of metabolite targets, and replication of significant bioactive ingredients, among other applications in the field of medicinal plant research. Metabolite profiling is a valuable technique that facilitates the identification and quantitative quantification of marker molecules that are naturally occurring within plants. On the other hand, dereplication involves the exploration of active constituents to distinguish novel compounds from previously researched active substances. The dereplication process offers significant benefits by circumventing the need for isolation or purification procedures based on existing knowledge of chemical compounds. Consequently, it presents a robust and time-efficient strategy in the realm of natural product-based drug development (Kumar, 2017; Kiani et al., 2023).

MS-based approaches have been identified as appropriate for investigating the structural characteristics of chemical constituents, as well as their qualitative as well as quantitative characterization. Considering the complicated structure of plant extracts, it is imperative to employ a chromatographic platform that effectively separates these chemicals according to their physiochemical characteristics. HPLC provides an adaptable approach for isolating natural substances. In the field of separation sciences, there has been significant progress in the development of ultra-high pressure liquid chromatography (UHPLC) with enhanced chromatographic resolution. This breakthrough aims to facilitate the identification of a wide range of metabolites found in plant samples (Wolfender et al., 2015). The conjunction of UHPLC with a MS) detector presents an appropriate methodology that effectively exceeds the fundamental criteria for analysis, including sensitivity, selectivity, and peak-assignment certainty. This approach enables the efficient identification of analytes at low concentrations inside intricate matrices. In addition, the UHPLC-TOF-MS and UHPLC-QToF-MS platforms provide enhanced resolution and enhanced sensitivity for the analysis of specific metabolites. Triple quadrupole (in SRM mode) paired with UHPLC is the most appropriate method for assessing target compounds, while TOF-MS analyzers are more

suitable for non-targeted analysis (Rathod et al., 2019). The present study employed the LC-QToF-MS-guided dereplication technology to efficiently identify the bioactive compounds that exhibit significant therapeutic activity. This approach is suitable for investigating lead molecules that have potential therapeutic activity from natural products. It may also be seen as a crucial stage in isolating and characterising compounds in the process of drug development based on natural products.

High-performance thin-layer chromatography (HPTLC) is a multifaceted and non-invasive method of chromatographic separation that operates on the principles of thin layer chromatography. This method is widely regarded as highly dependable for the purpose of separating, identifying, and determining the content uniformity and purity profile of convoluted herbal medicine. The utilisation of HPTLC fingerprint analysis facilitates the detection of marker compounds and adulterants that are found inside herbal medicine. The HPTLC method offers several notable advantages in comparison to conventional TLC methods include reproducibility, simplicity, quicker development time, lower solvent consumption, and increased resolution. HPTLC is widely employed in the herbal sector for accurately identifying plant materials used as raw materials, estimating the quantity of plant materials after harvesting, storage, and drying, and monitoring the fingerprint throughout the manufacturing process. In today's times, HPTLC has been enhanced by using MS, FTIR and Raman spectroscopy, among others, to provide enhanced sensitivity for the purposes of identification and characterization (Nile and Park, 2014). HPTLC employs smaller plates, often measuring 10 X 10 or 10 X 20 cm, resulting in a reduced development distance of 6 cm and a shorter analysis time of 7-20 mins. Twin-trough chambers, also known as horizontal development chambers, are utilised for the development of HPTLC plates. These chambers are equipped with filter paper to enhance repeatability. The identification of distinct substances on the sorbent layers is conducted within the ultraviolet (UV) spectrum (200-400 nm), specifically at wavelengths of 254 and 366 nm. The targeted substances are quantitatively estimated by measuring the zones of samples using densitometric scanning (Singha et al., 2020). A schematic diagram has been represented to understand workflow of HPTLC analysis (Figure 4.1).

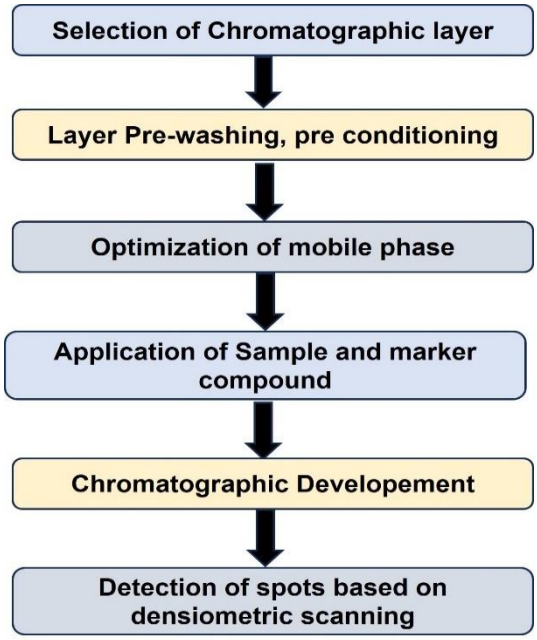


Figure 4.1. Workflow of HPTLC analysis

HPLC is a highly adaptable chromatographic separation method that enables the qualitative and quantitative assessment of specific marker compounds present in plant extracts. The main objective of HPLC is to achieve the separation, identification, and quantification of phytoconstituents found in plants. In simple terms, HPLC aims to comprehensively examine and characterise a combination of herbal components. The HPLC approach is extensively employed in the field of natural product research for the purposes of authentication, standardisation, and quality evaluation. This technique relies on the identification of marker compounds to assure the purity, safety, and efficacy of medicinal plants and herbal products. At present, HPLC has been enhanced by incorporating many detection techniques, including as UV-Vis, PDA, MS, and NMR, in order to achieve increased sensitivity and detection capacities for phyto-components. This advancement aims to improve the quality control of herbal medicines. Hyphenated chromatographic techniques, in conjunction with chemometric analysis, frequently provide a highly effective method for assessing the quality and effectiveness of medicinal plants. A high-performance liquid chromatography system comprises a pump for the transportation of the eluent and sample within the system, an injection device for the ingestion of the sample, one or more columns for the separation of solutes, a detector for the visualisation of the separated components, and a data collection device

to aid in the interpretation and storage of results (Figure 4.2). HPLC analysis can be categorised into two distinct phases, namely the normal-phase and reversed phase, depending on the specific characteristics of the stationary and mobile phases employed. The choice of a chromatographic mode is contingent upon the chemical identity of the analytes and their compatibility with both the stationary and mobile phases. RP-HPLC is the predominant method employed in HPLC. In addition to this, the separation process of HPLC relies on two distinct elution strategies. The isocratic method involves maintaining a constant mobile phase composition and flow rate during the whole run. Conversely, gradient elution involves altering the mobile phase composition over time. The isocratic approach is commonly employed for the purpose of identifying known or selective molecules within a mixture, while gradient elution is a valuable technique for the separation of complicated mixtures (Nag et al., 2020; Ooh et al., 2014).

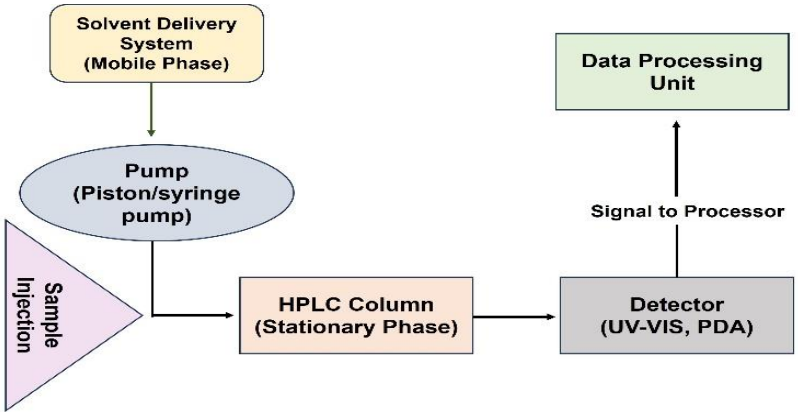


Figure 4.2. Workflow of HPLC study

4.2 Importance of Chlorogenic acid as marker compound

Chlorogenic acid (CA) is a phenolic secondary phytocomponent also known as 5-caffeoylquinic acid, which is formed through the esterification of caffeic acid and quinic acid. It is widely distributed in various plant species, fruits, and vegetables. It demonstrates substantial efficacy in mitigating cardiovascular diseases (CVDs), type 2 diabetes, and illnesses associated with inflammation. In recent times, CA has emerged as a viable alternative for the treatment of many neurological disorders, including Stress, Alzheimer's disease and also neuropathic pain (Pawar et al., 2023).

CAs are produced through the biosynthesis of phenylalanine via the phenylpropanoid reaction pathway. This process is widely recognised for its role in the synthesis of other significant chemicals, such as flavonoids, isoflavonoid phytoalexins, coumarins, and lignin (Clifford et al., 2017). The p-coumaroyl CoA has been found to exhibit three potential routes. The enzymatic processes involved in each route are consistent, including esterification and hydroxylation. These metabolites have the ability to safeguard plant tissues against harm caused by oxidative stress, pathogen infection, and wounds (Zhao et al., 2018). The putative biological properties of CA encompass a wider spectrum, offering potential health benefits. These features may include non-pharmacological and non-invasive techniques for hepatoprotection, antioxidant activity, anti-diabetic effects, antibacterial properties, anticarcinogenicity, anti-inflammatory responses, and anti-obesity effects (Telles et al., 2017). By improving memory function, improve attention, act as antidepressant, anxiolytic and prevents cerebral ischemia CA performed as neuroprotective agent (Kim and Park, 2019).

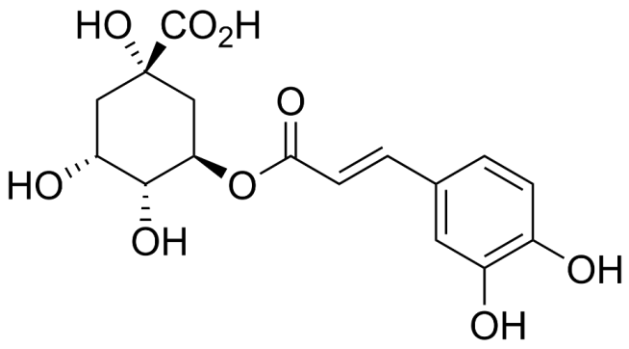


Figure 4.3. Chlorogenic Acid

4.3 LC-QToF-MS based metabolomics study of *Cucurbita maxima, Luffa acutangula, Momordica dioica* and *Trichosanthes dioica* fruit extracts

4.3.1 Materials & Methods

4.3.1.1 Chemicals, Reagents and Sample Preparation

Acetonitrile, ammonium acetate, Methanol, Water (LC-Ms Grade) were procured from Merck, Mumbai, India. The pH of the mobile phase was calibrated by using Mettler Toledo pH Meter. Additionally individual plant sample was dissolved in methanol to obtain a concentration of 100 µg/mL. The sample solutions then filtered using 0.22 µm syringe filters (Agilent) and then placed in high-recovery amber vial (Agilent Technologies, Santa Clara, CA, USA).

4.3.1.2 LC-QToF-MS method

The Liquid chromatography time-of-flight mass spectrometer (LC-QToF-MS) analysis was conducted using an Agilent 1260 Infinity II LC System coupled with Accurate-Mass (Q-ToF) Spectrometer. Agilent Zorbax Eclipse C18 column (50mm × 2.1mm, 1.7µm) was used for chromatographic separations, maintained at an isothermal temperature of 38 °C. 10mM ammonium acetate in water (A) and acetonitrile (B) were used as the mobile phase at a flow rate of 0.200 mL/min in a gradient mode. The gradient elution was started with 100% A at 0–2 min, 95%–100% A at 2–5 min, 85%–95% A at 5–10 min, 70%–85% A at 10–15 min, 5%–70% A at 15–25 min, 5–100% A at 25–35 min. The flow rate and injection volume were set to 0.5 mL/min and 5 µL respectively. For the mass spectrometry, an Agilent 6530 Accurate Mass Q-TOF mass spectrometer operating in positive ion mode was utilised, with a skimmer voltage of 60 V and a fragment voltage of 120 V. To investigate the structure of the prospective biomarkers, MS/MS analysis was done. Agilent MassHunter Qualitative Analysis v. 10.0 coupled with a customized database was used for data mining and identification of the phytoconstituent based on the Retention time, observed mass, major MS-MS fragments and error ppm.

4.4 HPTLC standardization of *Cucurbita maxima, Luffa acutangula, Momordica dioica* and *Trichosanthes dioica* fruit extracts

4.4.1 Materials and methods

4.4.1.1 Chemicals, reagents, instrumentation

Standard chlorogenic acid (> 98% purity) and Silica gel 60 F_{254} HPTLC plates were procured from Sisco Research Laboratories Pvt. Ltd., Mumbai and Merck, Mumbai, India respectively. A CAMAG (Muttenz, Switzerland) HPTLC system with Linomat V (sample applicator), Reprostar 3 (Photo-documentation chamber), TLC Scanner 3 (Densitometric scanner) and twin trough development chamber (were used for HPTLC analysis. A 100 µL Hamilton syringe (Bonaduz, Switzerland) was used for sample application in the HPTLC system. Methanol, ethyl acetate, chloroform, formic acid was purchased from Finar Limited, Ahmedabad.

4.4.1.2 Preparation of standard and sample solutions

The selected plant extract and reference standard chlorogenic acid were weighed in a microcentrifuge tube and dissolved in methanol to obtain 10 mg/mL extract solutions and 0.1 mg/mL standard solutions. The standard and extract solutions was vortexed until dissolved and then filtered through 0.45 µm syringe filter. Using five distinct amounts of the standard (CA, 1 mg/mL) in the range of 2–10 µL, a calibration curve was created to assess the linearity of the response generated by standards.

4.4.1.3 Chromatographic conditions

Standard and samples were applied by using Linomat V, band wise in HPTLC plates size of 10 cm × 10 cm dimension was used for stationary phase. Five bands of standard solutions (2-10 µL) and three sample bands for each plant extract (10, 12 and 14 µL) were prepared on each plate for the analysis. The plates were dried and developed in twin-trough chamber using the mobile phase ethyl acetate: chloroform: formic acid (6:4:0.5, V/V). Following development, the plates were dried, scanned at 254 nm using a Scanner 3, and the data were visualised employing the winCATS software.

4.4.2 HPTLC Method Validation

HPTLC method validation was performed as recommendation by the International Conference on Harmonization (ICH) guidelines (ICH 2005) in terms of assessing specificity, limits of quantification and limits of detection, accuracy, precision, and robustness.

4.4.2.1 Specificity

The results obtained from standardization were checked in terms of specificity according to the ICH guidelines to reducing errors due to the contamination of the sample. The specificity of the method was determined by analysing both standard and samples. The purity of the peaks was scrutinized by comparison of retention times and peak area of standard compound with extract by employing multivariate analysis.

4.4.2.2 Limits of quantification and limits of detection (LOD & LOQ)

The limits of quantification and limits of detection were determined by setting the standard deviation (σ) and the slope (S) of the calibration plot. Afterthat the LOD and LOQ were estimated by the formula LOD = 3:1 σ/S and LOQ = 10:1 σ/S, whereas, 'σ' is standard deviation of the response and 'S' is slope of the calibration plot.

4.4.2.3 Accuracy

The accuracy of the method was estimated by % recovery of the reference standard present in the plant extracts. By performing standard addition technique accuracy was studied, and expressed in % relative standard deviations (%RSD) (Mukherjee et al., 2015). The tests were first spiked with three known quantities of standard in triplicate prior to application on the plate. Analysis was performed under ambient conditions to calculate the total average recovery. Mean amounts of the standard achieved were considered as real values for spike recovery calculation.

4.4.2.4 Precision

The method precision was determined by applying six replicates at three different concentrations for the reference standard and the extracts. The values were represented as %RSD of intra-day and inter-day precision whereas the mean amount

and %RSD values were estimated. The intra-day precision was assessed by analysing 3 concentrations in 1 day and the inter-day precision was determined over three continuous days (n = 6).

4.4.2.5 Robustness

The robustness of the method was evaluated by analysis of the sample under different experiment condition including variation of flow rate, time variability, composition of mobile phase, wavelength detection and column temperature. The robustness of the method was measured as %RSD.

4.5 RP-HPLC standardization of *Cucurbita maxima, Luffa acutangula, Momordica dioica* and *Trichosanthes dioica* fruit extracts

4.5.1 Materials and methods

4.5.1.1 Chemicals, reagents, instrumentation

Standard chlorogenic acid (> 98% purity) was procured from Sisco Research Laboratories Pvt. Ltd., Mumbai, India. The RP-HPLC system (Shimadzu, Japan) included two pumps (Shimadzu LC-20 AD UFLC), a Prominence PDA detector (Shimadzu SPD-M20A), injector (Rheodyne 7725i, USA), and the LC-Solution software system for data processing. The C18 reverse phase column (Phenomenex-Luna C18, USA) was used as the stationary phase. HPLC grade methanol, glacial acetic acid and water were obtained from Finar chemicals, Ahmedabad, India.

4.5.1.2 Preparation of standard and sample solutions

Chlorogenic acid (standard) and each plant extracts were dissolved in methanol at a concentration of 1 mg/mL and 10 mg/mL respectively. Further the standard was diluted to 100, 200, 300, 400 and 500 µg/mL. Both the solutions were vortexed and sonicated, then filtered through 0.45 µ membrane filter (Millipore).

4.5.1.3 Chromatographic Conditions

For preparation of the mobile phase an isocratic solvent system of water, pH 3.8 adjusted with glacial acetic acid and methanol at the ratio of 25:75 was used. The temperature of column kept abiding at 25 °C. The isocratic elution of standard and

sample were employed as follows: volume of injection-20 µL; flow rate - 1 mL/minute; spectrum-190 nm and time of analysis-20 mins (Kar et al., 2015).

4.5.2 RP-HPLC Method Validation

RP-HPLC method validation was done as recommended by the International Conference on Harmonisation (ICH) guidelines (ICH, 2005) in terms of determining Specificity, Limits of detection (LOD) and Limit of quantification (LOQ), Accuracy, Precision, Robustness. Specificity of the method was assessed by comparison between standard and samples' retention time (Rt). The specificity mainly confirms the properties as well as characteristics of the analyte and minimizing the errors. By estimating LOD and LOQ sensitivity was evaluated. By using the formula LOD = 3:1 σ/S and LOQ = 10:1 σ/S LOD and LOQ were calculated, whereas, 'σ' is standard deviation of the response and 'S' is slope of the calibration plot. The accuracy of the method was determined by comparing the % recovery of reference standard in plant samples. Followed by the injection, the analysis samples have been spiked in triplicate by three distinct quantities of standard. Method precision was evaluated by applying six replicates at three different concentrations for the reference standard and the extracts to determine the repeatability of the method. The values were represented as %RSD of intra-day and inter-day precision whereas the mean amount and %RSD values were estimated. The intra-day precision was assessed by analysing 3 concentrations in 1 day and the inter-day precision was determined over three continuous days (n = 6). The robustness of the method was determined by evaluated by analysis of the sample under different experiment condition including variation of flow rate, time variability, composition of mobile phase, wavelength detection and column temperature. The robustness of the method was measured as %RSD.

4.6 Results and Discussions

4.6.1 LC-QToF-MS based metabolomics study of the selected fruit extracts

Plant metabolomic research have been employed to examine several characteristics, notably genetic diversity, composition of products, production techniques, sensory attributes, cultivation techniques, and environmental factors, among others. Metabolic profile techniques are commonly used to evaluate the nutritional value of a certain plant

cultivar for the aim of quality control. Due to recent technological improvements, the LC-MS technique has been widely applicable in metabolomics analysis. This technique offers a broad detection spectrum and demonstrates exceptional specificity and sensitivity (Patel et al., 2021). In the current investigation employed the LC-Q-ToF-MS method to evaluate the metabolic profiles of *C. maxima*, *L. acutangula, M. dioica*, and *T. dioica* extract. The results of this study revealed significant differences in the metabolic properties between the different fruits of Cucurbitaceae family. The molecular mass determination of the selected fruit extracts was conducted using a positive ionisation technique. The identification of the compounds was verified through the analysis of retention times, mol. wt. (m/z), mol. formula, error (in ppm), published data, and library search. The commonly acknowledged precision for verifying the authenticity of elemental compositions has been determined to be below 5 ppm. The combination of high-resolution Q-ToF-MS, chemical database, and Library searching approach enabled precise detection of bioactive ingredients in the food extract.

The LC-Q-ToF-MS analysis identified the presence of phytomolecules in the *C. maxima*, *L. acutangula, M. dioica*, and *T. dioica* extract represented in Table 4.1 – 4.4 respectively. In these tables' retention times, chemical formulas, ppm errors, major MS-MS fragments, and measured masses (both predicted and estimated m/z) was listed for all the plant extracts. Figure 4.4-4.7 represented "Positive mode LC-MS Chromatogram of *C. maxima*, *L. acutangula, M. dioica*, and *T. dioica* fruit extract respectively. Tryptophan, pentadecylic acid, catechin, β-sitosterol, linolenic acid, linoleic acid, stigmasterol, palmitic acid, stearic acid, oleic acid, 9,12-octadecadenoic acid, zeaxanthin, β-D-gentiobiosyl, eicosenoic acid, kaemperol, caffeic acid, karavilagenin C, balsaminoside A, cucurbitacin K, cucurbitacin J, α-spinasterol-3-O-β-D-glucoside, heptadecanol, oleic acid, β-Amyrine etc. major metabolites identified from the selected fruit extracts. The results revealed the presence of phenolic acid, flavonoid, triterpene, phytosterol, vitamin etc. class of compound.

Table 4.1. Compounds identified in *C. maxima* fruit extract by LC–QToF–MS

RT	m/z (Estimated)	m/z (Expected)	Chemical Formula	Error PPM	Major MS-MS Fragments	Name of Compounds	Class of Compound
1.231	204.0899	204.0898	C11H12N2O2	0.48	188, 146, 118	Tryptophan	Amino acid
1.9	430.3809	430.3810	C29H50O2	-0.23	230, 147	α-Tocopherol	Vitamin
7.237	305.2568	305.2566	C16H35NO4	0.65	200, 157, 129, 115	Lauric acid	Fatty acid
10.692	290.0797	290.0790	C15H14O6	2.41	236, 202, 166, 124, 109	Catechin	Flavonoid
13.791	164.0470	164.0473	C9H8O3	-1.82	119, 92, 90	*p*-coumaric acid	phenylpropanoids
16.4	556.3030	556.3036	C32H44O8	-1.07	518	Cucurbitacin E	Triterpene
17.3	272.0682	272.0684	C15H12O5	-0.73	153, 147	Naringenin	Flavonoids
19.3	414.3863	414.3861	C29H50O	0.48	379, 341, 287, 255, 213	β-sitosterol	Phytosterol
22.9	278.2245	278.2245	C18H30O2	0.00	261, 243, 205, 149, 123	Linolenic acid	Fatty acid
23.1	280.2400	280.2402	C18H32O2	-0.71	202, 165, 124, 100	Linoleic acid	Fatty acid
25.006	412.3705	412.3705	C29H48O	0.00	395, 315, 257, 199, 83	Stigmasterol	Fatty acid
25.2	256.2408	256.2402	C16H32O2	2.34	183, 149, 118, 75	Palmitic acid	Fatty acid
25.658	284.2715	284.2715	C18H36O2	0.00	275, 257, 241, 153, 125	Stearic acid	Fatty acid
26.4	256.2764	256.2766	C17H36O	-0.78	210, 135, 139, 112	Heptadecanol	Fatty alcohol
26.792	282.2555	282.2558	C18H34O2	-1.06	265, 205, 135, 107,	Oleic acid	Fatty acid

					83		
27.2	426.3867	426.3861	C30H50O	1.40	412, 342	β-Amyrine	Isoprenoid lipid
28.943	310.2874	310.2871	C20H38O2	0.96	290, 275, 227	Eicosenoic acid	Fatty Acid
29.2	594.1586	594.1584	C27H30O15	0.33	422, 304, 288, 256	kaemperol	Flavonoid

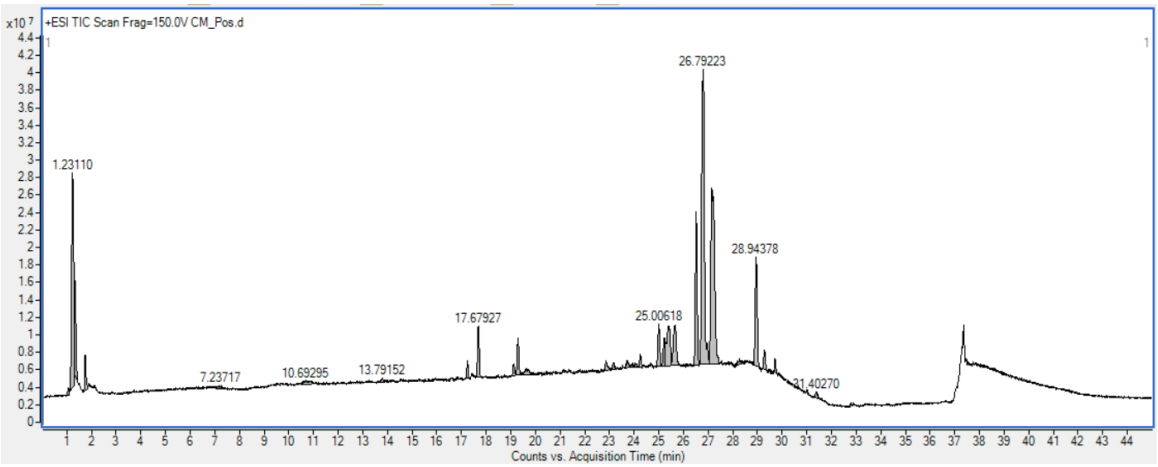


Figure 4.4. Positive mode LC-MS Chromatogram of *C. maxima* Fruit Extract

Table 4.2. Compounds identified in *L. acutangula* fruit extract by LC–QToF–MS

RT	m/z (Estimated)	m/z (Expected)	Chemical Formula	Error PPM	Major MS-MS Fragments	Name of Compounds	Class of Compound
1.231	204.0895	204.0898	C11H12N2O2	-1.46	188, 146, 118	Tryptophan	Amino acid
1.8	242.2245	242.2245	C15H30O2	0.00	365, 301, 171	Pentadecylic acid	Fatty acid
10.726	290.0799	290.0790	C15H14O6	3.10	236, 202, 166, 124, 109	Catechin	Polyphenol
19.3	414.3860	414.3861	C29H50O	-0.24	379, 341, 287, 255, 213	β-sitosterol	Phytosterols
22.9	278.2245	278.2245	C18H30O2	0.00	261, 243,	Linolenic acid	Fatty acid

					205, 149, 123		
23.1	280.2406	280.2402	C18H32O2	1.42	202, 165, 124, 100	Linoleic acid	Fatty acid
25.1	412.3705	412.3705	C29H48O	0.00	395, 315, 257, 199, 83	Stigmasterol	Phytosterols
25.2	256.2409	256.2402	C16H32O2	2.73	183, 149, 118, 75	Palmitic acid	Fatty acid
25.6	284.2714	284.2715	C18H36O2	0.35	275, 257, 241, 153, 125	Stearic acid	Fatty acid
26.6	282.2554	282.2558	C18H34O2	1.41	265, 205, 135, 107, 83	Oleic acid	Fatty acid
26.809	280.2401	280.2402	C18H32O2	0.35	278, 254	9,12-Octadecadenoic acid	Fatty acid
27.2	568.4289	568.4280	C40H56O2	1.58	551, 533, 463	Zeaxanthin	Carotenol
27.3	652.2733	652.2731	C32H44O14	0.30	564, 475, 313	β-D-gentiobiosyl	Dicarboxylic acid monoester
28.952	310.2875	310.2871	C20H38O2	1.28	290, 275, 227	Eicosenoic acid	Fatty acid
29.3	594.1587	594.1584	C27H30O15	0.50	422, 304, 288, 256	Kaempferol	Flavonoid

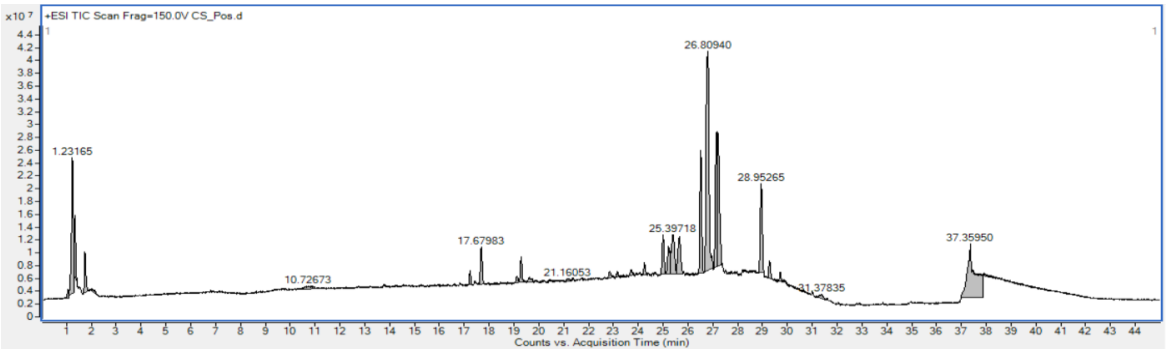


Figure 4.5. Positive mode LC-MS Chromatogram of *L. acutangula* Fruit Extract

Table 4.3. Compounds identified in *M. dioica* fruit extract by LC–QToF–MS

RT	m/z (Estimated)	m/z (Expected)	Chemical Formula	Error PPM	Major MS-MS Fragments	Name of Compounds	Class of Compound
1.233	204.0896	204.0898	C11H12N2O2	-0.97	188, 146, 118	Tryptophan	Amino acid
1.9	450.3495	450.3497	C31H46O2	-0.44	331, 315, 281, 257	Phytonadione	Vitamin
15.255	634.4440	634.4444	C37H62O8	-0.63	622, 576, 480	Balsaminoside A	Triterpene
17.672	376.1385	376.1382	C17H20N4O6	0.79	332, 284, 242, 197, 98	Riboflavin	Vitamin
19.29	414.3867	414.3861	C29H50O	1.44	379, 341, 287, 255, 213	β-sitosterol	Phytosterol
19.4	470.3398	470.3396	C30H46O4	0.42	456	Gypsogenin	Pentacyclic triterpenoid
22.9	278.2244	278.2245	C18H30O2	-0.35	261, 243, 205, 149, 123	Linolenic acid	Fatty acid
23.1	244.0888	244.0881	C10H16N2O3S	2.86	209, 199, 184, 166	Biotin	Heterobicyclic compound
24.3	219.1108	219.1106	C9H17NO5	0.91	146, 116, 99	Pantothenic acid	Vitamin
25.1	412.3704	412.3705	C29H48O	-0.24	395, 315, 257, 199, 83	Stigmasterol	Phytosterol
25.2	256.2400	256.2402	C16H32O2	-0.78	183, 149, 118, 75	Palmitic acid	Fatty acid
25.6	284.2710	284.2715	C18H36O2	-1.75	275, 257, 241, 153, 125	Stearic Acid	Fatty acid
26.6	282.2551	282.2558	C18H34O2	-2.48	265, 205, 135, 107, 83	Oleic acid	Fatty acid

26.794	280.2405	280.2402	C18H32O2	1.07	278, 254	9,12-Octadecadenoic acid	Fatty acid
27.2	574.4236	574.4233	C35H58O6	0.52	487, 356, 288	α-spinasterol-3-O-β-Dglucoside	Phytosterol
28.954	310.2877	310.2871	C20H38O2	1.93	290, 275, 227	Eicosenoic acid	Fatty Acid
29.3	594.1588	594.1584	C27H30O15	0.67	422, 304, 288, 256	Kaempferol	Flavonoid

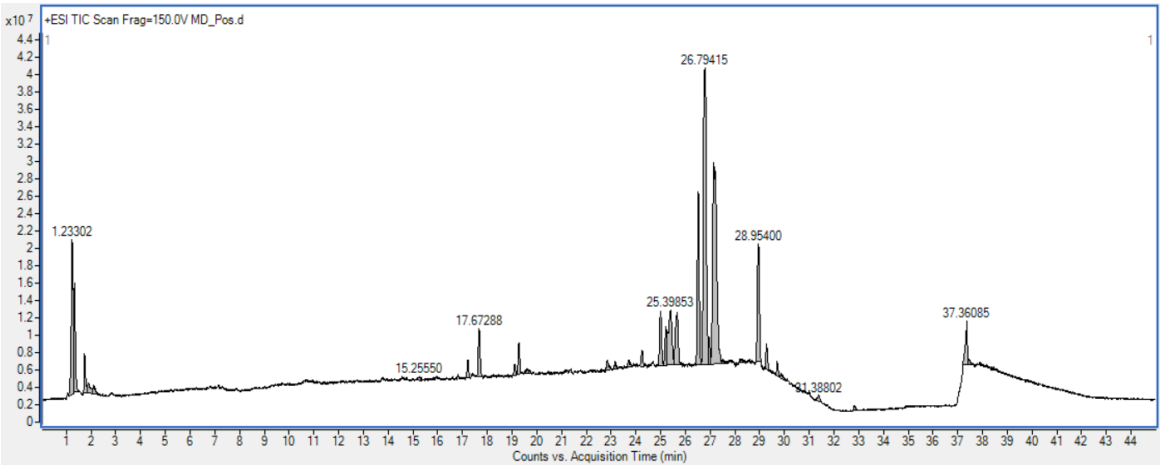


Figure 4.6. Positive mode LC-MS Chromatogram of *M. dioica* Fruit Extract

Table 4.4. Compounds identified in *T. dioica* fruit extract by LC–QToF–MS

RT	m/z (Estimated)	m/z (Expected)	Chemical Formula	Error PPM	Major MS-MS Fragments	Name of Compounds	Class of Compound
9.657	180.0426	180.0422	C9H8O4	2.22	162, 144, 115, 93, 88	Caffeic acid	Polyphenolic
12.4	620.4285	620.4288	C36H60O8	-0.48	402, 318	Karavilagenin C	Triterpenoid
13.9	165.0785	165.0789	C9H11NO2	-2.42	120	Phenylalanine	Amino acid
15.231	634.4445	634.4444	C37H62O8	0.15	622, 576, 480	Balsaminoside A	Triterpene
16.4	532.3032	532.3036	C30H44O8	-0.75	516, 362	Cucurbitacin K	Triterpenoid
17.474	532.3035	532.3036	C30H44O8	-0.18	518, 362	Cucurbitacin J	Triterpenoid
19.3	414.3855	414.3861	C29H50O	-1.44	379, 341, 287, 255, 213	β-sitosterol	Phytosterols

20.332	474.3709	474.3709	C30H50O4	0.00	414, 402, 386	Balsaminol A	Triterpenoid
25.0	412.3705	412.3705	C29H48O	0.00	395, 315, 257, 199, 83	Stigmasterol	Phytosterols
25.2	256.2402	256.2402	C16H32O2	0.00	183, 149, 118, 75	Palmitic acid	Fatty acid
25.7	284.2712	284.2715	C18H36O2	-1.05	275, 257, 241, 153, 125	Stearic acid	Fatty acid
26.5	282.2557	282.2558	C18H34O2	-0.35	265, 205, 135, 107, 83	oleic acid	Fatty acid
26.795	280.2404	280.2402	C18H32O2	0.71	278, 254	9,12-Octadecadenoic acid	Fatty acid
27.3	560.3340	560.3349	C32H48O8	-1.60	520, 442	Cucurbitacin C	Triterpenoid
28.955	310.2875	310.2871	C20H38O2	1.28	290, 275, 227	Eicosenoic acid	Fatty acid

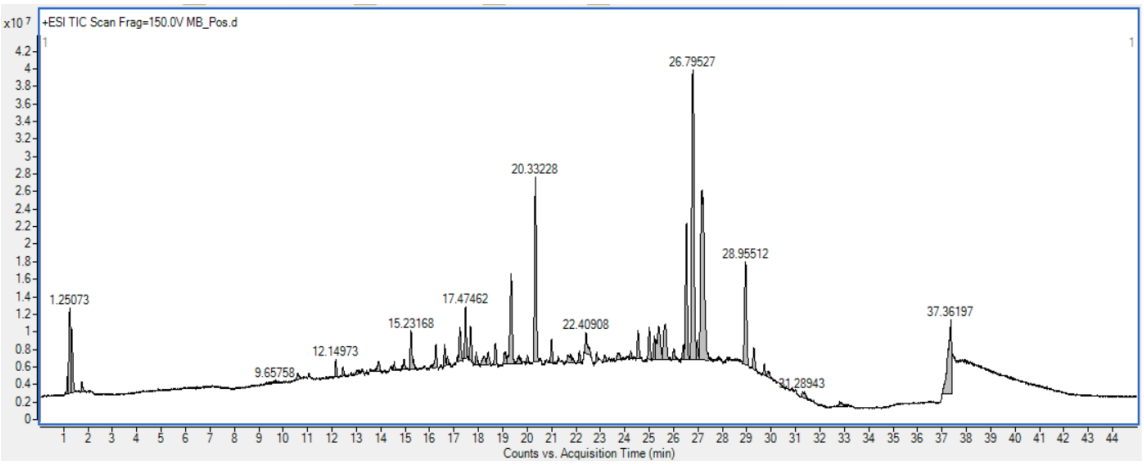


Figure 4.7. Positive mode LC-MS Chromatogram of *T. dioica* Fruit Extract

4.6.2 HPTLC standardization of *Cucurbita maxima, Luffa acutangula, Momordica dioica* and *Trichosanthes dioica* fruit extracts

The chlorogenic acid content in the selected plant extracts was evaluated using HPTLC analysis. In order to assess the adaptogenic activity of the plants, we have standardised the plants using chlorogenic acid, a widely recognised neuroprotective substance. The HPTLC analysis revealed that the *M. dioica* and L. acutangula extract exhibited the highest concentration of CA, measuring 1.543% and w/w. The CA content in *C. maxima* is 1.158% w/w, while in *T. dioica* it is 1.5% and 1.518 % w/w accordingly. The study

revealed that *C. maxima* and *T. dioica* had a comparatively lower concentration of CA in comparison to other extracts. The quantity of CA in the analysed fruit extract are displayed in Table 4.6. The validation of the procedures was conducted in accordance with the ICH Q2R1 recommendations. The experiment was replicated six times, yielding a statistically significant recovery rate ranging from 98.75% to 99.93%. Additionally, the low root mean square deviation (RMSD) value of 0.076-2.63% confirmed the method's high accuracy. In order to assess the precision, two distinct levels of standard chlorogenic acid were employed, specifically 200 µg and 400 µg. The average area of the chlorogenic acid was then calculated for both inter-day and intra-day precision. The six repetitions for each type of determination resulted in a RSD of 0.18-0.28% (intra-day) and 0.15-0.26% (inter-day), indicating a low level of precision for the procedure. The LOD was determined to be 623.20 ng per spot, while the LOQ was found to be 1950 ng per spot. The methodology also successfully underwent a robustness test. The findings suggest that the used methodology exhibits a high degree of robustness, accuracy, precision, and specificity in the analysis of chlorogenic acid using HPTLC. Table 4.5 illustrates the calibration curve for the fruit extracts and the corresponding correlation coefficients (r). Table 4.7 and 4.8 represented 'Recovery studies for determination of chlorogenic acid in plant extracts', 'Intra-day and Inter-day precision study for determination of chlorogenic acid in plant extracts (n=6) by HPTLC method' respectively for determining the accuracy of the HPTLC method. In figure 4.8 (i-x) Photograph of developed HPTLC plate and HPTLC chromatogram of standard chlorogenic acid with sample extracts under 254 nm were depicted.

Table 4.5. Regression equations and correlation coefficient (r) values for fruit extracts

Plant Sample	Regression equations	Correlation coefficient (r)
Cucurbita maxima	y= 887.9+ 1113x	0.99739
Luffa acutangula	y= 1426+ 1144X	0.99784
Momordica dioica	y= 371.1+ 737.3x	0.95409
Trichosanthes dioica	y= 998.4+ 816.3x	0.99583

Table 4.6. Content of chlorogenic acid in different fruit extract by HPTLC method

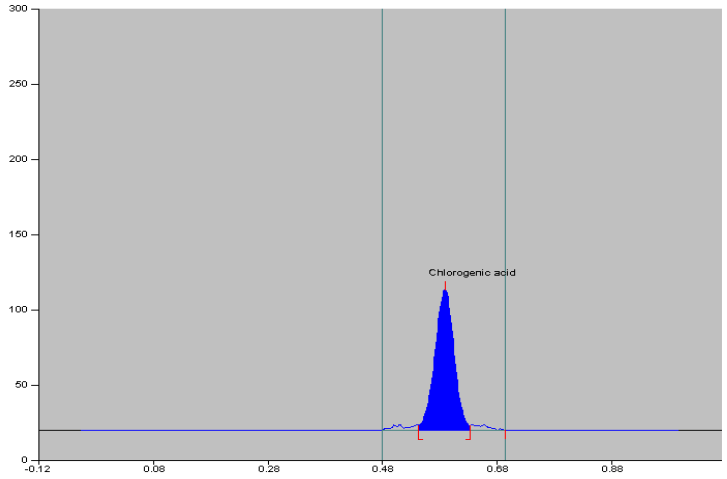
Plant Sample	Chlorogenic acid contain
Cucurbita maxima	1.158 % w/w
Luffa acutangula	1.543% w/w
Momordica dioica	1.50 % w/w
Trichosanthes dioica	1.16 % w/w

Table 4.7. Recovery studies for determination of chlorogenic acid in plant extracts

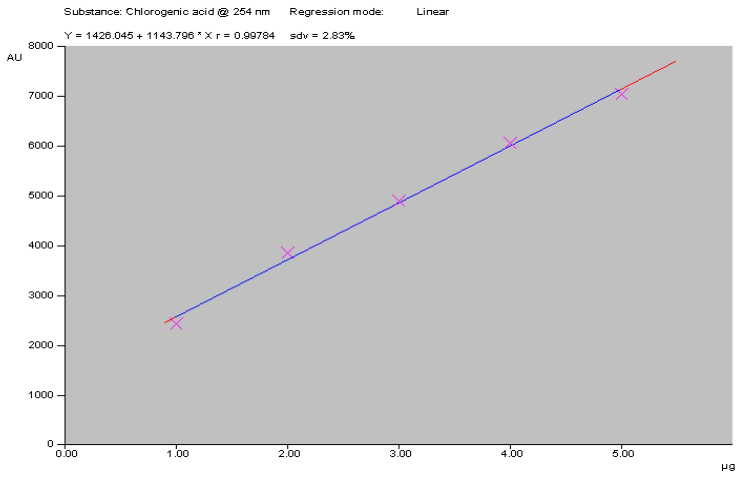
Excess Chlorogenic acid added to extract (ng)	Expected Chlorogenic acid (ng)	Chlorogenic acid found (ng)	Average Chlorogenic acid found (ng)	Average Recovery (%)	RSD (%)
0	1562.00	1558.60 1561.50 1561.30 1561.20 1560.50 1561.90	1560.83	99.93	0.08
200	1762.00	1756.50 1755.50 1760.30 1761.90 1758.00 1759.50	1758.62	99.81	0.14
400	1962.00	1956.00 1961.60 1960.50 1957.50 1961.90 1960.50	1959.67	99.88	0.12
600	2162.00	2150.00 2152.00 2156.00 2161.90 2160.00 2150.50	2155.06	99.68	0.23

Table 4.8. Intra - day & Inter-day precision study for determination of chlorogenic acid in plant extracts (n=6) by HPTLC method

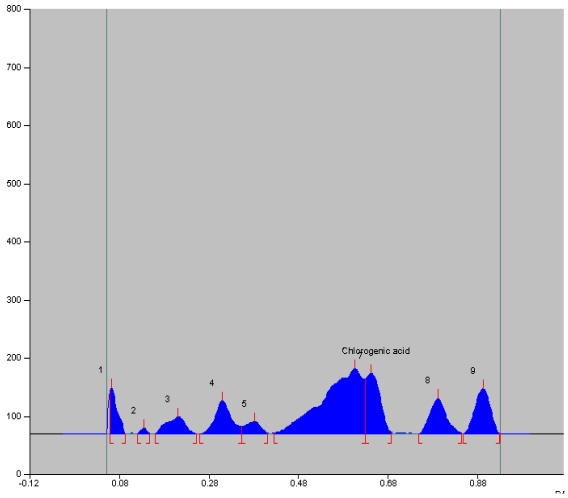
Amount of Chlorogenic acid applied [ng/spot]	Intra day			Inter day		
	Mean	S.D.	% RSD	Mean	S.D.	% RSD
200	9494.694	26.34	0.28	9499.134	24.00	0.26
400	18981.196	34.67	0.18	19000.258	29.10	0.15



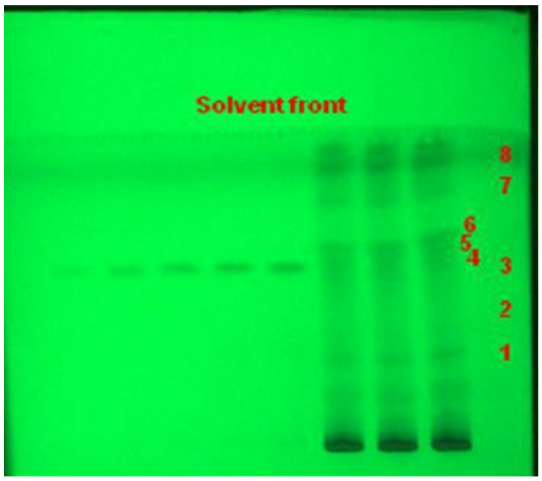
i - HPTLC Chromatogram of Chlorogenic acid



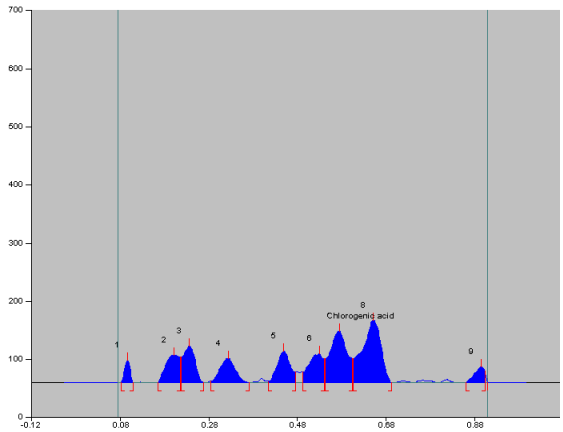
Ii - Calibration curve of Chlorogenic acid



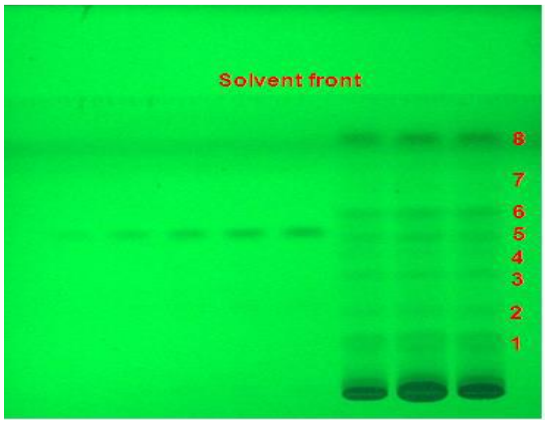
iii - HPTLC Chromatogram of *Cucurbita maxima*



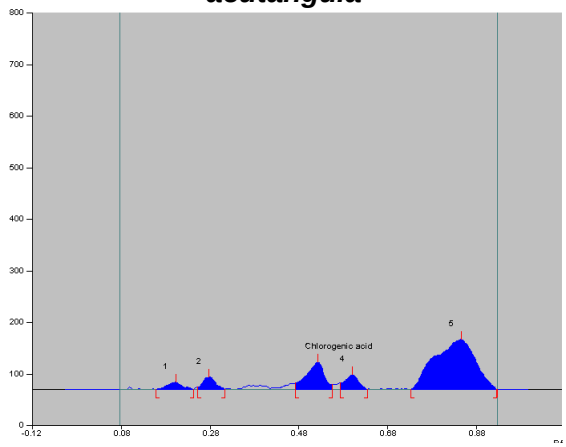
iv - Photo documentation of *Cucurbita maxima*



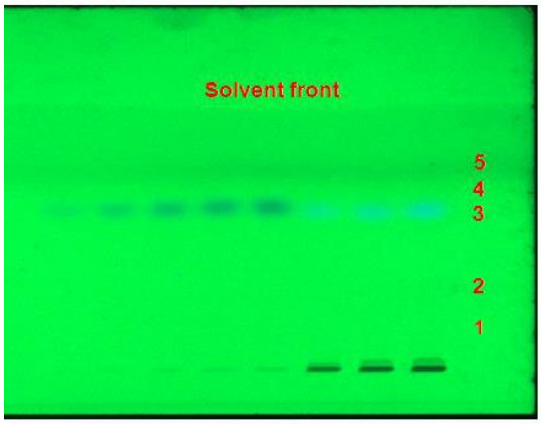
v - HPTLC Chromatogram of *Luffa acutangula*



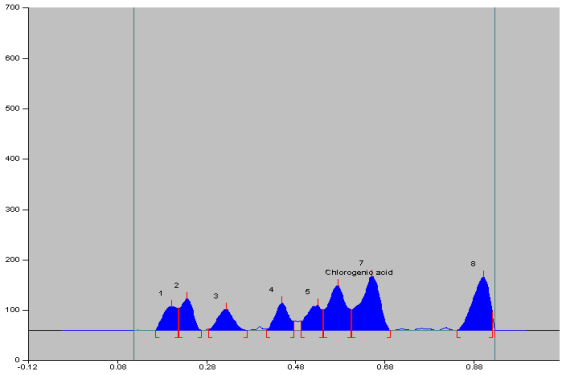
vi - Photo documentation of *Luffa acutangula*



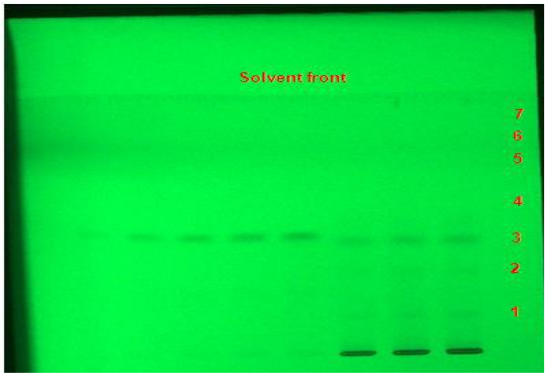
vii - HPTLC Chromatogram of *Momordica dioica*



viii - Photo documentation of *Momordica dioica*



ix - HPTLC Chromatogram of *Trichosanthes dioica*



x - Photo documentation of *Trichosanthes dioica*

Figure 4.8. (i-x) Photograph of developed HPTLC plate and HPTLC chromatogram of standard chlorogenic acid with sample extracts under 254 nm.

i - HPTLC Chromatogram of Chlorogenic acid, ii - Calibration curve of Chlorogenic acid, iii -HPTLC Chromatogram of *Cucurbita maxima*, iv - Photo documentation of *Cucurbita maxima*, v - HPTLC Chromatogram of *Luffa acutangula*, vi - Photo documentation of *Luffa acutangula*, vii - HPTLC Chromatogram of *Momordica dioica*, viii - Photo documentation of *Momordica dioica*, ix - HPTLC Chromatogram of *Trichosanthes dioica*, x - Photo documentation of *Trichosanthes dioica*

4.7.3 RP-HPLC standardization of *Cucurbita maxima, Luffa acutangula, Momordica dioica* and *Trichosanthes dioica* fruit extracts

The results of the RP-HPLC assessment indicate the presence of chlorogenic acid in all four medicinal plants that were selected for this study. The *M. dioica* exhibited the highest concentration of CA, measuring at 1.738% w/w. This was followed by *C. maxima* at 1.084% w/w, *T. dioica* at 0.791% w/w, and *L. acutangula* at 0.554% w/w (Table 4.9). The retention period of chlorogenic acid was determined to be 3.17±0.14 mins. In accordance with the ICH Q2R1 criteria, the procedures underwent validation. The experiment was conducted six times, and the statistical analysis revealed a recovery rate ranging from 98.74% to 99.78%. Additionally, the low root mean square deviation (RMSD) value of 0.60% to 2.24% proved the method's correctness. The precision was assessed by utilising standard chlorogenic acid at two distinct concentrations, namely 200 and 400 (Table 4.10). The average area was then

calculated for both inter-day and intra-day precision. The method's precision was indicated by the low percentage relative standard deviation (RSD) values of 0.02-0.03% (intra-day) and 0.02-0.04% (inter-day) obtained from six repetitions for each type of determination (Table 4.11). The LOD was determined to be 33 µg/mL, while the LOQ was discovered to be 105 µg/mL. The approach successfully passed the robustness test. The obtained results demonstrate that the method exhibits a high level of robustness, accuracy, precision, and specificity when applied to the HPTLC analysis of chlorogenic acid. Figure 4.9 and 4.10 (i-vi) represented calibration curve of CA and RP-HPLC chromatogram of standard CA and selected fruit extracts.

Table 4.9. Content of chlorogenic acid in different fruit extract by RP-HPLC method

Plant Sample	Chlorogenic acid contain
Cucurbita maxima	1.084 % w/w
Luffa acutangula	0.554 % w/w
Momordica dioica	1.738 % w/w
Trichosanthes dioica	0.791 % w/w

Table 4.10. Recovery studies for determination of chlorogenic acid in plant extracts (n=6) by RP-HPLC method

Excess Chlorogenic acid added to extract (ng)	Expected Chlorogenic acid (ng)	Chlorogenic acid found (ng)	Average Chlorogenic acid found (ng)	Average Recovery (%)	RSD (%)
0	156.00	157.50	154.03	98.74	1.41
		155.00			
		154.30			
		153.00			
		153.50			
		150.90			
100	256.00	255.40	254.20	99.30	2.24
		253.60			
		256.50			
		253.40			
		250.40			

		255.90			
200	356.00	355.50	355.20	99.78	0.60
		354.20			
		355.30			
		356.00			
		355.00			
		355.20			
400	556.00	556.10	554.78	99.78	1.38
		555.30			
		554.90			
		556.00			
		552.50			
		553.90			

Table 4.11. Intra - day & Inter-day precision study for determination of chlorogenic acid in plant extracts (n=6) by RP-HPLC method

Amount (ng/spot)	Intra-day precision				Inter-day precision			
	Retention time (minutes)		Response (AUC)		Retention time (minutes)		Response (AUC)	
	Mean	%RSD	Mean	%RSD	Mean	%RSD	Mean	%RSD
200	3.16	0.97	1104233.83	0.03	3.165	1.52	1104268.33	0.04
400	3.15	1.09	2532516.68	0.02	3.16	1.02	2532550.183	0.02

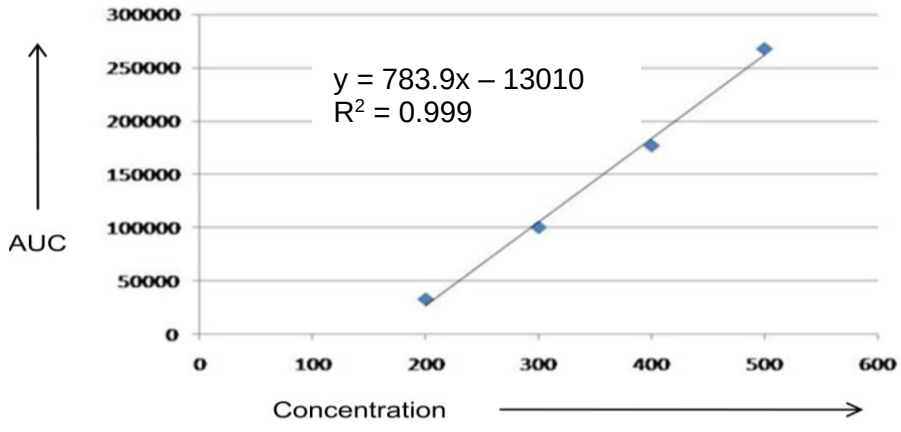
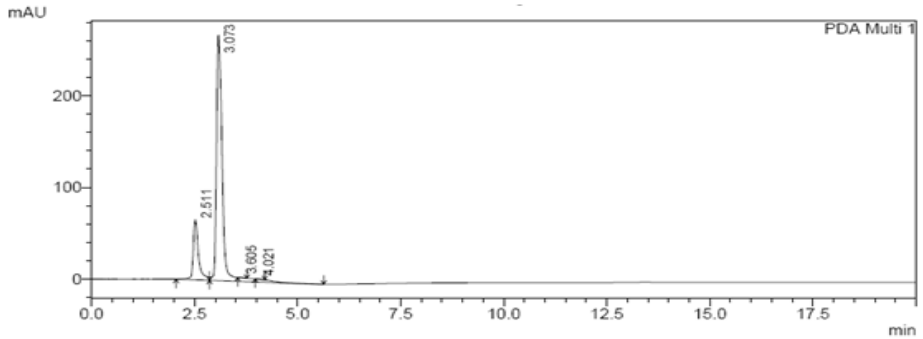
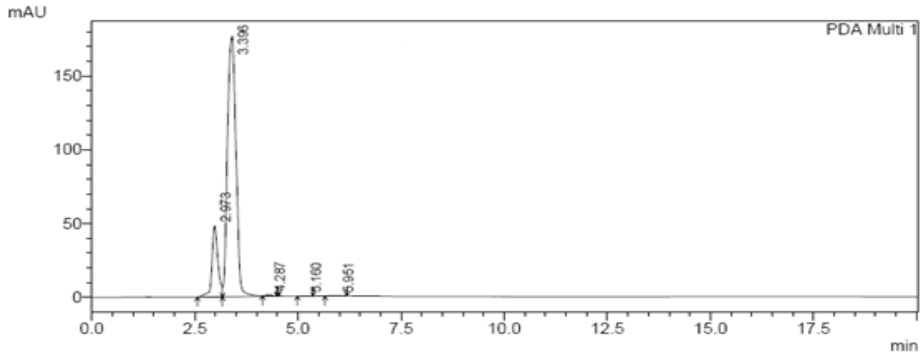


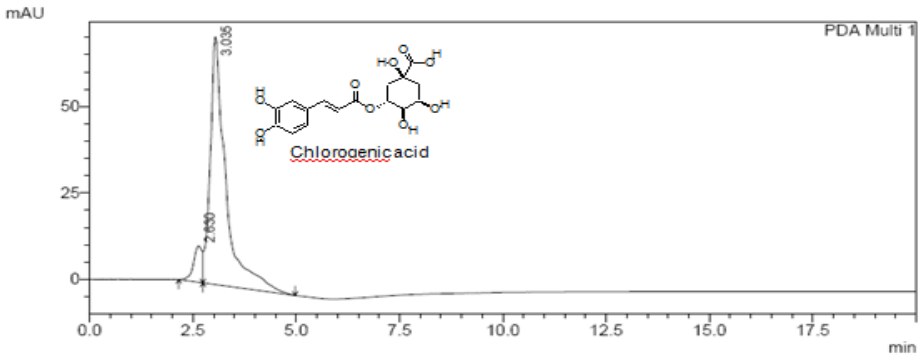
Figure 4.9. Calibration curve of chlorogenic acid



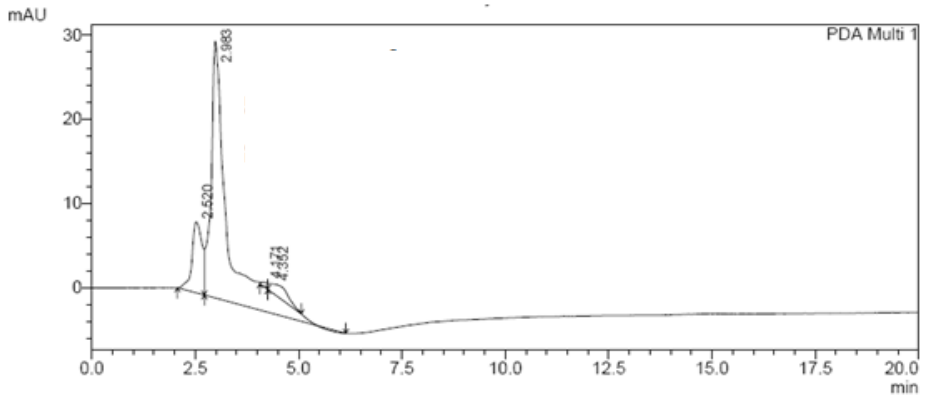
i. RP-HPLC chromatogram of chlorogenic acid (500 µg/mL)



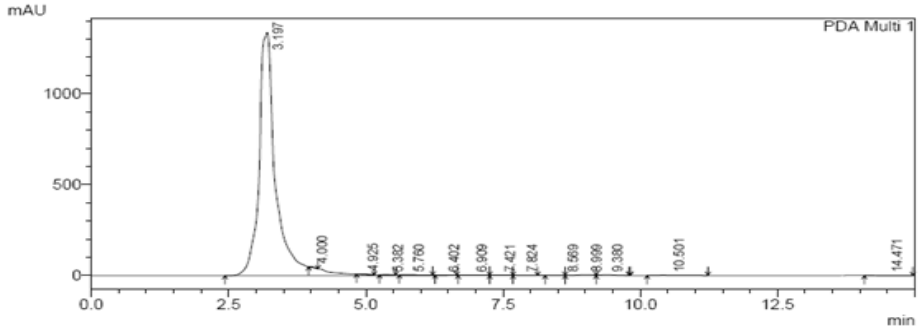
ii. RP-HPLC chromatogram of chlorogenic acid (300 µg/mL)



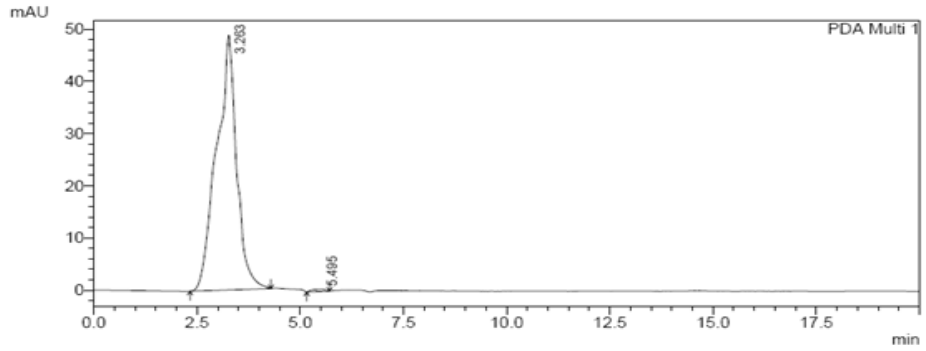
iii. RP-HPLC chromatogram of *Cucurbita maxima*



iv. RP-HPLC chromatogram of *Luffa acutangula*



v. RP-HPLC chromatogram of *Momordica dioica*



vi. RP-HPLC chromatogram of *Trichosanthes dioica*

Figure 4.10. RP-HPLC chromatogram of standard chlorogenic acid and selected fruit extracts. i. RP-HPLC chromatogram of chlorogenic acid (500 µg/mL), ii. RP-HPLC chromatogram of chlorogenic acid (300 µg/mL), iii. RP-HPLC chromatogram of *Cucurbita maxima*, iv. RP-HPLC chromatogram of *Luffa acutangula*, v. RP-HPLC chromatogram of *Momordica dioica*, vi. RP-HPLC chromatogram of *Trichosanthes dioica*

4.7 Conclusion

Phytochemical studies are highly valuable techniques for verifying and distinguishing alike plant species. Furthermore, it is crucial to conduct thorough chemical profiling, scientific validation, and documentation of food plants and herbal medical items in order to ensure quality assessment and widespread acceptability on a global scale. The utilisation of LC-Q-ToF-MS analysis has been established as a preferred approach for investigating the selected fruit extract from the Cucurbitaceae family. The findings of this study indicate that the metabolite composition of the various fruit species varies significantly. In this investigation, metabolites in extracts of *C. maxima, L. acutangula, M. dioica*, and *T. dioica* were potentially discovered by the utilisation of high-resolution mass and fragmentation data. The present UPLC-Q-ToF-MS analysis showcases the capacity of metabolic profiling methods to evaluate the nutraceutical properties of various plant types and foods. This implies a novel approach to the development of functional food or the exploration of new medications. Chlorogenic acid is one of the abundant bioactive phytochemicals present in the major plant family including Cucurbitaceae. This study dealt with the HPTLC method validation to justify the different contents of CA in extract as well as the reproducibility of the developed method. The HPTLC study found the different contents of CA among the different varieties of the selected fruit extracts. The RP-HPLC study explores the variation of CA content in the different Cucurbitaceae fruit extracts. The developed HPTLC and RP-HPLC method has been found to be simple, accurate, precise, robust and reproducible for quantification of CA with a narrow linear range. These methods may be supportive for the development of effective quality control and CA content analysis of extracts and herbal products of selected fruit. The HPTLC analysis revealed varying concentrations of CA amongst the chosen fruit extracts. The present work employs RP-HPLC to investigate the variability of CA content in several fruit extracts belonging to the Cucurbitaceae family. The HPTLC and RP-HPLC method that was developed has been determined to be straightforward, precise, accurate, reliable, and consistent for measuring the concentration of CA within a small linear range. These technologies have the potential to facilitate the advancement of efficient quality control and content analysis of extracts and herbal products derived from specified fruits.

Chapter 5

In-vitro enzyme inhibition assay of *Cucurbita maxima, Luffa acutangula, Momordica dioica* and *Trichosanthes dioica* fruits

5.1 Adaptogens in the context of oxidative stress and antioxidants

Oxidative stress is considered a key element in the advancement of various degenerative ailments, including metabolic disorder, cancer, hyperlipidemia, cardiovascular, neurodegenerative, inflammatory and renal disorder. A free radical possesses an unpaired electron, rendering it inherently unstable and resulted proclivity to achieve stability by engaging in electron pairing with biological macromolecules within the cells of healthy human beings, thereby resulting in the disruption of proteins and DNA. The cellular antioxidant defence systems can be compromised, leading to a more widespread occurrence of cell damage caused by free radicals. Antioxidants are essential compounds that have the capacity to shield the body from destruction caused by oxidative stress created by free radicals. It is essential to include antioxidants in human nutrition in order to safeguard cells from harm caused by free radicals. Theoretical mechanism by which adaptogens regulate the innate antioxidant system and prevent oxidative stress-induced ailments (Pizzino et al., 2017; Leyane et al., 2022). The formation of reactive oxygen species caused by stress leads to harmful interactions with several proteins involved in numerous cellular activities, including proteins that activate two genetic initiatives include apoptosis. Oxidative stress can initiate the activation of JNK kinase culminating to the activation of two signalling pathways. The equilibrium between the pro- and antiaging JNK-mediated processes is altered in favour of HSP70 during adolescence. Adaptogens may increase the activity of HSF1 and HSP70 in and also reduce the activity of JNK in living beings and prevent cell death (Panossian, 2017). The health advantages caused by antioxidant predominantly arise from the presence of polyphenolic compounds, carotenoids, and vitamins C and E which are abundantly present in both edible and non-edible plants, cereals, fruits, vegetables, oils, spices, and other plant components (Rahman et al., 2015). The current study intends to assess the *in-vitro* assays of the selected plant extracts for their ability to scavenge free radicals. The current investigation involved conducting DPPH, Nitric Oxide, Hydroxy and Superoxide radical scavenging experiments.

5.2 Role of α-amylase and α-glucosidase inhibition

Complex carbohydrates in the GI tract undergo a series of breakdown to convert them into monosaccharides. These simple sugars are then absorbed in the small intestine. The process of digestion initiates with the excretion of amylase enzyme synthesised by the pancreatic and salivary glands (Mandel and Breslin, 2012). The stomach's acidic environment suppresses the enzymatic function of salivary amylase, impeding the continued digestion of starch. When partially hydrolyzed starch enters the small intestine, it is further broken down by pancreatic amylases. The α-amylase (AA) enzyme is primarily involved for breaking down the α-1, 4 and α-1, 6-linked glucose subunits include maltose, maltotriose, and limit dextrins found in the dietary starch (Kalinovskii et al., 2023). α-glucosidase (AG) at the brush border of the enterocytes play a crucial role in the last stage of carbohydrate metabolism. The enzymes possess replicated glycoside hydrolases domains and they facilitate the breakdown of α-glucosidic connections in disaccharides and oligosaccharides (Lombard et al., 2014).

Glucocorticoids and catecholamines are the primary endocrine reactions to stress. While these hormones may not have immediate negative effects, but they can disrupt the balance of glucose regulation over time. The disruption of glucose regulation can result in persistent high blood sugar levels, which in turn can cause resistance to insulin and the development of type II diabetes (Kuo et al., 2015). Thus, the inhibition of both AA and AG resulted antihyperglycemic as well as antihyperlipidemic activities. The inhibitors impede the function of the enzymes, consequently decelerating the process of carbohydrate digestion and absorption by competitively obstructing the activity of AA and AG. Therefore, the optimum level of post prandial blood glucose reduces, leading to better regulation of blood sugar levels. When both the enzyme is inhibited, it slows down the metabolism, absorption, accumulation of carbohydrates into the body and resulted reducing the hyperglycemia as well as hyperlipidemia. As a result, a possible therapeutic advantage to metabolic syndrome and offering a valuable treatment option for diabetes mellitus and obesity (Oboh et al., 2012; Telagari and Hullatti, 2015). Figure 5.1 represents role of α-amylase and α-glucosidase in glucose absorption.

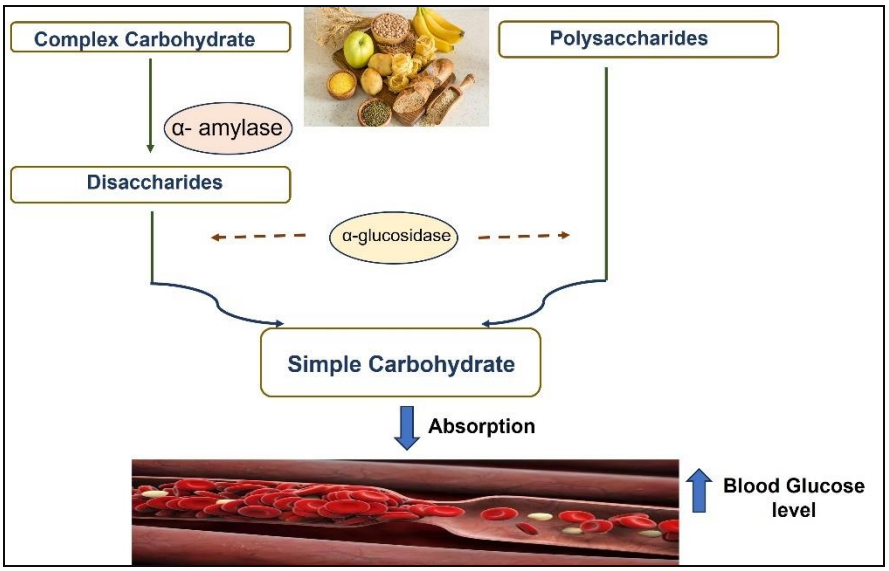


Figure 5.1. Role of α-amylase and α-glucosidase in glucose absorption

5.3 Role of carbonic anhydrase inhibition

Carbonic anhydrase, also known as EC 4.2.1.1 (bCA), contains Zn and is found in all animals and photosynthesizing organisms. The primary function of the enzyme is to convert carbon dioxide and water into bicarbonate ions and protons, or the other way around. Carboxylation and decarboxylation processes play a crucial role in numerous physiological functions. The bCA family has been categorized into three groups, namely α, ß, and γ, based on their structural and genetic diversities. bCA is found in various tissues outside of the kidneys, such as the eye, gastric mucosa, pancreas, central nervous system, and erythrocytes (Sentürk et al., 2011). Carbonic anhydrase plays a crucial role in managing several physiological and pathological functions. It facilitates the movement of CO_2 and bicarbonate ions between metabolising tissues and the lungs, hence regulating the pH of the blood and maintaining homeostasis. Additionally, it has a notable impact on the breakdown of bone, the regulation of blood flow in the kidneys, and the release of electrolytes in different tissues and organs. It also plays a function in several other biochemical activities such as the production of glucose, the synthesis of fats, and the formation of urea. It affects several neuronal cells in the brain, primarily through synapses in the hippocampus network that release GABA. The therapeutic functions of α-bCA isozymes play a significant role in the treatment of obesity, hypertension, cardiac hypertrophy, oedema, cerebral ischemia, glaucoma, and

osteoporosis (Occhipinti and Boron, 2019; Garcia-Llorca et a., 2024). The bCA inhibitor first binds to the active site of the enzyme by displacing the water or hydroxide ion that is bound to the zinc. Then, the inhibitor can also attach to the solvent molecule that is bound to the zinc. Finally, the inhibitor blocks the entrance to the cavity of the enzyme's active site (Karioti et al., 2016). The Physiological function of bCA in erythrocytes represented in Figure 5.2.

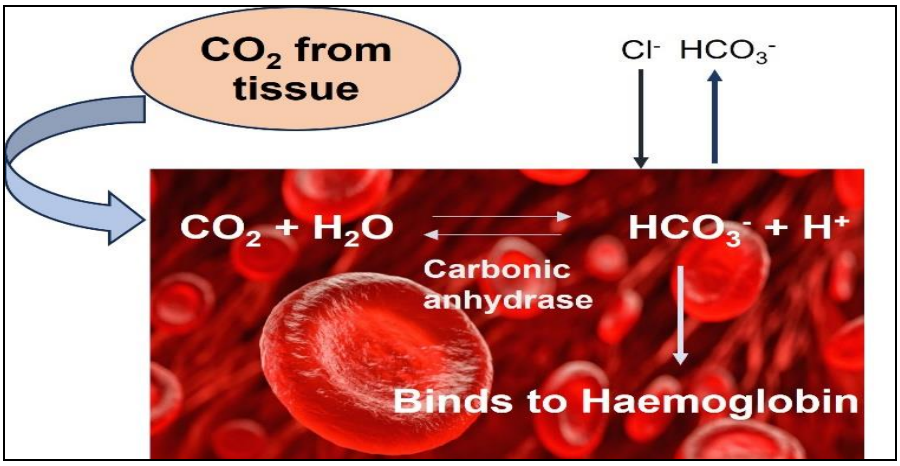


Figure 5.2. Physiological function of Carbonic anhydrase enzyme in Erythrocytes

5.4 Role of pancreatic lipase inhibition

Pancreatic lipases (PL) are carboxylesterases that play a vital role in fat metabolism throughout the human body. The PL is a glycoprotein composed of a single chain of 465 amino acids, with a molecular weight of 51,156 Da. The lipolytic action of PL is mediated by the presence of His 263, Asp-176, and Ser-152 at their catalytic site. The lipase gene family can be classified into three types: pancreatic lipase (PL), lipoprotein lipase, and hepatic lipase, based on their amino acid sequence and gene organisation. Two pancreatic lipase–related proteins, namely PLRP1 and PLRP2, were extracted from the human pancreas (Zhu et al., 2021). PL is accountable for the breakdown of 50–70% of the overall fats present in the diet through hydrolysis. It has a crucial function in the process of converting triglyceride into monoacylglycerol and free fatty acids, which are then transformed into mixed micelles in the presence of cholesterol and bile acids. After being absorbed as monoacylglycerol, it undergoes further

conversion into triglyceride, where it is stored as energy (Yen et al., 2015). The FFA are integrated into micelles composed of bile acids and phospholipids, taken up at the brush boundary of the small intestine, and ultimately transported into the peripheral circulation as chylomicrons. FFA is responsible for regulating the overall lipid content, which includes VLDL, LDL, and HDL. Hyperlipidemia, caused by an excessive amount of triglyceride, is a significant factor in the development of cardiovascular illnesses, atherosclerosis, hypertension, diabetes, and other organ dysfunctions (Wang et al., 2013).

One of the main mechanisms believed to cause obesity and high lipid levels is pancreatic lipase. Therefore, developing drugs that target pancreatic lipase is considered crucial in the fight against obesity and high lipid levels. By suppressing the action of pancreatic lipase, the conversion of triglyceride is prevented, leading to a decrease in the reabsorption of triglyceride in adipose tissue and ultimately resulting in hypolipidemia (Lunagariya et al., 2014). Psychological stress has a significant impact on the human body, particularly on specific organs and physiological parameters. One of these factors affected by stress is the lipid profile. Engaging in physical labour can also impact lipid profiles due to the physical demands it imposes. Researchers demonstrated a correlation between stress and dyslipidemia, which encompasses elevated levels of total cholesterol and LDL, as well as reduced levels of HDL (Assadi, 2017). In figure 5.3, physiological function of PL was depicted.

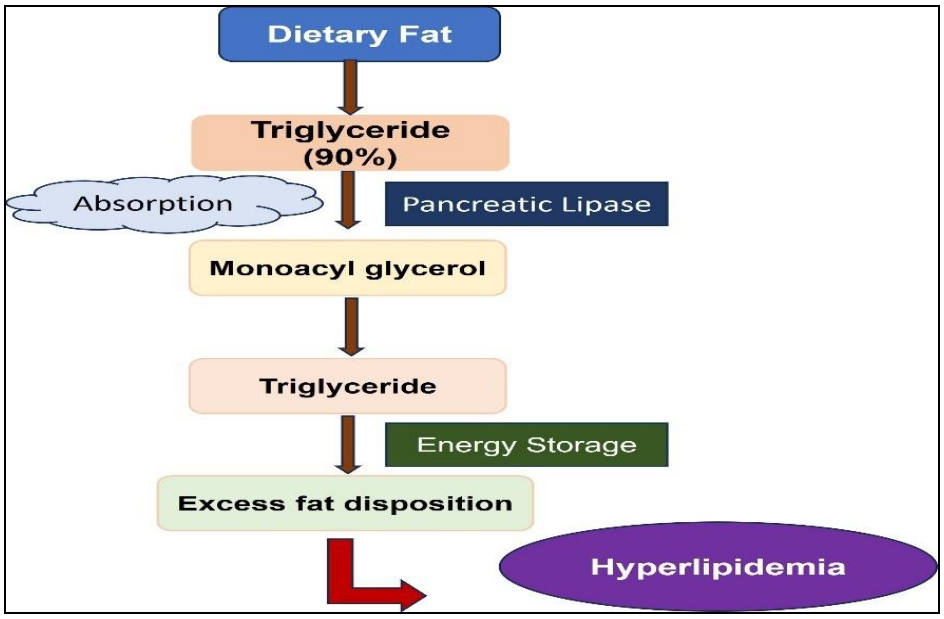


Figure 5.3. Physiological function of pancreatic lipase enzyme

5.5 *In-vitro* Antioxidant activity

In-vitro antioxidant was performed by employing DPPH, Nitric Oxide, Superoxide and Hydroxyl radical scavenging assays. Selected fruit extract of *Cucurbita maxima* Duchesne (Pumpkin), *Luffa acutangula* (L.) Roxb. (Ridge Gourd), *Momordica dioica* Roxb. ex Willd. (Spine Gourd) and *Trichosanthes dioica* Roxb. (Pointed Gourd) was studied above mentioning radical scavenging assay and ascorbic acid was used as reference standard.

5.5.1 Chemicals & Reagents

2,2-diphenyl-1-picrylhydrazyl, Ascorbic acid was obtained from Sigma Aldrich, Bangalore, India. From Sisco Research Laboratory, Mumbai, India sodium nitroprusside, sulfanilic acid, glacial acetic, napthylethylenediamine, nitro blue tetrazolium, phenazine methosulfate, NADH, Tris-HCl buffer, Phosphate buffer, Deoxyribose, H_2O_2, EDTA, thiobarbituric acid, NaOH, trichloroacetic acid, Methanol was procured. All aqueous solutions were prepared using purified water (resistivity of 18.2MΩ cm at 25°C) from a Mili-Q filtration system.

5.5.2 DPPH Radical Scavenging Assay

The DPPH free radical scavenging assay of all selected fruit extract of Cucurbitaceae family was estimated by earlier described methods (Banerjee et al., 2023, Baliyan et al., 2022). The decolorization of a 2,2-diphenyl-1-picrylhydrazyl (DPPH) methanol solution was used to assess the plant extracts' capacity to donate hydrogen atoms. In methanol solution, DPPH generates a violet or purple colour that fades to shades of yellow when antioxidants are present. Methanol was used to prepare each plant extracts solution, as well as a 0.1 mM DPPH (prepared in methanol). Ascorbic acid was used as the standard drug for the determination of the antioxidant activity. The combination was allowed to sit in the dark for 10 minutes at room temperature (rt). Spectramax ID3 (Molecular Devices LLC, United States) was used to measure the absorbance at 517 nm.

5.5.3 Nitric Oxide (NO) Scavenging Assay

Nitric oxide (NO) scavenging assay was measured spectrophotometrically with the selected fruit extract. According to the Griess-Illosvoy reaction, nitrite ions are produced at physiological pH when nitric oxide from an aqueous sodium nitroprusside solution reacts with oxygen. 50 µL of samples at various concentrations was mixed with 50 µL 0.2% sodium nitroprusside (prepared in Phosphate buffer at pH 7.4) with 150 minutes of incubation at 25°C. Following incubation 50 µL of 0.2% sulfanilic acid in 20% glacial acetic acid was added to the reaction admixture and let stand for 5 min at rt for completing diazotization. After that 50 µL 0.1% napthylethylenediamine dihydrochloride was added and incubated at room temperature for another 30 min. The absorbance of the reaction mixture was measured at 540 nm (Al Mahmud et al., 2017).

5.5.4 Superoxide radical scavenging activity

The superoxide radical scavenging assay was based on the reduction of nitro blue tetrazolium (NBT) in the presence of phenazine methosulfate (PMS) and NADH in an aerobic environment. PMS/NADH system, which is not enzymatic, produces superoxide radicals. NBT is then transformed into a purple formazan by the superoxide radicals. 50 µL of 0.2mM NBT (prepared in distilled water), 50 µL of 0.5mM NADH (prepared in 0.1 M Tris-HCl at pH 8) and 50 µL of 25 µM PMS (prepared in distilled water) were

mixed. In the reaction mixture samples were added and further incubated at room temperature for 10 minutes. Ascorbic acid was used as reference standard. At 570 nm the absorbance of reaction mixture was determined (Sangameswaran et al., 2009).

5.5.5 Hydroxyl radical scavenging activity

This assay was performed as per the methodology described in Jing et al. 2015, with slight modifications. The antioxidant activity of all the extracts and ascorbic acid (reference standard) prepared in distilled water was determined by hydroxyl radical scavenging activity. 10 µL of 2.8 mM deoxyribose (prepared in phosphate buffer at pH 7.4) mixed with each10 µL of 1mM $FeCl_3$, 1 mM of H_2O_2, 1mM of EDTA and 100 µL sample at various concentrations. Further 10 µL of 1mg/mL ascorbic acid added to the admixture to initiate the reaction. After incubation of 1 hour at 37°C, 500 µL of 10 % trichloroacetic acid was added to end the reaction. A pink chromogen was appeared when 500 µL of 0.5 % thiobarbituric acid (prepared in 50mM NaOH) added to the mixture and there after it heated in water bath at 42°C for 30 mins. The absorbance was measured at 532 nm by employing Spectramax ID3 (Molecular Devices LLC, United States).

Using GraphPad Prism version 8.0.2 software, nonlinear regression was performed to obtain the IC_{50} value. Plotting the % relative activity versus concentration yielded the concentration-response curve. A one-way ANOVA was used to assess the differences between and within the groups. A multi-comparison Dunnett test was then performed in comparison with the positive control. Antioxidant activity was expressed as IC_{50} (mg/mL) (Chanda et al., 2019). % scavenging assay of was calculated by:

% scavenging assay: $[(A_1-A_0)/A_1] \times 100$

Where, A_1 = absorbance of control

A_0 = absorbance of sample at different concentrations

5.6 *In-vitro* enzyme inhibition assay

5.6.1 *In-vitro* α-amylase inhibitory activity of the selected fruit extracts

5.6.1.1 Chemicals & Reagents

AA (from Aspergillus oryzae, powder- 30U/mg, CAS: 9001-19-8), 3,5-Dinitro salicylic acid (DNSA) (purity ≥99%, CAS: 6381-59-5), starch soluble extra pure (purity ≥99%, CAS: 9005-84-9), potassium sodium tartrate tetrahydrate (purity ≥99%, CAS: 6381-59-5), chlorogenic acid (≥99%), were obtained from Sigma-Aldrich, Bangalore, India (St. Louis, MO, USA). Chlorogenic acid (> 98% purity, catalogue no. 40881), Acarbose extra pure (purity ≥ 95%, Catalogue no. 65457) was purchased from Sisco Research Laboratories Pvt. Ltd., Mumbai, India. Sodium dihydrogen phosphate (NaH_2PO_4) and disodium hydrogen phosphate (Na_2HPO_4) were obtained from Merck, Mumbai, India. The ultra-pure water from a Milli-Q water filter (Bedford, USA) was used to prepare other aqueous solutions.

5.6.1.2 α-amylase inhibition assay

The *in-vitro* AA inhibition assay was performed using a 96-well plate method reported earlier by Nasab et al., 2020 and Muritala et al., 2018 with slight modifications. AA enzyme, DNSA, and sample solutions were prepared in 20 mM sodium phosphate buffer at pH 6.9. 30 µL of AA (1 U/mL) and 30 µL of different concentration of sample solutions (0.5-7 mg/mL) was mixed. 40 µL of starch substrate (1 mg/mL) was added into the reaction mixture and incubated at 37°C for 10 mins. 20 µL of DNSA was added to the mixture and warmed up using a water bath for 10 mins to conclude the reaction. Lastly, 80 µL of buffer solution was added to dilute the mixture and absorbance was measured at 540 nm using a spectrophotometer (SpectraMAx iD3, LLC, USA). The assay was performed in triplicate and the results were expressed as IC_{50} value.

5.6.2 *In-vitro* α-glucosidase inhibitory activity of the selected fruit extracts

5.6.2.1 Chemicals & Reagents

AG (from Saccharomyces cerevisiae, Type I, ≥10 units/mg protein, catalogue no. G5003), 4-Nitrophenyl-α-D-glucopyranoside (p- NPG, purity ≥99%, catalogue no. N1377), were procured from Sigma Aldrich, Bangalore, India. Chlorogenic acid (> 98%

purity, catalogue no. 40881), Acarbose extra pure (purity ≥ 95%, Catalogue no. 65457) was purchased from Sisco Research Laboratories Pvt. Ltd., Mumbai, India. Na_2CO_3, NaH_2PO_4 and Na_2HPO_4 were acquired from Merck, Mumbai, India. All aqueous solutions were prepared using purified water (resistivity of 18.2MΩ cm at 25°C) from a Mili-Q filtration system.

5.6.2.2 α-glucosidase inhibition assay

The *in-vitro* AG inhibition assay was conducted in a 96-well plate following a slightly modified methodology described in Chanda et al., 2020 and Beidokhti et al., 2017. AG enzyme, *p*-NPG and sample solutions were prepared in 50 mM phosphate buffer at pH 6.8. The reaction mixture was prepared by using 150 µL of 0.1 M sodium phosphate buffer, 20 µL of AG enzyme (0.5 U/mL) and 10 µL of different concentration of sample solution (0.5-7 mg/mL) including positive control. Then the microplate was preincubated at 37 °C for 8-10 mins consequently 20 µL of p-NPG was added and the reaction admixture. The OD was measured at 405 nm using spectrophotometer, acarbose was employed as positive control. The assay was performed in triplicate (n = 3) and the results were presented as IC_{50} value.

5.6.3 *In-vitro* carbonic anhydrase inhibitory activity of the selected fruit extracts

5.6.3.1 Chemicals & Reagents

Carbonic anhydrase isozyme II from bovine erythrocytes (bCA) (EC-232-576-6), 4-nitrophenyl acetate (4-NPA) were procured from Sigma-Aldrich, Bangalore, India. We received a gift sample of acetazolamide IP (Batch No. AZM-V-P/131107) from Mangalam Drugs and Organics Ltd. in Mumbai, India. Purified water from a Mili-Q filtering system (resistivity of 18.2MΩ cm at 25°C) was used to prepare all aqueous solutions. Tris buffer GR, sulphuric acid (purity 98%), acetone (HPLC grade), dimethyl sulfoxide, petroleum ether, dichloromethane, ethyl acetate, ethanol (synthesis grade) was purchased from Merck, Mumbai, India. From Sisco Research Laboratory, Mumbai, India Acetonitrile, anhydrous acetonitrile (99.8%) and trifluoroacetic acid (HPLC grade ≥ 99.0%) were purchased.

5.6.3.2 Carbonic anhydrase inhibition assay

The enzyme inhibitory activity of the chosen plant extracts was assayed based on method described by Chanda et al., 2021 utilising 4-NPA as substrate. Based on the shift in absorbance carried on by the breakdown product of 4-NPA, 4-nitrophenol was released, causing inhibition. 30 µL of bCA II (115 U/mL) prepared in Tris buffer (50 mM) mixed with 20 µl of test sample at different concentrations and incubated for 15 mins at rt. By adding 10 mM 4-NPA prepared in anhydrous alcohol in the incubated admixture and the absorbance was measured at 400 nm by utilising Spectramax ID3. Acetazolamide used as standard inhibitor and the study procedure carried through in Triplicate (n = 3) and the results were presented as IC_{50} value.

5.6.4 *In-vitro* pancreatic lipase inhibitory activity of the selected fruit extracts

5.6.4.1 Chemicals & Reagents

Lipase from Porcine pancreas Type II (activity 59 U/mg protein) (PL), p-nitrophenyl caprylate (p-NPC), Orlistat (PHR1445-1G) were obtained from Sigma Aldrich, Bangalore, India. Hexane, ethyl acetate, ethanol was procured from Sisco Research Laboratory, Mumbai, India. All aqueous solutions were prepared using purified water from Mili-Q filtration system (resistivity of 18.2MΩ cm at 25°C).

5.6.4.2 Pancreatic lipase inhibition assay

The *in-vitro* pancreatic lipase inhibition assay was performed with the hydroalcohol extract of selected Cucurbitaceae fruit extract, based on the methodology published by Chanda and his co-researchers with slight alteration (Chanda et al., 2019). The enzyme (5.67 U/mL) was prepared in 50 mM phosphate buffer at pH 7.0 and p-NPC was used as a substrate in a concentration of 200 µM (prepared in phosphate buffer). p-NPC was added in the enzyme- inhibitor mixture to initiate the inhibition assay. Orlistat was used as a positive control. The rate of change of absorbance of the reaction mixture in each well was determined at 405 nm using a UV–visible spectrophotometer. The assay procedure was carried out in triplicate (n = 3). The results stated as IC_{50} values of the extracts as well as the standard inhibitor.

5.7 Statistical analysis

All the experiments were performed in triplicate and results are shown as mean ± standard deviation. Graph pad prism 8.0.2 software (Boston, MA, USA) was used for statistical calculation. The statistical significance of the difference and the significance level at which it was determined (P-value < 0.05) were assessed using Tukey's multiple comparison test. The Pearson correlation coefficient was computed to assess the association between the enzymes and their phenolic, flavonoid, and antioxidant activity.

5.8 Results and Discussions

5.8.1 *In-vitro* antioxidant activity

To assess the free radical scavenging assay four selected plants (C*. maxima, L. acutangula, M. dioica, T. dioica*) from Cucurbitaceae family was studied by using DPPH, NO, superoxide along with hydroxyl radical scavenging assay. Ascorbic acid was used as the standard compound in all *in-vitro* antioxidant studies. As compared to the standard *L. acutangula* and *T. dioica* exerted modest antioxidant activity whereas, *M. dioica* and *C. maxima* showed prominent *in-vitro* antioxidant activity in all performed free radical scavenging assays. The IC_{50} value of Ascorbic acid, *C. maxima, L. acutangula, M. dioica* and *T. dioica* expressed as µg/mL in Table 5.1. In DPPH radical scavenging assay, the IC_{50} of C*. maxima, L. acutangula, M. dioica, T. dioica* and ascorbic acid was 142.61 ± 0.314, 190.76 ± 0.262, 130.16 ± 0.385, 173.46 ± 0.427 and 113.69 ± 0.094 µg/mL accordingly. In the NO antioxidant assay, *L. acutangula* (183.39 ± 0.334 µg/mL) and *T. dioica* (178.49± 0.363 µg/mL) possesses minimised activity and *M. dioica* (118.54 ± 0.313 µg/mL) and *C. maxima* (137.35 ± 0.275 µg/mL) demonstrated good activity in compare to ascorbic acid (105.12 ± 0.013 µg/mL).

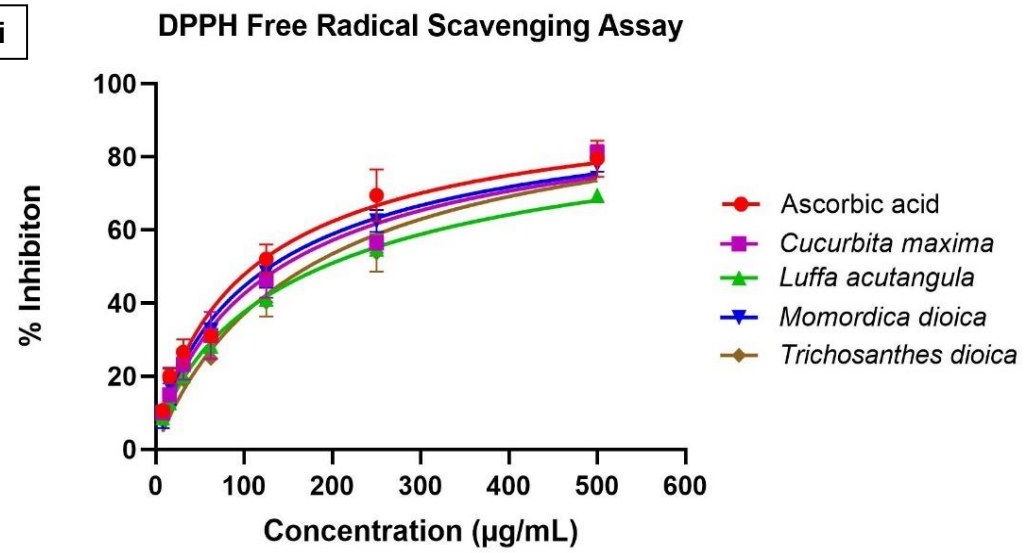
M. dioica exhibits notable antioxidant activity in the superoxide and hydroxyl scavenging assay (superoxide: 107.24 ± 0.222 µg/ mL; hydroxyl: 103.21 ± 0.184 µg/mL) in compared to the standard compound. Whereas, *C. maxima* (superoxide: 116.8 ± 0.216 µg/mL; hydroxyl: 119.23 ± 0.171 µg/mL) showed moderate and *L. acutangula* (superoxide: 131.43 ± 0.269 µg/mL; hydroxyl: 144.46 ± 0.402 µg/mL) and *T. dioica* (superoxide: 141.57 ± 0.422 µg/mL; hydroxyl: 125.89 ± 0.392 µg/mL) exhibited inadequate antioxidant activity. The IC_{50} value of ascorbic acid for superoxide assay as

100.09 ± 0.143 µg/mL; whereas for hydroxyl assay: 97.49 ± 0.108 µg/mL). The figure 5.4 represents the Concentration response curve of DPPH (i), NO (ii), Superoxide (iii), and Hydroxyl (iv) free radical scavenging experiment of *C. maxima, L. acutangula*, *M. dioica*, and *T. dioica* fruit extract, as well as the Standard Ascorbic acid.

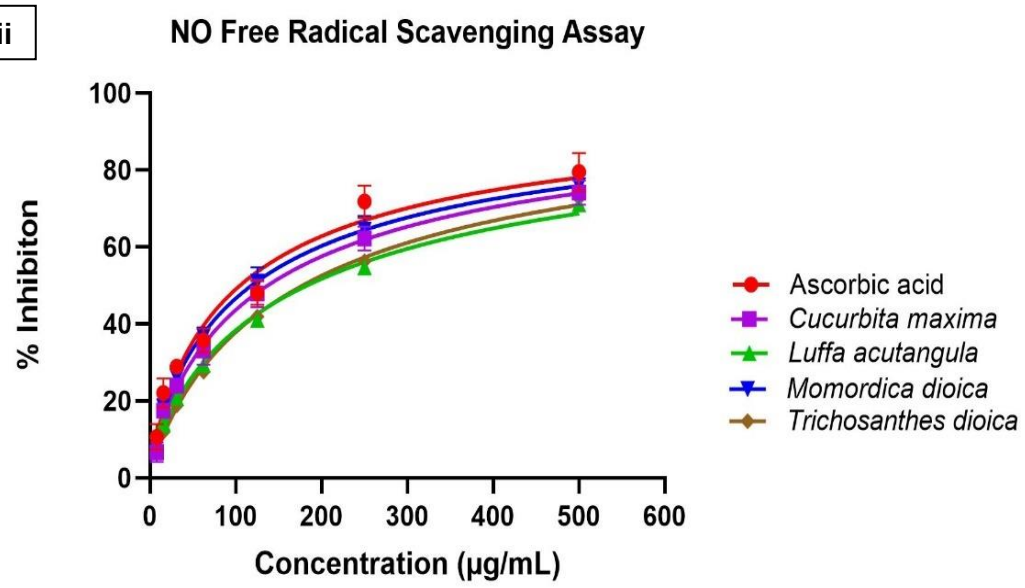
Table 5.1. IC_{50} values (µg/mL) of Ascorbic acid, *C. maxima, L. acutangula, M. dioica* and *T. dioica* fruit extract by DPPH, NO, Superoxide and Hydroxyl free radical scavenging assay

***In-vitro* antioxidant assay**	**IC_{50} value of Extract/ Sample**				
	Ascorbic acid (µg/ mL)	*C. maxima* (µg/ mL)	*L. acutangula* (µg/ mL)	*M. dioica* (µg/ mL)	*T. dioica* (µg/ mL)
DPPH	113.69 ± 0.094	142.61 ± 0.314	190.76 ± 0.262	130.16 ± 0.385	173.46 ± 0.427
NO	105.12 ± 0.013	137.35 ± 0.275	183.39 ± 0.334	118.54 ± 0.313	178.49± 0.363
Superoxide	100.09 ± 0.143	116.8 ± 0.216	131.43 ± 0.269	107.24 ± 0.222	141.57 ± 0.422
Hydroxyl	97.49 ± 0.108	119.23 ± 0.171	144.46 ± 0.402	103.21 ± 0.184	125.89 ± 0.392

i


DPPH Free Radical Scavenging Assay
% Inhibiton
Concentration (µg/mL)
Ascorbic acid
Cucurbita maxima
Luffa acutangula
Momordica dioica
Trichosanthes dioica

ii


NO Free Radical Scavenging Assay
% Inhibiton
Concentration (µg/mL)
Ascorbic acid
Cucurbita maxima
Luffa acutangula
Momordica dioica
Trichosanthes dioica

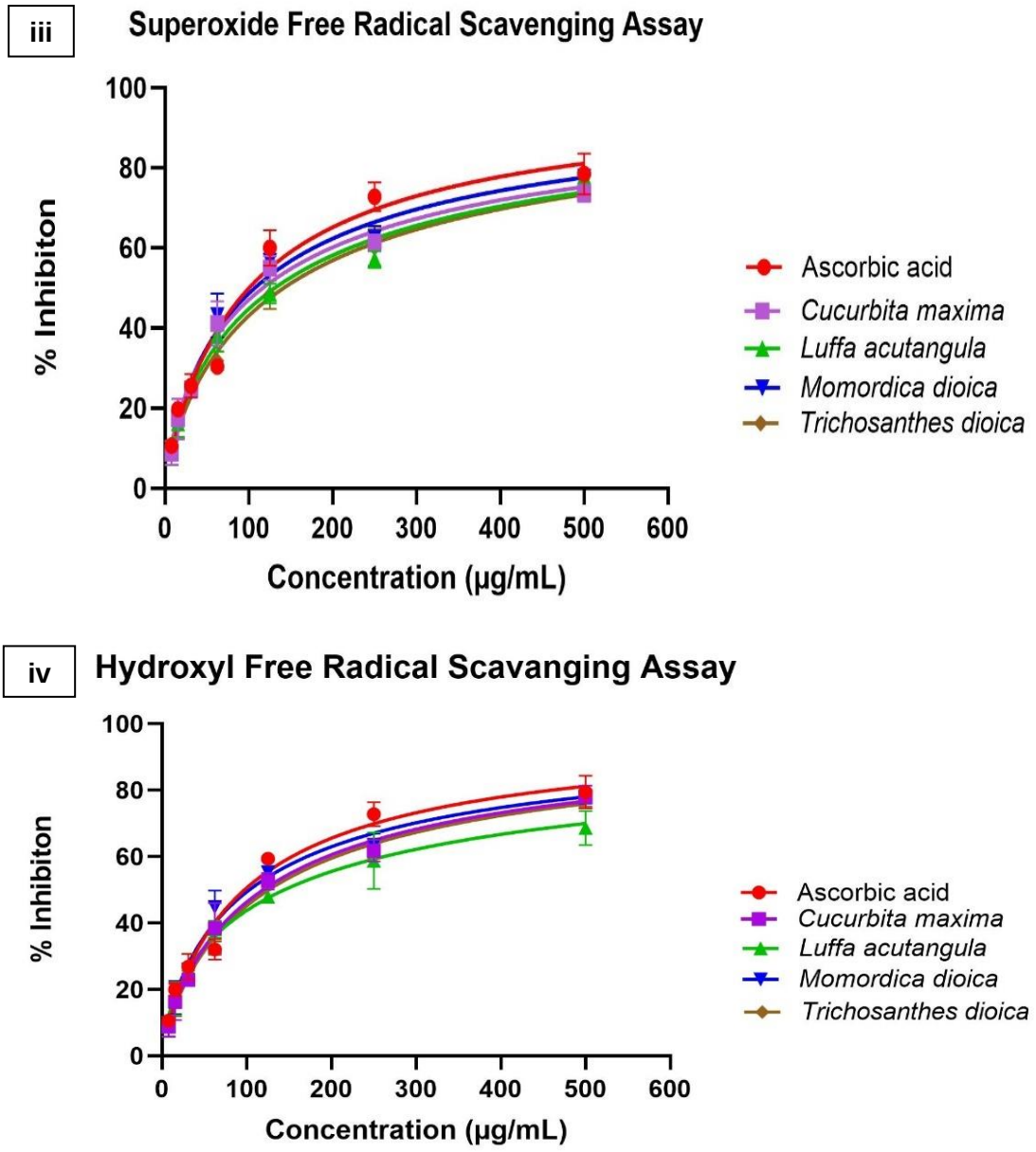


Figure 5.4. Concentration response curve of DPPH (i), NO (ii), Superoxide (iii), and Hydroxyl (iv) free radical scavenging assay of Ascorbic acid, *C. maxima*, *L. acutangula*, *M. dioica*, and *T. dioica* fruit extract

5.8.1 *In-vitro* enzyme inhibition activity

The therapeutic activity of *C. maxima, L. acutangula, M. dioica*, and *T. dioica* against AA, AG, bCA, and PL enzymes was examined *in-vitro* manner to establish their effectiveness in treating numerous metabolic disorders. In the AA enzyme inhibition assay, the plant extracts of *C. maxima* and *M. dioica* demonstrated significant enzyme inhibition compared to the plant extracts of *L. acutangula* and *T. dioica*. The positive control Acarbose exhibited an IC_{50} value of 97.27 ± 0.141 µg/mL. The fruit extract of *M. dioica* had strong inhibitory action in the AG inhibition assay, with an IC_{50} value of 89.32 ± 0.214 µg/mL. This activity was compared to the positive control Acarbose, which had an IC_{50} value of 66.62 ± 0.138 µg/mL. The IC_{50} values (µg/mL) of *C. maxima, L. acutangula, M. dioica, T. dioica* fruit extract, and Acarbose were provided in Table 5.2, as determined by an *in-vitro* enzyme inhibition investigation using AA and AG. The figure 5.5 illustrates the dose response curve for the inhibition of the AA and AG enzymes. Figure 5.6 displays a bar diagram representing the IC_{50} values of *C. maxima, L. acutangula, M. dioica, T. dioica*, and Acarbose in the context of AA and AG enzyme inhibition assays.

Table 5.2. IC_{50} values (µg/mL) of *C. maxima, L. acutangula, M. dioica*, *T. dioica* fruit extract and Acarbose by *In-vitro* AA and AG enzyme inhibition assay

Sample/Extract	*In-vitro* enzyme inhibition study (µg/ mL)	
	α-amylase	**α-glucosidase**
C. maxima	122.44 ± 0.184	96.17 ± 0.159
L. acutangula	146.53 ± 0.278	105.65 ± 0.251
M. dioica	133.53 ± 0.112	89.32 ± 0.214
T. dioica	152.51 ± 0.298	111.26 ± 0.272
Acarbose	97.27 ± 0.141	66.62 ± 0.138

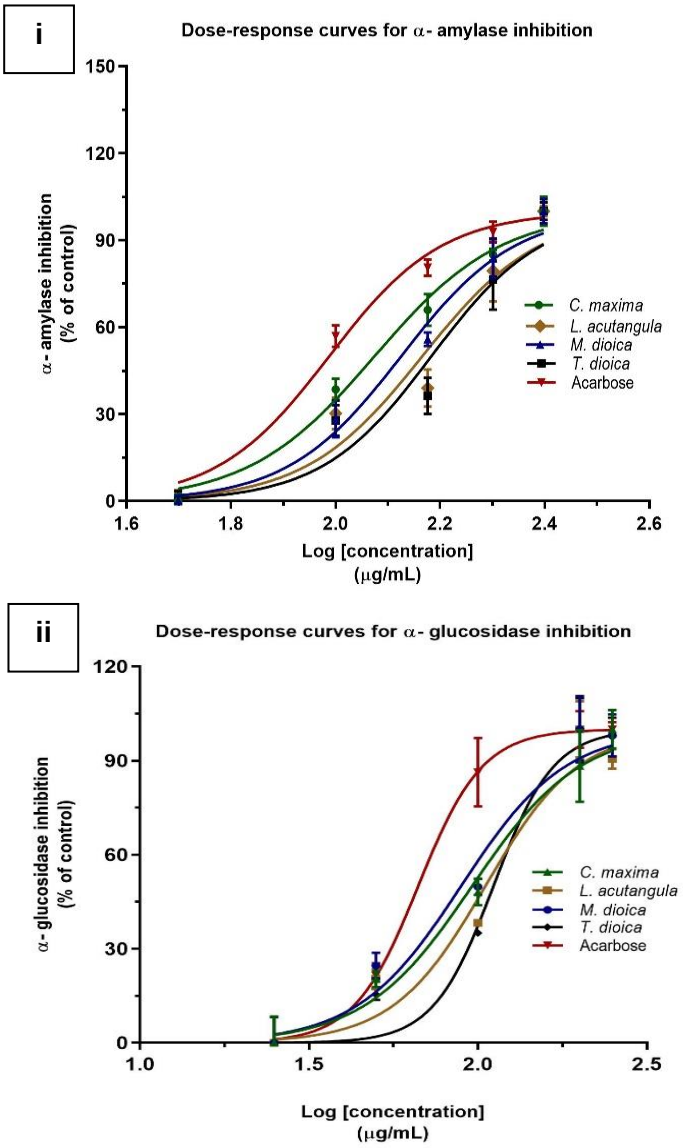


Figure 5.5. Dose response curve of α-amylase (i), α-glucosidase (ii) enzyme inhibitory effects of *C. maxima, L. acutangula, M. dioica*, *T. dioica* fruit extract and Standard Acarbose

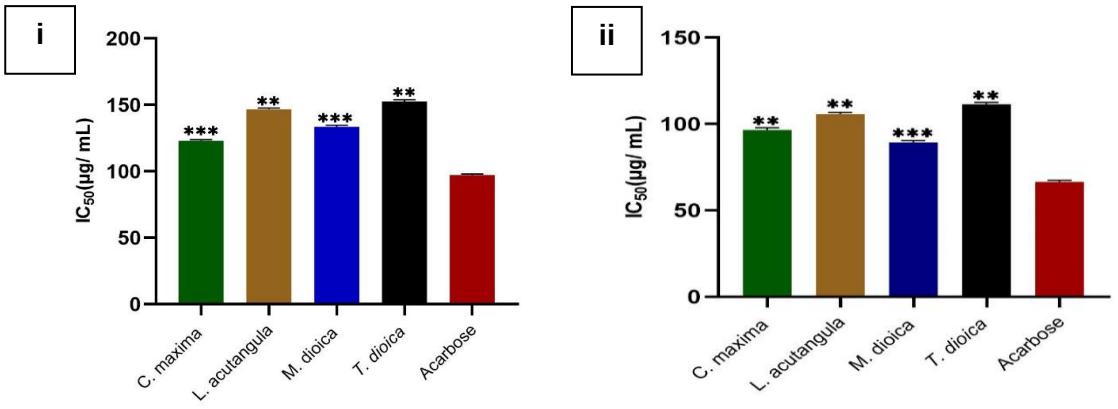


Figure 5.6. IC_{50} value of *C. maxima, L. acutangula, M. dioica*, *T. dioica* fruit extract and Standard Acarbose against α-amylase (i), α-glucosidase (ii) enzyme inhibitory study [results are represented as mean ± SD (n = 3); One-way ANOVA with Dunnett's multiple comparisons, at *p value* <0.0001] Significant values are represented with ***

The fruit extract of *M. dioica* shown strong inhibitory activity in the *in-vitro* investigation of bCA and PL enzymes, with IC_{50} values of 179.41 ± 0.171 µg/mL and 120.28 ± 0.228 µg/mL, respectively. Acetazolamide, which is a positive control for bCA enzyme, had an IC_{50} value of 166.61 ± 0.163 µg/mL. Orlistat, a positive control for PL enzyme, had an IC_{50} value of 90.34 ± 0.857 µg/mL. The fruit extract of *C. maxima* showed considerable inhibitory action against both enzymes, with IC_{50} values of 186.32 ± 0.197 µg/mL for bCA and 133.79 ± 0.175 µg/mL for PL. The bCA and PL enzymes are not well suppressed by the fruit extracts of *T. dioica* and L. acutangula. Table 5.3 and 5.4 display the IC_{50} values (µg/mL) of *C. maxima, L. acutangula, M. dioica, T. dioica* fruit extract, as well as Acetazolamide and Orlistat, determined using *in-vitro* bCA and PL enzyme inhibition experiment. Figure 5.7 depicts the dose-response curve of *in-vitro* bCA and PL enzymes. Figure 5.8 displays a bar diagram illustrating the IC_{50} values of *C. maxima, L. acutangula, M. dioica, T. dioica* fruit extract, as well as the standard drugs Acetazolamide and Orlistat, in their inhibitory effects on bCA and PL enzymes.

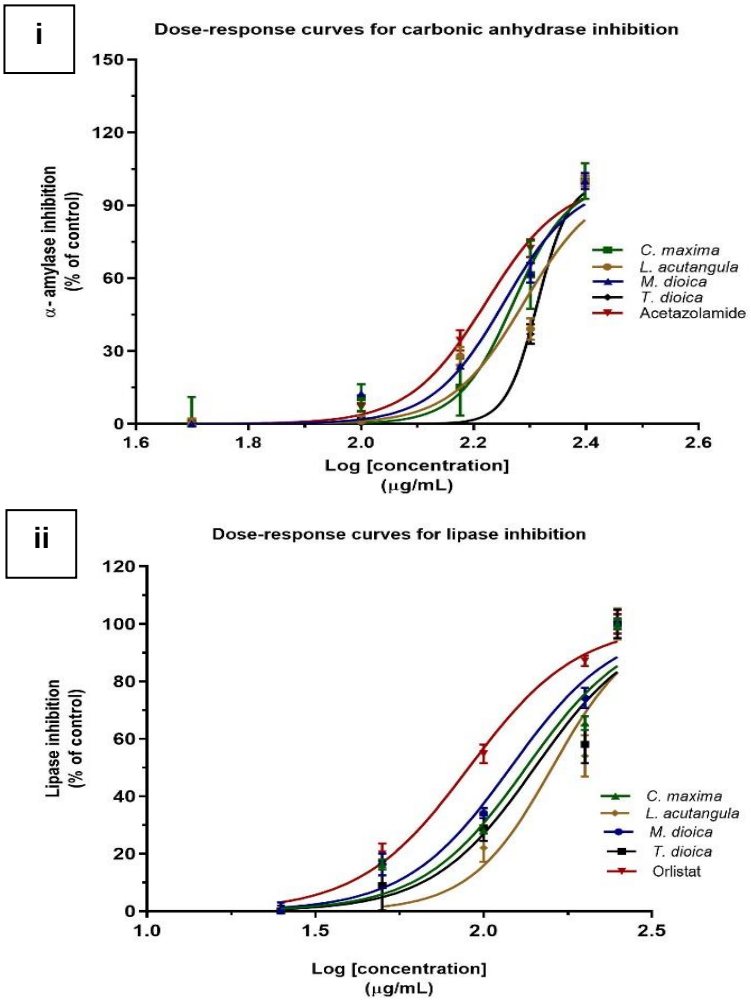


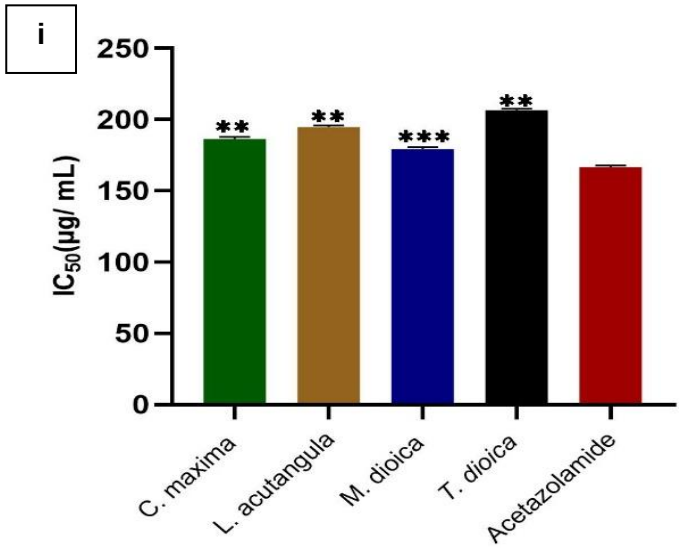
Figure 5.7. Dose response curve of Carbonic anhydrase (i), Pancreatic lipase (ii) enzyme inhibitory effects of *C. maxima, L. acutangula, M. dioica*, *T. dioica* fruit extract and Standard Acetazolamide and Orlistat respectively

Table 5.3. IC_{50} values (µg/mL) of *C. maxima, L. acutangula, M. dioica*, *T. dioica* fruit extract and Acetazolamide (Positive Control) by *In-vitro* bCA enzyme inhibition assay

Sample/Extract	IC_{50} (µg/mL)
C. maxima	186.32 ± 0.197
L. acutangula	194.81 ± 0.151
M. dioica	179.41 ± 0.171
T. dioica	206.14 ± 0.197
Acetazolamide	166.61 ± 0.163

Table 5.4. IC_{50} values (µg/mL) of *C. maxima, L. acutangula, M. dioica*, *T. dioica* fruit extract and Orlistat (Positive Control) by *In-vitro* PL enzyme inhibition assay

Sample/Extract	IC_{50} (µg/mL)
C. maxima	133.79 ± 0.175
L. acutangula	159.92 ± 0.253
M. dioica	120.28 ± 0.228
T. dioica	142.46 ± 0.360
Orlistat	90.34 ± 0.085



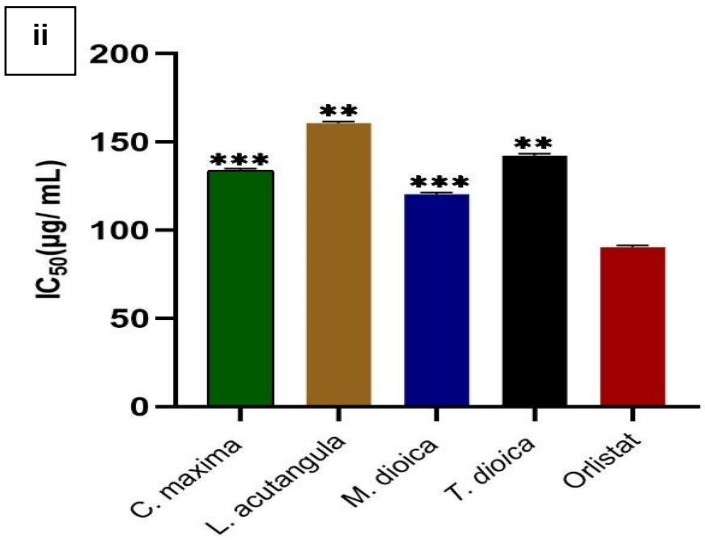


Figure 5.8. IC_{50} value of *C. maxima, L. acutangula, M. dioica*, *T. dioica* fruit extract and Standard Acetazolamide and Orlistat against Carbonic anhydrase (i), Pancreatic lipase (ii) enzyme inhibitory study [results are represented as mean ± SD (n = 3); One-way ANOVA with Dunnett's multiple comparisons, at *p value* <0.0001] Significant values are represented with ***

Conclusion

Stress has a negative impact on both metabolic health and oxidative stress, which in turn leads to the development or aggravation of metabolic disorders including diabetes, hyperlipidemia, hypertension, cardiovascular disorder etc. The hydroalcoholic extract of all the selected plants from the Cucurbitaceae family exhibits antioxidant properties and also inhibits the activity of AA, AG, bCA, and PL enzymes in *in-vitro* manner. Compared to all other plant extracts, the antioxidant activity of the fruit extract from M. dioica is more potent. In AG, bCA and PL enzyme inhibitory potential, compared with the positive control Acarbose, Acetazolamide and Orlistat, the inhibitory potential of *M. dioica* is more substantial. *C. maxima* extract exhibited considerable inhibitory action against AA enzymes and modest inhibitory activity against AG, bCA, and PL enzymes. Therefore, it may be inferred that the fruit extract of M. dioica possesses strong antioxidant properties and has the potential to be utilised in the treatment of metabolic disorders caused by stress. This is achieved by prohibiting the enzymes AA, AG, bCA, and PL. Based on the acquired result, *M. dioica* has been chosen for further evaluation of its *in-vivo* adaptogenic activity.

Chapter 6

Evaluation of *in-vivo* adaptogenic potential of selected fruit extract

6.1 Role of Adaptogens to Combat Stress and Promote Well-being

Stress is a general reflex of the body that disrupts the normal balance of the organism's physiological functioning, leading to numerous problems in the nervous system, hormonal system, and internal organs. Survival in the human race depends on one's capacity to build and sustain resistance against a wide range of stressors. In contemporary culture, individuals are consistently subjected to stress-inducing factors such as atmospheric pollution, food contamination, an excessively ambitious and competitive way of life, antagonism, and synthetic medications. Enhanced stress levels can lead to the onset of illness in an organism. Stress is a fundamental contributor to various ailments, including diabetes, hyperlipidemia, atherosclerosis, coronary heart disease, ageing, and liver disease. The pursuit of regulating coping mechanisms has given rise to the emergence of the field of adaption science (Yaribeygi et al., 2017). The HPA axis and the sympatho-adrenal system (SAS) are the two major systems responsible for sustaining and managing homeostasis. The adrenal cortex is prompted to release corticosterone when the HPA axis is triggered due to stress. Conversely, the autonomic response of the SAS is accountable for triggering fight-or-flight reactions (Herman et al., 2016). The pathological circumstances of stress arise due to changes in the aforementioned homeostatic systems. The reciprocal modulation of corticotropin-releasing hormone and norepinephrine systems is a firmly documented relationship between the CNS and the hypothalamic-pituitary-adrenal axis. This interaction primarily governs the organism's biological reactions to external stresses. If there is any disruption in the functioning of these systems, it would result in the failure of the stress responses and make the organism more vulnerable to stress diseases. In response to such a circumstance, the body triggers a range of autonomic, endocrine, and visceral responses, including the production of hormones such as cortisol and adrenaline (Chaves et al., 2021). Both peripheral and central processes appear to govern these alterations. Stress causes the breakdown of the protective lining of the body's mucous membranes, as well as an elevation in heart rate, blood pressure, and metabolic rate. These physiological responses are aimed at enhancing the body's overall performance and capacity to face the task at hand (Godoy et al., 2018).

Adaptogens are plants or substances that seem to enhance an organism's ability to withstand and cope with stressful inputs that could disrupt its internal equilibrium. A number of researchers in this area have proposed specific criteria that must be met in order to be classified as adaptogens. These criteria include the ability to elicit a non-specific response, such as increasing resistance to various physical, chemical, or biological stressors. Additionally, an adaptogen must restore equilibrium to any malfunctioning bodily systems and must be harmless, meaning it should not have a greater impact on normal bodily functions than necessary. It has been asserted that they can halt the process of ageing and the decline in physical and mental function caused by age related issues (Todorova et al., 2021).

Ashwagandha, a well-known adaptogenic plant, has garnered considerable interest due to its alleged therapeutic advantages in traditional medical practices. As an adaptogen, it is purported to enhance the body's ability to manage stress and support general wellness. Nevertheless, it is crucial to acknowledge that ashwagandha may develop adverse effects including stomach discomfort, nausea, diarrhoea, and allergic responses. In addition, ashwagandha has the potential to interact with drugs such as sedatives, thyroid hormone therapies, and immunosuppressants, which could result in adverse responses (Singh et al., 2011). Owing to the negative impact of Ashwagandha, other phytocomponents must be investigated in order to assess their adaptogenic potential. The purpose of this study is to assess the adaptogenic activity of selected plant extract of Cucurbitaceae family.

6.2 Evaluation of *In-vivo* adaptogenic activity

6.2.1 Chemicals, reagents and instruments

The Diazepam was acquired from USV Pvt. Ltd., an Indian company. All other reagents utilised were of the utmost quality and met the highest standards accessible in the commercial market. The Milli-Q water filter from Bedford, USA, was utilised to make additional aqueous solutions using ultra-pure water. The SpectraMax iD3, LLC, USA, device was utilised to quantify the absorbance of the biological compound present in the blood serum. Cellenium 5D Retic, India haematology analyser was employed for estimation of haematological levels.

6.2.2 Experimental animals

Adult male Albino mice (Swiss strain) weighing 20-25 gm were procured from from a registered breeder, West Bengal Livestock Development Corp. Ltd., SCLAB, Kalyani, Nadia, India (FSSAI REG. NO. 10012031000104). Before the study began, the animals were housed in an animal housing with a temperature of 25±2°C and a humidity level of 40–50%. The animals had unfettered accessibility to drinking water and dried pellet meal (Hindustan Unilever, India). Following acquiring, the animals underwent a seven-day acclimatisation phase in a conventional husbandry setting with a 12-hour light/dark cycle with adequate ventilation and hygienic conditions. A group of animals was kept in a polypropylene cage with a paddy husk bed coated in stainless steel wire mesh, along with a feeding and watering station. This study was conducted in conformance with the ethical standards set by the Institutional Animal Ethical Committee (IAEC Protocol approval No. **JU/IAEC-22-16**), which was authorised by the Central Committee for the Control and Supervision of Animal Experiments (CPCSEA), India.

6.2.3 Acute toxicity study

Acute oral toxicity studies of the selected fruit extracts belonging to the Cucurbitaceae family were conducted in female swiss albino mice according to the Organization for Economic Co-operation and Development (OECD) guidelines, the sequential analysis that employed a maximum of five animals (Anonymous 2008). In situations when there is experimental evidence suggesting that the test material is unlikely to be hazardous, a test dose of 2000 or exceptionally 5000 mg/kg may be administered. The test protocol aims to reduce the number of animals needed to determine the oral acute toxicity of a sample, as well as estimate the LD_{50} and confidence intervals.

As per the protocol, the study was conducted after allowing the animals to acclimatise for a period of 4-5 days. This study utilised healthy, young adult mice weighing between 20 and 25 gm. The mice were nulliparous (never having given birth) and non-pregnant. After the administration of the sample under study, food was withheld for a duration of primarily 3-4 hrs and additionally 1-2 hrs, while water was not withheld. A single animal was administered plant extract under study, orally. Following the survival of the first experimental animal, four further animals were administered oral doses on successive

days, resulting in a total of five animals being tested. The experimental animals were subjected to individual observations at regular intervals of 5 mins throughout the initially 30 mins period following dosing. Subsequently, periodic observations were conducted per 2 hrs for the first 24 hrs, with particular emphasis on the first four hrs. Following this, daily observations were conducted for a duration of 14 days.

6.2.4 Experimental Pharmacology

6.2.4.1 Animal Grouping

The Swiss albino mice, weighing between 20 and 25 gm, were randomised into five groups, with each group including six experimental animals. Group I was treated with only saline water (1 mL p.o). Group II to IV was taken as test groups, group II, III and IV was treated with *M. dioica* at 100 mg/Kg, 200mg/ Kg and 400 mg/Kg respectively. Group V was treated with standard, Diazepam 2 mg/Kg. Forced swimming endurance stress test, anoxic stress tolerance test, tail suspension test and elevated plus maze test was performed on all the groups. Detailed animal grouping is representing in the table 6.1.

Table 6.1. Experimental Design and Treatment schedule

Group I:	Control (normal saline (1 mL) p.o.), subjected to stress
Group II:	*M. dioica* dose I (100 mg/Kg)
Group III:	*M. dioica* dose II (200 mg/Kg)
Group IV:	*M. dioica* dose III (400 mg/Kg)
Group V:	Diazepam 2 mg/kg (Standard drug) with stress

6.2.4.2 Forced Swimming Endurance Stress Test

The forced swim endurance stress test was conducted using Swiss albino mice. The animals were divided into five groups and each group was treated according to the conditions specified in the table above. The drug treatment was administered concurrently for a duration of seven days, with a consistent daily dosage. On the last

day (8th day) the experimental animals were subjected to swimming stress test. Polypropylene tank with dimension of 37 × 37 × 30 cm filled with water to a height of 25 cm was used for the study. The mice were permitted to swim until they reached a state of total exhaustion, and the point at which the animal began to drown was recorded. The mean duration of swimming for each group was calculated. After the study, animals were removing from water and pat dried with towel and placed in the cage and the polypropylene tank was cleaned thoroughly to remove any residual or debris that could affect following analysis (Can et al., 2012; Li et al., 2022).

6.2.4.3 Anoxic Stress Tolerance Test

The swiss albino mice were segregated into 5 groups and all were treated as mentioned above. For the study, conical flasks with a capacity of 250 mL were utilised. The flasks were sealed with rubber cork prior to commencing the experiment. Each of the animal was confined in a hermetically sealed container, and the duration of time was recorded using a stopwatch. Upon the onset of an animal's initial convulsion or signs of distress, it was promptly withdrawn from the container and, if necessary, resurrected. The duration between the animal's entry into the airtight vessel (conical flask) and the onset of the initial convulsion was labelled as the period of "anoxic stress tolerance." The mean time till the commencement of convulsion was measured and the animal was immediately removing when the convulsion occurred (Chikkamath et al., 2023).

6.2.4.4 Tail suspension test

This method relies on the observation that animals, when hanging by their tails, exhibit alternating periods of agitation and immobility. The state of immobility serves as a sign of a depressive state. The categorization of animals was identical to the prior testing procedure used to determine the duration of immobility. The relevant groups were provided a vehicle, extract, and positive control orally, once day for 8th days. Following a one-hour treatment on the eighth day, the animals were individually suspended upside-down on a wire. Following initial forceful movements, the animal adopts a motionless position, and the duration of immobility over a 5 mins observation period was documented. Animals were deemed immobile if they maintained full motionlessness for a minimum of one minute. The term 'immobility' was defined as the inability to make any

motions, such as attempting to catch the adhesive tape, or any bodily torsions or jerks (Singh and Patra, 2019).

6.2.4.5 Elevated Plus maze Test

The Elevated Plus Maze is comprised of a configuration consisting of two open arms and two enclosed arms, placed in a plus-shaped arrangement, which is raised above the ground level. The structure was made of black wooden planks, with two closed arms measuring 50 cm x 10 cm x 40 cm, and two open arms measuring 50 cm x 10 cm. These planks were attached to a central platform measuring 10 cm x 10 cm and elevated to a standard height of 50 cm above the floor. The mice were placed in the centre of the maze facing any open arm and allow the experimental animal to explore the maze. Number of entries into the open arms and closed arms, time spent in the open arms and closed arms and total distance covered was recorded. A total of 5 minutes was allocated for recording the number of entrances and the duration of time spent in open arms. Each entry was recorded when the mouse's four paws made contact with either an open or closed arm. Video camera was utilised to record all assessment procedures (Gamberini et al., 2015; Gasper et al., 2023).

6.2.4.6 Estimation of haematological and serum biochemical parameters

For estimation of haematological and serum biochemical parameters blood samples were withdrawn through retroorbital puncture of experimental animals after completion of the *in-vivo* studies. Haematological parameters including neutrophil, lymphocyte, monocyte and Eosinophil as well as RBCs was estimated employing automated haematology analysers (Cellenium 5D Retic, Make: Trivitron Healthcare, India). Serum biochemical parameters such as fasting blood glucose (FBG), Total cholesterol (TC), Triglyceride (TG), Serum Glutamic Pyruvic Transaminase (SGPT), Serum glutamic oxaloacetic transaminase (SGOT), Serum Alkaline phosphate (SALP), Blood Urea Nitrogen (BuN), Total Protein (TP), Corticosterone (Cort.) was estimated by separating serum from blood employing centrifuge.

6.2.4.7 Estimation of antioxidant biomarkers in brain

All of the mice were sacrificed by administration of pentobarbitone sodium (60 mg/kg) at the end of the *in-vivo* study. The entire brain was promptly anatomized and then

rinsed with ice-cold saline solution and also 0.15 M Tris-HCl buffer to eliminate any traces of blood. The lipid peroxidation assay (LPO) was performed by using thiobarbituric acid reactive substances (TBARS) method. Samples were mixed with 15% trichloroacetic acid and 0.38% thiobarbituric acid and further incubated. The combination was subjected to heat for a duration of one hr using water bath and measuring the absorbance produced compound at a wavelength of 532 nm (Nunes et al., 2015). Measurement of superoxide dismutase (SOD) activity was conducted using a reaction mixture consisting of 0.1 mL of phenazine methosulfate, 1.2 mL of sodium pyrophosphate buffer (pH 7.0), and 0.3 mL of the tissue homogenate. The enzyme reaction began by introducing 0.2 mL of 780 µM NADH and aborted after 1 minute by adding 1 mL of glacial acetic acid. The quantity of chromogen produced was quantified by measuring the intensity of colour at 560 nm. The results were quantified in units/ mg of protein (Maryam et al., 2018). Assay for catalase (CAT) contained the composition of the reaction solution consisted of 0.1 mL of tissue homogenate, 2.5 mL of 0.05 M phosphate buffers and 0.7 mL of 0.2 M Hydrogen peroxide. After a minute, the reaction solution's changes in absorbance at 570 nm were measured and the results were measured in units/mg of protein (Patro et al., 2016). To estimate the reduced glutathione (GSH) dithiobisnitro-benzoate was employed as a substrate. The yellow hue was observed and measured promptly at a wavelength of 412 nm, and the results were reported as µg GSH/mg tissue (Salbitani et al., 2017).

6.3 Statistical Analysis

All values were represented as mean ± standard error of the mean. The statistical analysis of the results involved the utilisation of a one-way analysis of variance (ANOVA) followed by Dunnet's multiple range test multiple comparison test against the corresponding control group. $P < 0.05$ were considered to be significant. The statistical analyses were conducted using GraphPad Prism 8.0.2.

6.4 Results and Discussions

6.4.1 Acute toxicity study

All of the animals were in good physical condition, and there were no apparent symptoms of poisoning, not even a fatality in a mouse that had received oral therapy of

the *M. dioica* fruit extract up to 2000 mg/kg after 48 hr. The study demonstrated the safety of the extracts, which can be regarded as a therapeutic benefit.

6.4.2 *In-vivo* adaptogenic activity

In the forced swim endurance test, sequential treatment with *M. dioica* dosages and diazepam significantly increased swimming time compared to the control group. The swimming survival time for groups II to IV was recorded at 139, 202, and 215 seconds, respectively. Comparing the swimming survival time of Groups III, IV, and V with the control group revealed a significant increase in the survival time. The higher dose (400 mg/Kg) of *M. dioica* extract showed a significant increase in swimming survival time compared to the positive control. Thus, higher dose of *M. dioica* is more effective compared to a lesser dose of the extract. All of the administered doses demonstrated the ability to enhance swimming endurance in comparison to the animals who were given normal saline water. Table 6.2 and Figure 6.1 depict the impact of extract and positive control on the duration of survival during forced swimming.

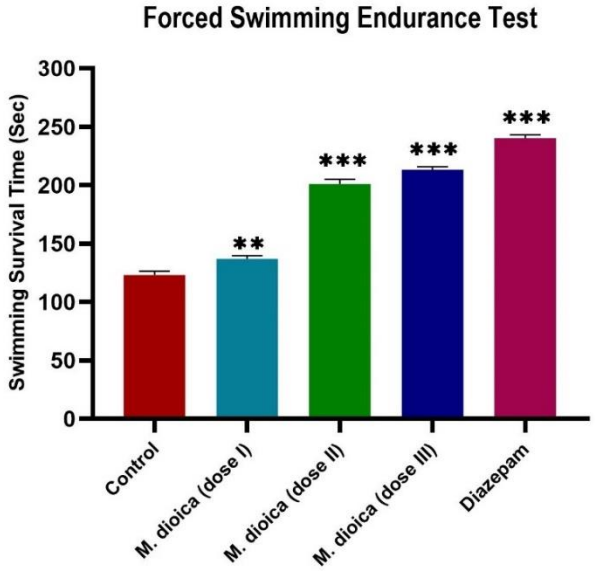


Figure 6.1. Effect of *M. dioica* extracts and positive control (Diazepam) on swimming survival time, represents as sec for Forced Swimming Endurance Test (** $P < 0.5$; *** $P < 0.01$)

Table 6.2. Effect of *M. dioica* extracts and positive control (Diazepam) on swimming survival time, represents as sec for Forced Swimming Endurance Test
(** $P < 0.5$; *** $P < 0.01$)

Groups	Treatment Dose (mg/Kg) p.o.	Swimming Survival Time (sec)
Group I: Control (subjected with stress)	Saline (1 mL)	124.94 ± 2.58
Group II: *M. dioica* dose I	100 mg/Kg	139.16 ± 1.17**
Group III: *M. dioica* dose II	200 mg/Kg	202.39 ± 1.73***
Group IV: *M. dioica* dose III	400 mg/Kg	215.05 ± 1.54***
Group V: Diazepam (Positive Control)	2 mg/ Kg	241.96 ± 1.94***

In anoxic stress tolerance test, group I was placed in hermetic vessel for a duration of 19.54 min. Group V considered as positive control, treated with diazepam survived for 53 min in the vessel; The animals in groups II, III, and IV persisted for 27, 35 and 39 min, respectively in the same sealed container. The anoxia tolerance test was assessed by observing the occurrence of convulsions as the endpoint. The two doses of *M. dioica* (200 mg/Kg and 400 mg/Kg) demonstrated a notable increase in tolerance stress time in 8th day when compared with the control group. In table 6.3 and figure 6.2 the anoxic stress tolerance time of test as well as positive control was depicted.

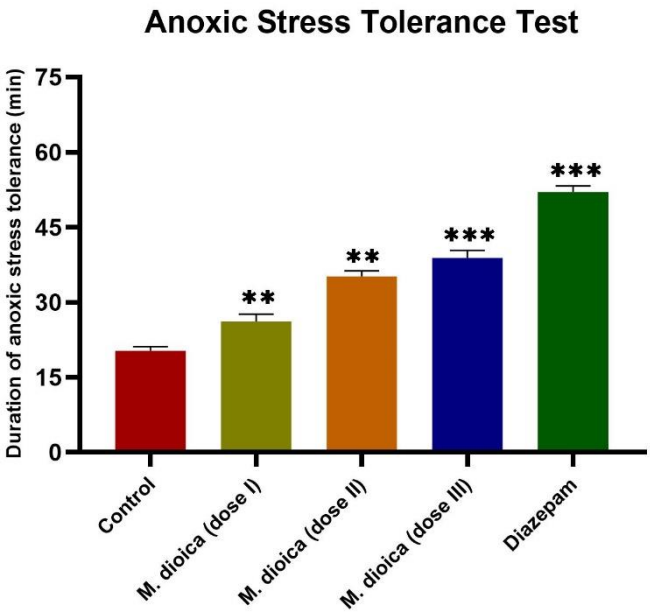


Figure 6.2. Effect of *M. dioica* extracts and positive control (Diazepam) on anoxic stress tolerance time, represents as min for Anoxic Stress Tolerance Test (** $P < 0.5$; *** $P < 0.01$)

Table 6.3. Effect of *M. dioica* extracts and positive control (Diazepam) on duration of anoxic stress tolerance time, represents as min for Anoxic Stress Tolerance Test (** $P < 0.5$; *** $P < 0.01$)

Groups	Treatment Dose (mg/Kg) p.o.	Anoxic Stress Tolerance Time (min)
Group I: Control (subjected with stress)	Saline (1 mL)	19.54 ± 0.47
Group II: *M. dioica* dose I	100 mg/Kg	27.11 ± 0.36**
Group III: *M. dioica* dose II	200 mg/Kg	35.31 ± 0.32**
Group IV: *M. dioica* dose III	400 mg/Kg	39.48 ± 0.28***
Group V: Diazepam (Positive Control)	2 mg/ Kg	53.47 ± 0.37***

In tail suspension test, group I, II, III, IV and V exerted immobility time at a duration of 245, 193, 177, 142 and 99 secs respectively. *M. dioica* at 200 and 400 mg/Kg doses and diazepam (2 mg/Kg) exhibited potent adaptogenic activity in compare to the control

group by decreasing the immobility time. In table 6.4 and figure 6.3 the immobility time of test as well as positive control was showed.

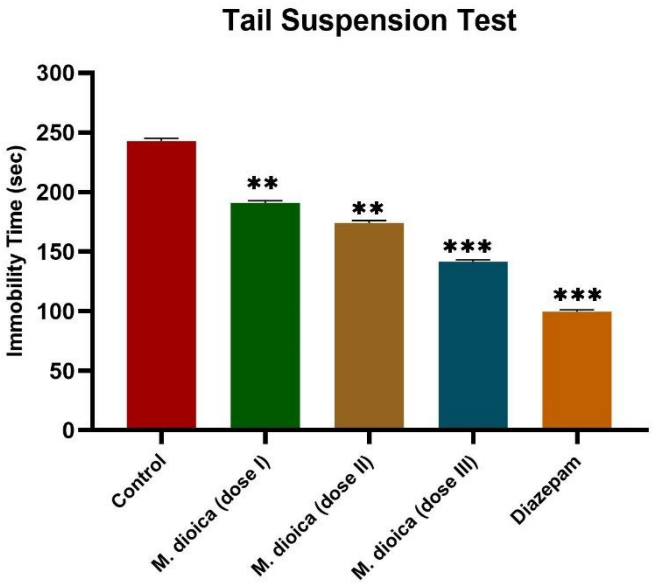


Figure 6.3. Effect of *M. dioica* extracts and positive control (Diazepam) on immobility time, represents as sec for Tail Suspension Test (** $P < 0.5$; *** $P < 0.01$)

Table 6.4. Effect of *M. dioica* extracts and positive control (Diazepam) on immobility time, represents as sec for Tail Suspension Test (** $P < 0.5$; *** $P < 0.01$)

Groups	Treatment Dose (mg/Kg) p.o.	Immobility Time (sec)
Group I: Control (subjected with stress)	Saline (1 mL)	245.53 ± 0.41
Group II: *M. dioica* dose I	100 mg/Kg	193.28 ± 0.36**
Group III: *M. dioica* dose II	200 mg/Kg	176.81 ± 0.19**
Group IV: *M. dioica* dose III	400 mg/Kg	141.84 ± 0.25***
Group V: Diazepam (Positive Control)	2 mg/ Kg	98.94 ± 0.13***

During the EPM test, the number of entries into the open arm increases, whereas the number of entries into the closed arm decreases after seven consecutive days of

treatment with the extracts and positive control. Following the course of treatment, the amount of time spent in the open arm increased, group II-V exhibited durations of 54, 101, 142, 149, and 168 seconds, respectively. The duration of time spent in the closed arm was recorded as 252, 181, 168, 160, and 120 seconds for groups II-V, correspondingly. The administration of *M. dioica* at doses of 200 and 400 mg/Kg, as well as the treatment with diazepam, resulted in a significant increase in the time spent in the open arm and a significant decrease in the time spent in the closed arm. The mice's movement within the apparatus increased after being treated with the extracts and positive control. Figures 6.4 and 6.5 depict the number of entries in the open and closed arms, as well as the duration spent in the open and closed arms, during the elevated plus maze test. The distance measured during the study is depicted in figure 6.6. Table 6.5 displays the count of entries in both arms and the duration of time spent in each arm.

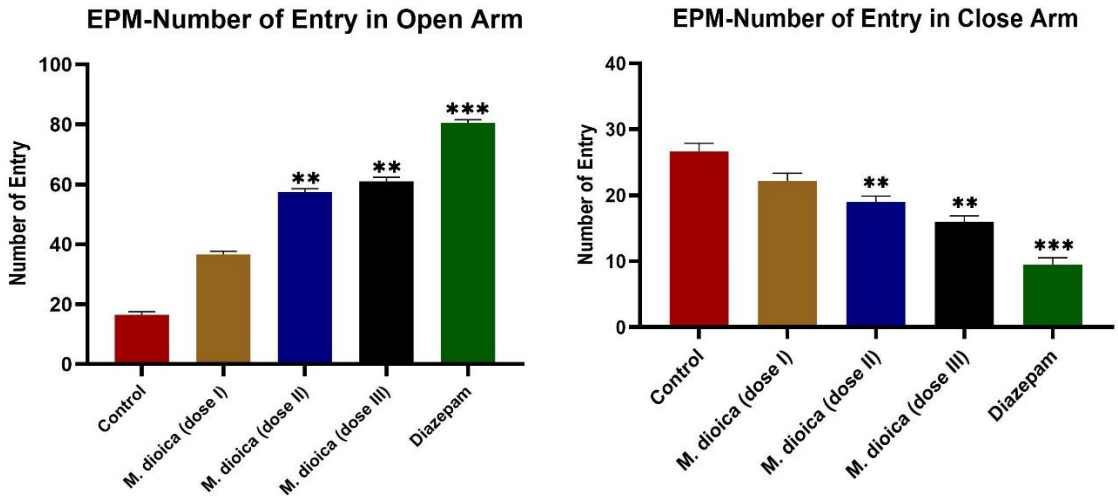


Figure 6.4. Effect of *M. dioica* extracts and positive control (Diazepam) on number of entries in open and closed arm in Elevated Plus Maze test (** $P < 0.5$; *** $P < 0.01$)

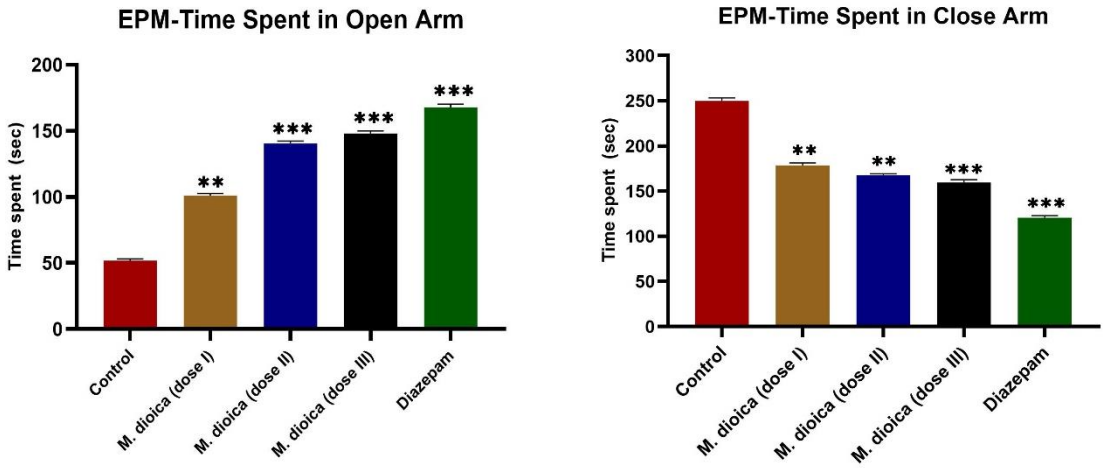


Figure 6.5. Effect of *M. dioica* extracts and positive control (Diazepam) on time spent (sec) in open and closed arm in Elevated Plus Maze test (** $P < 0.5$; *** $P < 0.01$)

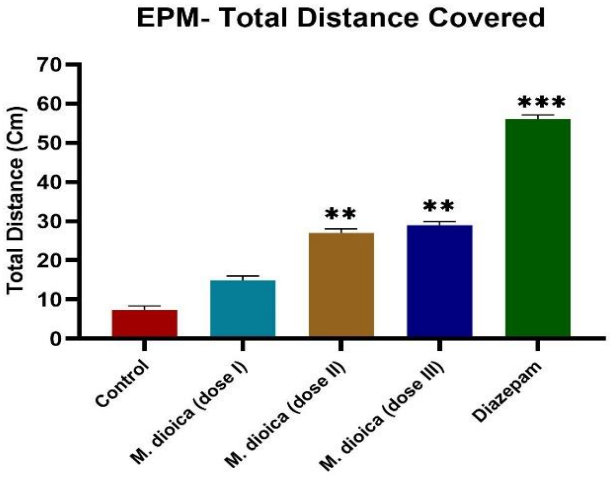


Figure 6.6. Effect of *M. dioica* extracts and positive control (Diazepam) on total distance covered (cm) in Elevated Plus Maze test (** $P < 0.5$; *** $P < 0.01$)

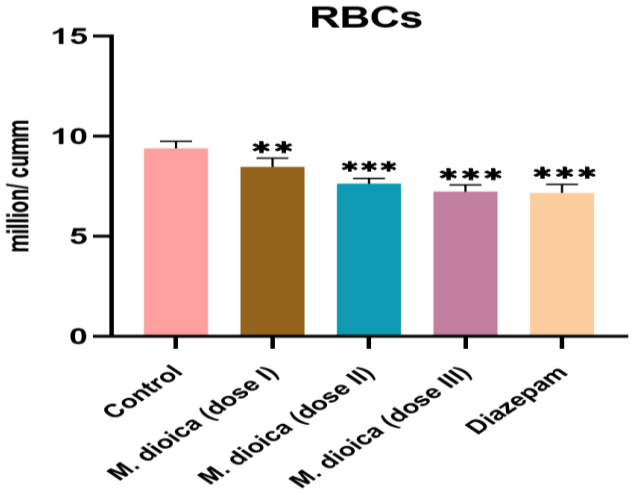
Table 6.5. Effect of *M. dioica* extracts and positive control (Diazepam) on number of entries in open and closed arm, time spent (sec) in open and closed arm for Elevated Plus Maze Test (** $P < 0.5$; *** $P < 0.01$)

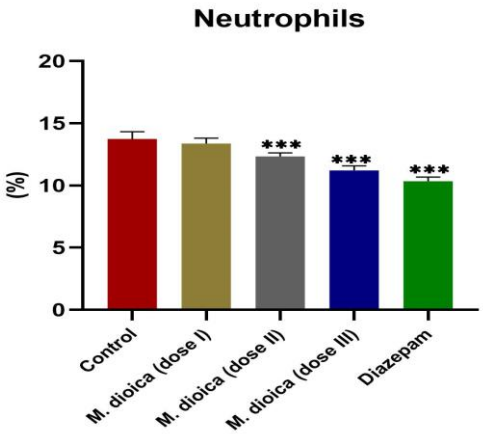
Groups	Number of Entry in Open Arm	Number of Entry in Closed Arm	Time Spent in Open Arm (Sec)	Time spent in Closed Arm (Sec)
Group I: Control (subjected with stress)	16.3 ± 0.47	28.4 ± 0.32	54.20 ± 0.24	251.96 ± 0.05
Group II: *M. dioica* dose I (100 mg/Kg)	37.8 ± 0.21	23.8 ± 0.09	100.91 ± 0.11**	180.94 ± 0.13**
Group III: *M. dioica* dose II (200 mg/Kg)	58.7 ± 0.20**	19.8 ± 0.16**	141.59 ± 0.32***	167.76 ± 0.17**
Group IV: *M. dioica* dose III (400 mg/Kg)	61.1 ± 0.26**	16.9 ± 0.11**	149.55 ± 0.17***	159.85 ± 0.13***
Group V: Diazepam (2 mg/Kg)	80.5 ± 0.37***	9.4 ± 0.36***	168.08 ± 0.16***	120.55 ± 0.24***

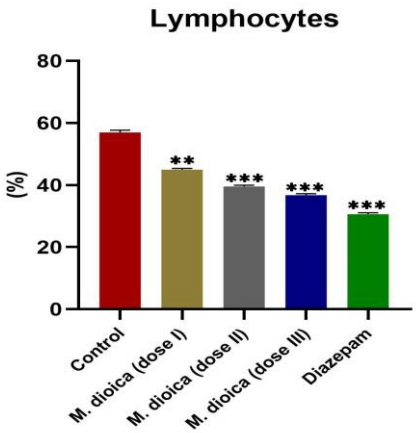
6.4.3 Estimation of haematological and serum biochemical parameters

On the 8th day, all animal groups were examined for haematological parameters. Group I, which served as the control, was treated with saline water. Groups II to IV served as the test groups, while Group V functioned as the positive control. The RBC count parameter was measured to be 8.06, 7.71, 7.08, and 7.57 million/ cumm in both the test groups and the positive control group. The control group had a red blood cell density of 9.7 million/ cumm. The count of RBCs was consistently decreased as a result of pretreatment with extracts and the positive control. The neutrophil counts of animals in Groups I, II, III, IV, and V were determined to be 14.03%, 13.24%, 12.7%, 11.83%, and 10.27%, respectively. The lymphocyte counts of animals in Groups I, II, III, IV, and V was measured as 57.1%, 44.5%, 39.6%, 37.28%, and 30.6%, respectively. The

monocyte counts of animals in Groups I, II, III, and IV was determined to be 3.7%, 2.1%, 1.9%, 1.2%, and 1.1%, respectively. The eosinophil count of animals in Group I, II, III, IV, and V was 3.5, 2.1, 1.8, 1.5, and 1.3 respectively. The neutrophils, lymphocytes, monocytes, and eosinophils count in Groups II, III, and IV decreased as a result of pretreating with the extracts compared to control group. The administration of diazepam and higher dosages of *M. dioica* resulted in a notable reduction in red blood cell (RBC) and differential leukocyte counts. This reduction effectively prevented the stress-induced alterations in the haematological parameters. However, lower dose of *M. dioica* also has a considerable adaptogenic activity. In the table 6.6 the haematological parameters represented. The haematological parameters were illustrated in figure 6.7.







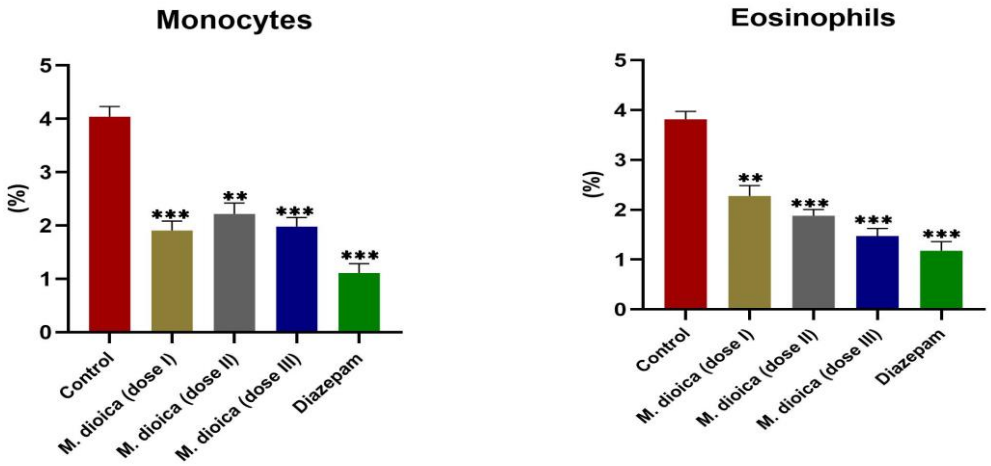


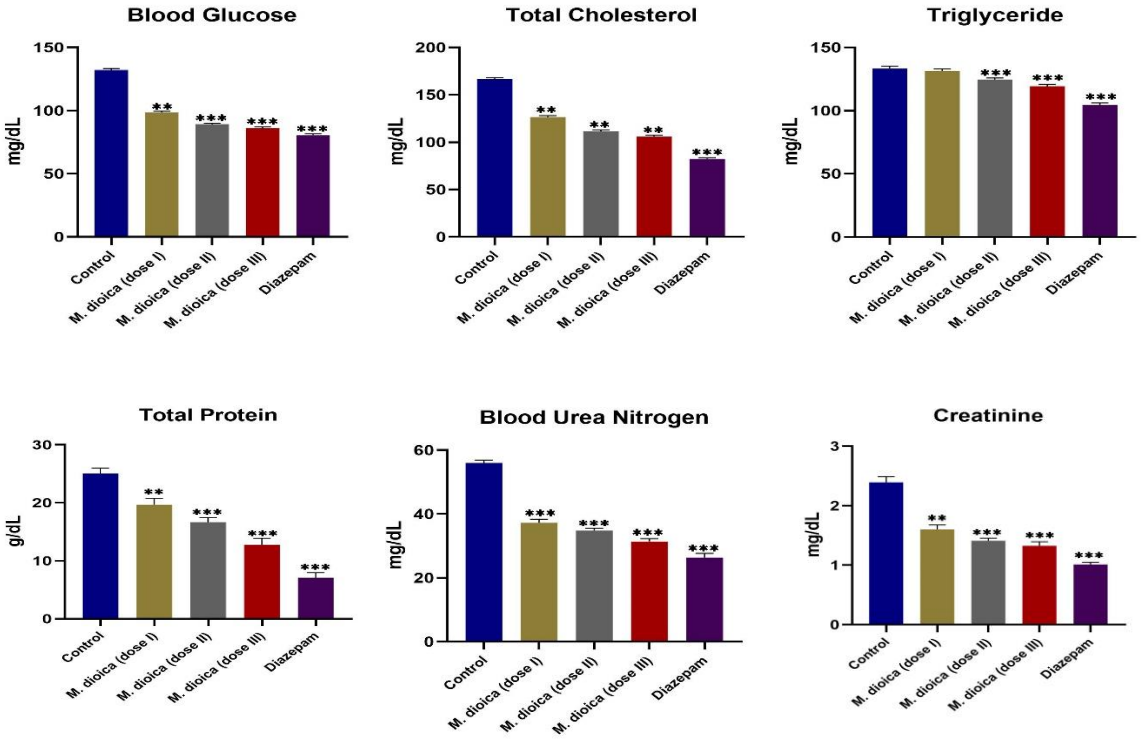
Figure 6.7. Effect of *M. dioica* extracts and positive control (Diazepam) on Haematological Parameters (** $P < 0.5$; *** $P < 0.01$)

Table 6.6. Effect of *M. dioica* extracts and positive control (Diazepam) on Haematological parameters (** $P < 0.5$; *** $P < 0.01$)

Groups	RBCs (million/ cumm)	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)
Group I: Control (subjected with stress) Saline (1 mL)	9.7 ± 0.32	14.03 ± 0.20	57.1 ± 1.22	3.7 ± 0.09	3.5 ± 0.16
Group II: *M. dioica* dose I 100 mg/Kg	8.06 ± 0.24**	13.2 ± 0.17	44.5 ± 0.28**	1.9 ± 0.04***	2.1 ± 0.17**
Group III: *M. dioica* dose II 200 mg/Kg	7.71 ± 0.18***	12.7 ± 0.11***	39.6 ± 0.37***	2.1 ± 0.14**	1.8 ± 0.05***
Group IV: *M. dioica* dose III 400 mg/Kg	7.57 ± 0.26***	11.8 ± 0.12***	37.2 ± 0.31***	1.2 ± 0.07***	1.5 ± 0.04***

Group V: Diazepam (Positive Control) 2 mg/ Kg	7.06 ± 0.06***	10.2 ± 0.13***	30.67 ± 0.24***	1.1 ± 0.08***	1.3 ± 0.03***

On the eighth day, the levels of glucose, total cholesterol, triglyceride, SGOT, SGPT, SALP, total protein, and blood urea nitrogen were evaluated. The biochemical parameters exhibited an elevation during the period of stress, but subsequently decreased as a result of the pre-treatment with *M. dioica* extracts. The stress caused an increase in the amount of corticosterone. The control group had a corticosterone level of 140.46 ± 0.32 mg/dL, while group II-V had levels of 130.04 ± 0.07, 124.24 ± 0.12, 118.17 ± 0.16, and 106.22 ± 0.0557 mg/dL respectively. *M. dioica* doses at 200 and 400 mg/Kg shown strong therapeutic effects by decreasing increased biochemical parameters. The table 6.7 and figure 6.8 presents the biochemical parameters and the corresponding level of corticosterone.



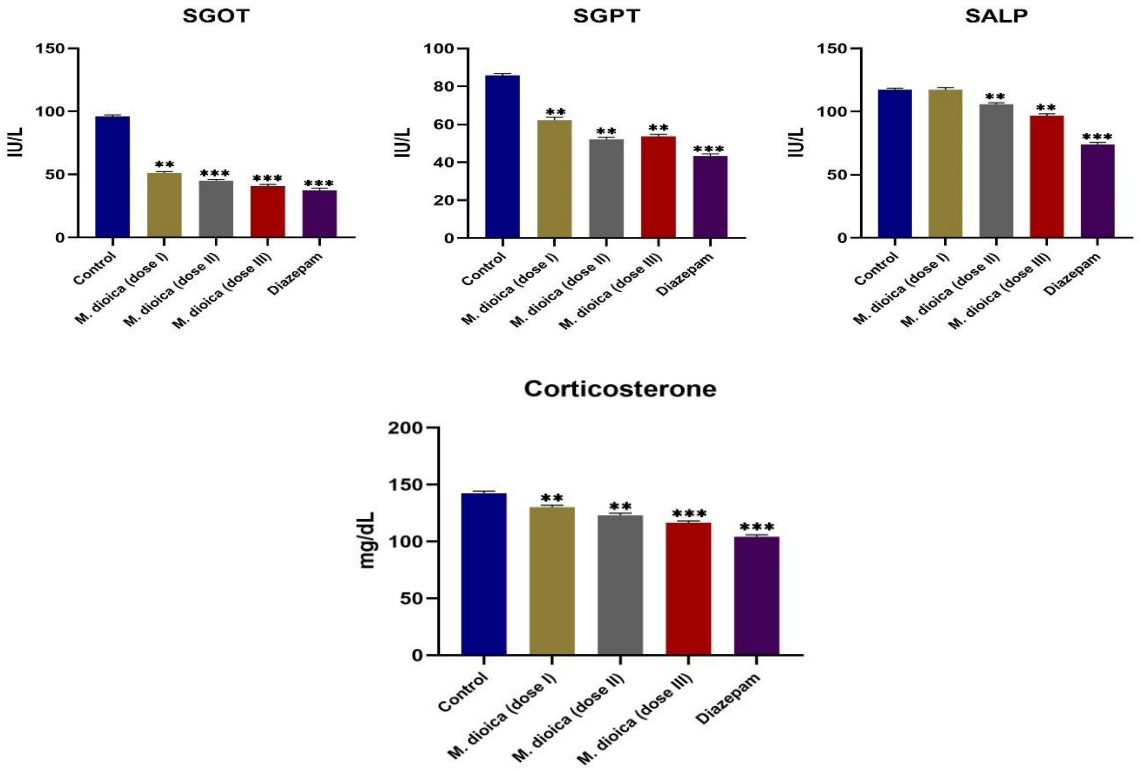


Figure 6.8. Effect of *M. dioica* extracts and positive control (Diazepam) on Biochemical Parameters (** $P < 0.5$; *** $P < 0.01$)

Table 6.7. Effect of *M. dioica* extracts and positive control (Diazepam) on Biochemical Parameters (** $P < 0.5$; *** $P < 0.01$)

Biochemical Parameters	**Group I: Control (subjected with stress) Saline (1 mL)**	**Group II: *M. dioica* dose I 100 mg/Kg**	**Group III: *M. dioica* dose II 200 mg/Kg**	**Group IV: *M. dioica* dose III 400 mg/Kg**	**Group V: Diazepam (Positive Control) 2 mg/ Kg**
Blood Glucose (mg/dL)	130.8 ± 0.16	98.59 ± 0.36**	89.46 ± 0.23***	86.8 ± 0.21***	81.55 ± 0.33***
Total Cholesterol (mg/dL)	166.50 ± 0.33	126.72 ± 0.23**	112.57 ± 0.22**	106.82 ± 0.26**	83.08 ± 0.12***
Triglyceride (mg/dL)	132.29 ± 0.28	131.4 ± 0.29	125.33 ± 0.24***	120.91 ± 0.10***	105.86 ± 0.12***

Total Protein (g/dL)	23.83 ± 0.16	19.32 ± 0.19**	17.62 ± 0.20***	14.46 ± 0.34***	7.56 ± 0.23***
BuN (mg/dL)	55.64 ± 0.18	37.53 ± 0.17***	34.71 ± 0.27***	31.26 ± 0.20***	27.22 ± 0.08***
Creatinine (mg/dL)	2.26 ± 0.14	1.63 ± 0.01**	1.42 ± 0.01***	1.33 ± 0.01***	1.02 ± 0.02***
SGOT (IU/L)	95.03 ± 0.13	51.48 ± 0.18**	45.53 ± 0.25***	40.77 ± 0.11***	38.68 ± 0.14***
SGPT (IU/L)	85.33 ± 0.25	62.49 ± 0.26**	52.64 ± 0.21**	53.97 ± 0.09**	44.00 ± 0.16***
SALP (IU/L)	116.72 ± 0.22	117.43 ± 0.25	107.58 ± 0.21**	98.45 ± 0.09**	74.88 ± 0.11***
Corticosterone (mg/dL)	140.46 ± 0.32	130.04 ± 0.07**	124.24 ± 0.12**	118.17 ± 0.16***	106.22 ± 0.05***

6.4.4 Estimation of antioxidant biomarkers in brain

On the eighth day of the study, estimation of antioxidant biomarkers in brain of experimental animals were performed. The brain antioxidant activities of TBARS, SOD, GSH and also CAT potently altered with the treatment of plant extract in a dose dependent manner. Table 6.8 and figure 6.9 elucidated the changes in brain activities of antioxidant enzymes in experimental animals. In LPO assay, higher dose of *M. dioica* extract treated group possesses significantly lower the content of TBARS (1.11 µM/100gm Tissue) whereas, other two groups of *M. dioica* consisted 100 and 200 mg/Kg exhibited modest TBARS lowering activity. In both SOD and GSH assay groups treated with *M. dioica* at doses of 200 mg/Kg and 400 mg/Kg showed the alteration of the SOD and GSH concentration. The content of CAT at 100, 200 and 400 mg/Kg of *M. dioica* extract treated groups exhibited 1.8, 3.53 and 4.54 Unit/Min respectively. With the treatment of standard drug diazepam reduced level of TBARS and elevated level of other enzymes were observed.

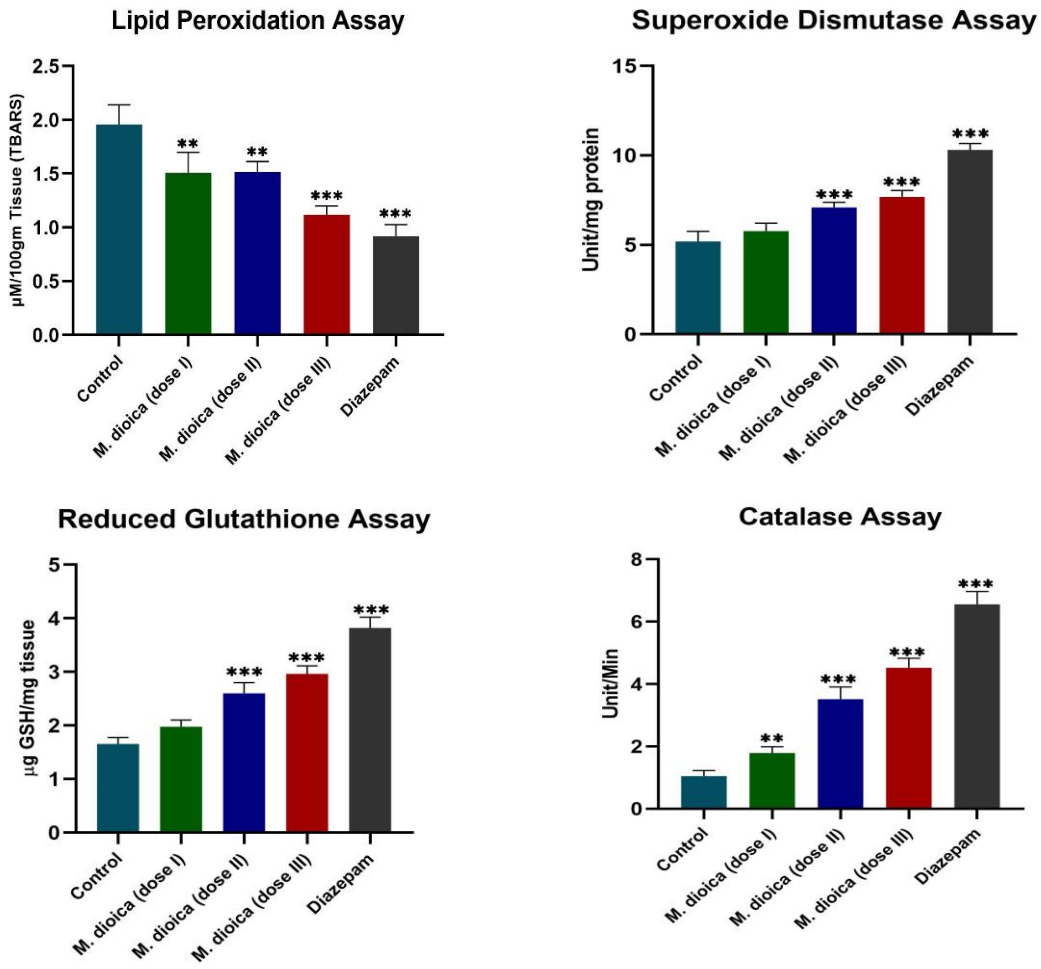


Figure 6.9. Effect of *M. dioica* extracts and positive control (Diazepam) on antioxidant biomarkers in brain tissue (** $P < 0.5$; *** $P < 0.01$)

Table 6.8. Effect of *M. dioica* extracts and positive control (Diazepam) on antioxidant biomarkers in brain tissue (** $P < 0.5$; *** $P < 0.01$)

Groups	LPO (µM/100gm Tissue)	SOD (Unit/mg protein)	GSH (µg/mg tissue)	CAT (Unit/Min)
Group I: Control (subjected with stress) Saline (1 mL)	1.94 ± 0.03	5.26 ± 0.06	1.67 ± 0.02	1.06 ± 0.01
Group II: *M. dioica* dose I 100 mg/Kg	1.63 ± 0.13**	5.69 ± 0.12	1.99 ± 0.15	1.8 ± 0.02**

Group III: *M. dioica* dose II 200 mg/Kg	1.56 ± 0.04**	7.21 ± 0.06***	2.55 ± 0.12***	3.53 ± 0.04***
Group IV: *M. dioica* dose III 400 mg/Kg	1.11 ± 0.01***	7.84 ± 0.12***	2.96 ± 0.05***	4.54 ± 0.06***
Group V: Diazepam (Positive Control) 2 mg/ Kg	0.99 ± 0.06***	10.31 ± 0.08***	3.87 ± 0.08***	6.58 ± 0.09***

6.5 Conclusion

To assess the *in-vivo* adaptogenic activity of *M. dioica* fruit extract was studied employing forced swim endurance test, anoxic stress tolerance test, tail suspension test and elevated plus maze test. The doses of *M. dioica* shown resilient adaptogenic activity in all the conducted tests by altering the stress response. The extract also prevents the changes in haematological and biochemical parameters that occur as a result of stress. The antioxidant activity in brain tissue was assessed by increasing the levels of SOD, GSH, and CAT, while simultaneously reducing the levels of TBARS also observed in a dose-dependent manner. The medicinal potential of this fruit may be attributed to the synergistic action of the phytoconstituents it contains. These first findings show promise, and additional research on different doses would be beneficial in confirming the drug's adaptogenic effects. However, additional research is required to identify and separate the specific biomarker present in this fruit that is being investigated in this study. The results of the acute toxicity investigation indicate that the selected fruit extract appears to be non-toxic at doses up to 2000 mg/kg. The fruit extract exhibited significant adaptogenic effect in Swiss albino mice in a dose-dependent manner. Both the 200 mg/Kg and 400 mg/Kg doses of *M. dioica* demonstrated a significant impact on haematological markers, liver enzymes, renal function, and other biochemical parameters. The brain tissue antioxidant properties of *M. dioica* extracts at 200 mg/Kg and 400 mg/Kg also altered the oxidative stress related response and promote adaptogenic activity of the plant extract. The current study suggests that the chosen fruit is efficacious and can be regarded as an adaptogen. Furthermore, these findings provide further evidence to support the long-standing assertion that this fruit of *M. dioica* is effective in the treatment of neurological disorders.

Nevertheless, it is necessary to conduct an inquiry into the primary compounds that are accountable for adaptogenic activity in order to ascertain the specific component responsible for these activities.

Chapter 7

Summary & Conclusion

7.1 Summary

Plant remedies have a rich historical tradition in treating stress and are widely favoured by the general public, with over 35% of Americans reporting the usage of at least one herbal remedy (Burns, 2023). The accessibility, perceived safety, and diverse delivery methods of herbal medicines make them a convenient self-care intervention, especially when combined with a comprehensive awareness of the scientific principles and strategic use of these remedies (Ekor, 2013). Medicinal food plants have been historically utilised for disease prevention and treatment, and they are currently being assessed as a potential avenue for therapeutic advancements (Mukherjee et al., 2015). It is also essential for maintaining a healthy lifestyle and mitigating stress induced metabolic risk factors like as diabetes, hypertension, dyslipidemia, and obesity (Rippe, 2019). The % yield value of plant bioactive substances often referred to as marker compounds, is a crucial aspect in accurately identifying or verifying the specific plant's relevant connection (Mukherjee et al., 2019). The variation in phytomarker content among different plant species is influenced by genetic differences, external growth factors such as climate, temperature, soil composition, fertilisers, and irrigation, as well as the specific conditions under which the plants are grown (Pregitzer et al., 2013).

According to the World Health Organisation (WHO), stress can be described as a psychological state characterised by anxiety or mental strain resulting from a challenging circumstance. Stress is an innate physiological and psychological reaction that compels us to confront and overcome difficulties and dangers that arise in our lives (Anonymous, 2023). Chronic stress impairs the body's ability to protect itself from reactive oxygen species (ROS), leads to an imbalance between the production of ROS and the body's ability to remove them. The lack of symmetry in this situation results in oxidative stress, which in turn causes damage to cells and organs, including those involved in metabolic regulation (Pizzino et al., 2017). Oxidative stress can impede the pathways via which insulin signals are transmitted, exacerbate inflammation, and promote the degradation of lipids. These combined effects contribute to the development of insulin resistance and metabolic dysfunction. Furthermore, the stress-induced oxidative damage may impair the pancreatic beta cells' capacity to produce

insulin, hence exacerbating the control of glucose levels. Hence, the intricate correlation between stress, oxidative stress, and metabolic dysfunction underscores the importance of holistic approaches that address both psychological stressors and oxidative imbalance in the management of metabolic-related diseases (Tangvarasittichai, 2015; Matough et al., 2012). Due to the significant connections between oxidative stress, aand ailments, there is a growing fascination with the biomolecular impacts of herbs, which could be linked to their antioxidant properties, contain phenolic groups. The phytocomponent have the ability to impede or hinder the process of lipid oxidation or oxidation of other molecules by obstructing the progression of oxidative chain reactions. The production of an excessive quantity of free radicals and reactive species during metabolic processes results in oxidative harm to biomolecules within the body, leading to apoptosis and degeneration of vital macromolecules such as lipids, proteins, and DNA (Kasote et al., 2015).

The study was designed to screen four Cucurbitaceae family plants *viz. Cucurbita maxima* (Pumpkin), *Luffa acutangula* (Ridge Gourd), *Momordica dioica* (Spine Gourd) and *Trichosanthes dioica* (Pointed Gourd) based on their ethnopharmacological relevances as documented in several literatures to assess their adaptogenic activity in *in-vivo* model and also enzyme inhibition *viz.* α-amylase, α -glucosidase, carbonic anhydrase, and pancreatic lipase linked with several metabolic disorders. The work in this thesis also focuses to assess the marker profiling via HPTLC, HPLC and determining metabolite content using LC-MS as well as determination of antioxidant activities of the plant extracts. This study may be helpful for the scientific community to establish the quality, therapeutic effectiveness of the selected food plants.

In Chapter 1, the brief introduction based on scientific reports on different aspects such as background and perspective of adaptogen, physiological aspect of stress related to this study work have been described. Along with the overview of Cucurbitaceae plant family including geographical distribution, phytochemistry, pharmacological activity, nutritional importance etc. has been discussed. The literature review was carried out from several search engines, e.g. PubMed, ScienceDirect, Google Scholar, Scopus, Web of Science, ResearchGate and Google Books up to early 2024. Later on, the list

herbs compounds with adaptogenic potential have been mentioned. Furthermore, geographical distribution of Cucurbitaceae food plant and phytochemistry and pharmacology aspect listed in tables.

Chapter 2, emphasize an overview of the scope and rationale, as well as the specific objective and plan of work study. A study framework was developed implementing a work plan for this current study.

Chapter 3, outlines the rationale for the selection of four species from the Cucurbitaceae family viz. *Cucurbita maxima*, *Luffa acutangula*, *Momordica dioica* and *Trichosanthes dioica*. This selection was made based on documented pharmacological activity and diverse ethnomedicinal practices, with the goal of validating their quality and effectiveness. The available literature has been used to emphasise the botanical taxonomy, plant profile, morphological characteristics, geographical distribution, ethnopharmacological relevance, phytochemical profile, and pharmacological properties of each selected food plant. Additionally, a thorough discussion of the process of collection, authentication, extraction have been discussed. The % yield values of individual fruits varied from one another. Furthermore, the plant extracts are subjected to quantitative analysis using Total phenolic and flavonoid content assay.

Chapter 4, involved conducting Liquid Chromatography-Quadrupole Time of Fly-Mass Spectrometry (LC-QToF-MS) analysis to analyse the metabolic profile of the plants being studied. The goal was to identify any variations or similarities in the composition of metabolites in the fruit extracts. The identification of the compounds was verified based on their respective retention times, molecular weight (m/z), molecular formula, error (in ppm), published data, and a library search conducted using the Agilent 1260 Infinity II LC System. The LC-QToF-MS analysis detected a total of 18 compounds in *C. maxima*, 16 compounds in *L. acutangula*, 17 compounds in *M. dioica*, and 15 compounds in *T. dioica*. These compounds include phenolic acid, flavonoid, fatty acid, phytosterol, vitamins, amino acids, and other substances.

The utilization of HPTLC and RP-HPLC in medicinal plant analysis and the development of a validated HPTLC as well as RP-HPLC method was discussed in order

to standardize the hydroalcohol extract of the selected food plants by using chlorogenic acid as a marker compound. The quantification was performed by HPTLC and utilising calibration curve of chlorogenic acid, constructed by correlating the peak area of chlorogenic acid with its corresponding concentration. *M. dioica* and *L. acutangula* had the highest chlorogenic acid content measuring 1.5% and 1.54% w/w, respectively. However, *C. maxima* and *T. dioica* contain 1.158% and 1.16% of the marker compound, respectively. The HPTLC method that was developed has been determined to be uncomplicated, exact, reliable, and capable of being reproduced for the quantification of chlorogenic acid within a limited linear range. This method can be beneficial for enhancing the development of efficient quality control and analysis of chlorogenic acid content in extracts and herbal products. The HPTLC method that was developed has been determined to be simple, precise, reliable, and capable of being reproduced for the quantification of chlorogenic acid within a limited linear range. This method can be beneficial for enhancing the development of efficient quality control and analysis of chlorogenic acid content in extracts and herbal products. RP-HPLC analysis was carried out using a mobile phase consisting of 75% methanol and 25% water, pH of the water was adjusted to 3.8 by adding glacial acetic acid. The validation of the RP-HPLC technique was also conducted according to the ICH Q2R1 criteria, assessing linearity, specificity, accuracy, precision, limit of quantification (LOQ), and limit of detection (LOD). The quantification was performed using a calibration curve of chlorogenic acid, which was created by graphing the area under the curve (AUC) against the concentration of chlorogenic acid. *M. dioica* exhibited the greatest concentration of chlorogenic acid, measuring at 1.738% w/w. The chlorogenic acid content of the other plants was determined to be as follows, *C. maxima* (1.084% w/w), *L. acutangula* (0.554% w/w), and *T. dioica* (0.791% w/w).

Chapter 5, explores the antioxidant properties of selected food plant extracts and focusing on their ability to inhibit enzymes such as α-amylase, α-glucosidase, carbonic anhydrase, and pancreatic lipase. The antioxidant activity of *C. maxima, L. acutangula, M. dioica*, and *T. dioica* was evaluated using the DPPH, Nitric oxide, Superoxide, and Hydroxyl free radical scavenging assays. The plants exhibited antioxidant activities that were comparable with the positive control, Ascorbic acid. Compared to other plant

extracts, *M. dioica* demonstrates a noteworthy *in-vitro* antioxidant activity. It exhibits an IC_{50} value of 130.16 ± 0.385 µg/mL for the DPPH assay, 118.54 ± 0.313 µg/mL for the NO assay, and 107.24 ± 0.222 µg/mL and 103.21 ± 0.184 µg/mL for the Superoxide and Hydroxyl assays, respectively. On the other hand, *C. maxima* demonstrated a moderate level of antioxidant activity for DPPH, NO, Superoxide, and Hydroxyl, with IC_{50} values of 142.61 ± 0.314, 137.35 ± 0.275, 116.8 ± 0.216, and 119.23 ± 0.171 µg/mL, respectively. *L. acutangula* and *T. dioica* displayed relatively lower *in-vitro* antioxidant activity compared to the positive control Ascorbic acid. In this investigation, the IC_{50} values for ascorbic acid were found to be 113.69 ± 0.094 µg/mL for DPPH, 105.12 ± 0.013 µg/mL for NO, 100.09 ± 0.143 µg/mL for Superoxide, and 97.49 ± 0.108 µg/mL for Hydroxyl free radical scavenging assay. Acarbose was employed as a positive control for the inhibition of α-amylase and α-glucosidase enzymes in *in-vitro* manner. In the present study, acetazolamide and orlistat were utilised as positive controls to inhibit carbonic anhydrase and pancreatic lipase enzyme, respectively. Stress, as a non-specific response, can reduce metabolic syndrome, which includes conditions such as hyperglycemia, hyperlipidemia, hypertension, and cardiac disorders.

By suppressing these four enzymes associated with the metabolic process of the human body, the probability of many ailments can be minimised. The hydroalcohol extract of *C. maxima, L. acutangula, M. dioica*, and *T. dioica* fruit was employed for *in-vitro* enzyme inhibition investigations. The results showed that *M. dioica* fruit exhibited a substantial inhibitory effect on α-amylase and α-glucosidase enzymes, with IC_{50} of 133.53 ± 0.112 and 89.32 ± 0.214 µg/mL, correspondingly. These values were compared to the positive control acarbose, which had IC_{50} of 97.27 ± 0.141 µg/mL for α-amylase and 66.62 ± 0.138 µg/mL for α-glucosidase. Moderate to lower inhibition of the α-amylase and α-glucosidase enzyme was seen in *C. maxima*, L. acutangula, and *T. dioica* fruit extract. The investigation of the inhibitory ability of selected fruit extracts on carbonic anhydrase enzyme was conducted. The positive control acetazolamide demonstrated an IC_{50} value of 166.61 ± 0.163 µg/mL. When compared to the positive control, *M. dioica* showed an IC_{50} value of 179.41 ± 0.171 µg/mL. On the contrary, *C. maxima, L. acutangula*, and *T. dioica* had IC_{50} values of 186.32 ± 0.197, 194.81 ± 0.151, and 206.14 ± 0.197 µg/mL, respectively. An assessment was conducted to determine

the inhibitory action of pancreatic lipase in selected plants from the Cucurbitaceae family. By inhibiting this enzyme, the absorption of food substances in the intestine is prevented, resulting in an anti-obesity effect. The hydroethanolic extract of *C. maxima, L. acutangula*, *M. dioica*, and *T. dioica* fruit was used to perform the *in-vitro* pancreatic lipase inhibition assay. It was found that *M. dioica* had the strongest inhibitory activity with a value of 120.28 ± 0.228 µg/mL. The IC_{50} values for the remaining extracts were 133.79 ± 0.175 µg/mL for *C. maxima*, 159.92 ± 0.253 µg/mL for *L. acutangula*, and 142.46 ± 0.360 µg/mL for *T. dioica*. The positive control, orlistat, demonstrated inhibitory action with an IC_{50} value of 90.34 ± 0.085 µg/mL. Through the use of One-way ANOVA with Dunnett's multiple comparisons at a significance level of $p < 0.0001$, it was shown that *M. dioica* fruit extract exhibited significant inhibitory capabilities against all of the aforementioned enzymes. This extract has the potential to be beneficial in mitigating the risks associated with metabolic disorders. In light of this reason, *M. dioica* has been chosen for further assessment of adaptogenic activity in *in-vivo* models.

Chapter 6, focuses on evaluating the adaptogenic activity of *M. dioica* fruit extract as the extract has been found to possess strong antioxidant characteristics, in addition to the ability to inhibit α-amylase, α-glucosidase, carbonic anhydrase, and pancreatic lipase enzymes in *in-vitro* manner. An acute toxicity study was conducted to assess the safety profile of the test sample. The selected fruit extract was determined to be non-toxic in acute oral toxicity testing, even at a level of 2000 mg/kg. Plant adaptogens mitigate the reactivity of the host defence system and attenuate the harmful effects of different stressors by increasing the levels of baseline level mediators implicated in the stress response. In order to assess the adaptogenic potential of *M. dioica* extract at various doses, several *in-vivo* experiments were conducted on Swiss albino mice including forced swimming endurance, anoxic stress tolerance, tail suspension, and elevated plus maze test. In these experiments, *M. dioica* was administered at doses of 100, 200, and 400 mg/Kg, whereas Diazepam was utilised as a positive control at a dose of 2mg/Kg. In FST model, observations have shown that mice treated with *M. dioica* extracts have a longer swimming time and improved physical performance compared to the untreated or control group. The adaptogenic effect in the FST was found in a dose-dependent approach. More precisely, a dose of 100mg/kg of *M. dioica*

resulted in a swimming survival time of 139.16 ± 1.17 secs, while the other two doses resulted in swimming survival times of 202.39 ± 1.73 secs and 215.05 ± 1.54 secs, respectively. The study on anoxic stress tolerance found that when oxygen levels were reduced in the hermetic container, convulsions occurred. However, when the animals were treated with *M. dioica* extract beforehand, their stress tolerance significantly improved. This confirms that the extracts have adaptogenic properties and are effective antioxidants that can scavenge free radicals. The control group has a stress tolerance time of 19.54 ± 0.47 mins, while the three doses of *M. dioica* plus diazepam showed stress tolerance times of 27.11 ± 0.36, 35.31 ± 0.32, 39.48 ± 0.28, and 53.47 ± 0.37 mins, respectively. The *M. dioica* extract demonstrated a reduction in immobility time in TST models, indicating enhanced physical endurance in animals and a stress management characteristic. When compared to the positive control diazepam, the doses of *M. dioica* at 200 and 400 mg/Kg resulted in a considerable reduction in immobility time, with times of 176.81 ± 0.19 and 141.84 ± 0.25 secs, respectively. The findings of the EPM test indicated that the extract of *M. dioica* led to an increase in both the number of entries and the amount of time spent by the experimental animals in the open arms of the maze. On the contrary, the animals that were already treated also exhibited a reduction in both the number of times they entered and the amount of time they spent in the closed arms of the maze. The overall distance traversed throughout the maze is also augmented following the application of extracts and the positive control. More precisely, *M. dioica* at doses of 200 and 400 mg/Kg exhibited notable adaptogenic effects when compared to the lower dose of *M. dioica*. The outcomes of the FST, Anoxic stress, TST, and EPM tests indicate that the mice who received prior treatment with *M. dioica* extracts shown resilience to stress in dose dependent manner. Extracts of *M. dioica* at dosages of 200 and 400 mg/Kg demonstrated notable adaptogenic effect, potentially attributed to their polyphenolic composition. When animals are stressed, their spleen contracts and releases a larger quantity of blood cells, including both red and white blood cells, into bloodstream. *M. dioica* reduces the count of RBCs and WBCs generated by stress. Stress leads to an elevation in blood glucose level, total cholesterol, and triglyceride content. The biochemical analysis revealed that the mice who received pretreatment exhibited a reduction in the biochemical markers associated with stress, indicating the adaptogenic action of the *M.*

dioica extract. Administration of *M. dioica* fruit extracts via oral route effectively restored various serum biochemical parameters, such as total protein, BuN, Creatinine, SGOT, SGPT, and SALP concentration, in animals that were subjected to stress. Adrenocorticotropic hormone is released as a reaction to stress. This hormone then operates on the adrenal cortex to encourage the production and release of cortisol, a stress hormone. Elevated levels of plasma cortisol impact the release of stored fat and glucose reserves, hence increasing the likelihood of developing metabolic disorders. The extract of *M. dioica* effectively reduces the enhanced levels of cortisol generated by stress. Oxidative stress plays a crucial role in the progression and development of neurological disorders, especially those associated to stress. It causes damage between cells by accumulating oxidation products and increasing metabolic dysfunctions. The decrease in TBARS content and an upsurge in the brain activities of CAT, GSH, and SOD, leading to enhanced tissue antioxidant activity. Treatment with *M. dioica* fruit extract can modulate the levels of tissue antioxidant markers and defend the brain tissue against pathological impairments caused by oxidative stress.

7.2 Conclusion

The Cucurbitaceae family include diverse plants that can be both consumed as food and also used for medicinal purposes. The need to confirm the traditional claims of several Cucurbitaceae plants regarding their quality, safety, and effectiveness arises due to the reports highlighting their ethnopharmacological significance. Stress is the physiological response of the body to a stressor, which can be either actual or imagined. Acute stressors have a short-term impact on an organism, while chronic stressors have a longer-term effect as it causes the body and immune system to become depleted and operate inadequately, leading in decompensation. The outcome can present itself as evident ailments such as ulcers, depression, diabetes, and gastrointestinal issues, as well as cardiovascular difficulties and other psychological disorders. Adaptogens are compounds that aid organisms in adjusting to adverse and challenging settings, encompassing physical, chemical, biological, and mental circumstances. Certain pioneering researchers in this field have established precise criteria that must be met in order to be classified as adaptogens. These criteria include

the ability to generate a non-specific response, meaning an increase in resistance to various physical, chemical, or biological stresses.

This thesis focuses on evaluating the adaptogenic efficiency, metabolite profile using LC-MS, and marker analysis using HPTLC and HPLC of selected fruit extract from the Cucurbitaceae plant family, based on their ethnopharmacological significance. The study aims to assess the antioxidant activity and inhibition of enzymes such as α-amylase, α-glucosidase, carbonic anhydrase, and pancreatic lipase in *in-vitro* approaches. In addition, the extracts were also assessed for the determination of the overall phenolic and flavonoid content. The adaptogenic activity of the specified plant extract from the Cucurbitaceae plant family was assessed using tests for forced swimming endurance, anoxic stress tolerance, tail suspension, and elevated plus maze. Ensuring quality control of food plants relies substantially on marker analysis and standardisation for quality evaluation. The plant extracts from *Cucurbita maxima, Luffa acutangula, Momordica dioica* and *Trichosanthes dioica* fruit showed the presence of polyphenolic substances. The LC-Q-TOF-MS analysis successfully detected a comprehensive range of phytochemicals in several species of the plant being investigated. This work illustrates the potential of metabolic profiling methods to evaluate characteristics of various fruit kinds, so proposing a novel approach to the creation of functional foods or the identification of medications.

The HPTLC and RP-HPLC analysis revealed variations in the chlorogenic acid composition among the chosen plant extracts. The *in-vitro* study on antioxidant and enzymes inhibition demonstrated that *Momordica dioica* had strong antioxidant properties and the ability to hinder enzymes. This suggests that it has the potential to reduce the risk of developing metabolic disorders caused by oxidative stress. The *in-vivo* investigation showed significant adaptogenic activity in a dose-dependent manner in Swiss albino mice. Higher doses of *M. dioica* extract had a promising impact on haematological parameters, blood glucose, lipid profile, as well as enzymatic liver and kidney biochemical markers in stress-induced experimental animals. The extract also shown a significant decrease in the elevated corticosterone levels resulting from stress. *M. dioica* administered at doses of 200 and 400 mg/Kg have demonstrated a considerable ability to prevent stress-induced alterations. Both doses of *M. dioica*, 200

mg/Kg and 400 mg/Kg, have the ability to exhibit antioxidant activity in brain tissue by reducing the quantity of TBARS and increasing the levels of SOD, GSH, and CAT in the brain tissue. These first findings show promise, and additional research on different doses would be beneficial in providing evidence for the drug's adaptogenic effects. Polyphenolic compounds are crucial for stabilising lipid oxidation and are linked to antioxidant action. Phenolic and flavonoids have been found to exhibit a range of biological actions, including adaptogenic activity. The observed adaptogenic activity of the extracts may be ascribed to the synergistic actions of various phytocompounds. The study's evaluation of the adaptogenic potential of the extract clearly demonstrates that it enhances physical endurance and overall performance in animals, while also exhibiting considerable anti-stress activity.

The current study focuses on the quality, safety, and efficacy of plants belonging to the Cucurbitaceae family, which are commonly consumed as food in the Indian subcontinent. The utilisation of metabolomics analysis was beneficial in identifying the functional constituents for enhancing the therapeutic efficacy of Cucurbitaceae family food plants. In addition, the findings will compel the researchers to further investigate the synergistic effects of the identified metabolites found in the selected plants. Therefore, the current study using Cucurbitaceae food plants confirm the therapeutic benefits of various conventional medicinal uses. This work will contribute to the future development of the notion of using food as medicine to create "new generation therapeutics" that may effectively address the growing problem of stress-related metabolic disorders.

7.3 Publications and Presentations

7.3.1 List of Publication (s)

- **Singha S**, Biswas S, Das Gupta B, Kar A, Mukherjee P K, 2020. Standardization of some plants of the Cucurbitaceae family by a validated high-performance thin-layer chromatography method. JPC-Planar Chromatography – Modern TLC, 33(5), 463-472.

- **Singha S**, Das Gupta B, Sarkar A, Jana S, Bharadwaj P K, Sharma N, 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.

- Mukherjee P K, **Singha S**, Kar A, Chanda J, Banerjee S, Dasgupta B, Haldar P K, Sharma N, 2022. Therapeutic importance of Cucurbitaceae: A medicinally important family. Journal of Ethnopharmacology, 282, 114599. doi: 10.1016/j.jep.2021.114599.

7.3.2 List of Presentations in National and International Conference

- **Singha S**, Dasgupta B, Biswas S, Mukherjee P K. *In-vitro* α-amylase enzyme potential of some food plants belongs to Cucurbitaceae family. 6th Convention & National seminar on “Translational Research of Traditionally used Indian Medicinal Plants with special reference to “*Tinospora cordifolia”*: Jadavpur University, Kolkata, India during September 07-08, 2019.

- **Singha S**, Dasgupta B, Biswas S, Das B, Jana S, Kar A, Mukherjee P K. Marker profiling and *in-vitro* α-glucosidase & α-amylase inhibitory potential of *Cucurbita maxima* fruits – A food plant. 7th Convention & International

symposium on “Combating COVID-19 – Ethnopharmacology & Traditional Food and Medicine”: during December 17-19, 2020.

- **Singha S**, Dasgupta B, Jana S, Debnath P, Kar A, Mukherjee P K. Metabolite analysis and *in-vitro* α-glucosidase enzyme inhibitory potential of *Trichosanthes dioica* Roxb. fruits. 8th Convention & National Seminar on “Ethnopharmacology for wellness: Tradition to Transition”: CSIR-IICB, Kolkata, India, on December 10, 2021.

- **Singha S**, Dasgupta B, Jana S, Gayen S, Chowdhury S, Kar A, Haldar P K, Mandal S C, Mukherjee P K. Assessment of *In-vivo* Adaptogenic Activity of *Momordica dioica* Fruit Extract. 22nd International Congress of International Society for Ethnopharmacology (ISE) & 10th International Congress of the Society for Ethnopharmacology (SFE), India (ISE-SFEC, 2023): Imphal, Manipur, India during February 24- 26, 2023.

Chapter 8

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ORIGINAL RESEARCH PAPER

Standardization of some plants of the *Cucurbitaceae* family by a validated high-performance thin-layer chromatography method

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Abstract

Medicinal plants of the *Cucurbitaceae* family are widely consumed as foods in human diet. In the present study, a simple, rapid, quantitative, and validated high-performance thin-layer chromatographic (HPTLC) method has been developed for the estimation of chlorogenic acid (CA) in the hydroalcoholic fruit extracts of *Luffa acutangula* (sponge gourd), *Sechium edule* (chayote squash), *Trichosanthes cucumerina* (snake gourd) and *Trichosanthes dioica* (pointed gourd) belonging to the *Cucurbitaceae* family. Densitometric analysis of CA was carried out in the absorbance mode at 254 nm. The method gave spot at $R_F = 0.55 \pm 0.04$, corresponding to CA in different samples. The limit of detection and limit of quantification per spot were confirmed with the mobile phase ethyl acetate−chloroform−formic acid (6:4:0.5, *V*/*V*). Linear regression analysis data for the calibration plot for CA showed a good linear relationship with a correlation coefficient (*r*) of 0.9997 in the concentration range of 200–1000 ng per spot. The method was validated for sensitivity, linearity, accuracy, precision and specificity as per the international conference on harmonization guidelines. The proposed validated HPTLC method provides a novel approach for the quality control and standardization of some selected medicinal food plants of the *Cucurbitaceae* family.

Keywords Cucurbitaceae · Chlorogenic acid · Medicinal food plant · Standardization · High-performance thin-layer chromatography (HPTLC) · Validation

List of abbreviations

CA	Chlorogenic acid
ICH	International Conference on Harmonization
r	Correlation coefficient
R_F	Retardation factor
µm	Micrometre
mg	Milligram
mL	Millilitre
µL	Microlitre
ng	Nanogram
HPTLC	High-performance thin-layer chromatography
LOD	Limit of detection
LOQ	Limit of quantification
σ	Standard deviation
S	Slope
RSD	Relative standard deviation
%	Percentage
LAHE	*Luffa acutangula* hydroalcoholic extract
SEHE	*Sechium edule* hydroalcoholic extract
TCHE	*Tricosanthes cucumerina* hydroalcoholic extract
TDHE	*Tricosanthes dioica* hydroalcoholic extract
LQC	Low quality control
MQC	Medium quality control
HQC	High quality control

1 Introduction

Cucurbitaceae is the largest family of vegetable and edible crops, which includes approximately 125 genera and 960 species. The plants belonging to this family are further classified into two major subfamilies, *Cucurbitoideae* and *Zanonioideae,* based on their morphological, cytological and floral characteristics [1]. Various parts including fruits, seeds, stems, roots, flowers and leaves are very popular for their uses in culinary purposes from the ancient times. The

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ancient literature, including Ayurveda, describes several therapeutic importance of this plant family for the management of human health [2]. *Trichosanthes cucumerina* (snake gourd) is widely distributed in south eastern Asian countries. *Trichosanthes cucumerina* is known to be a great source of fiber, carbohydrates, vitamins, etc. and also of phosphorus, sodium, magnesium and zinc. The snake gourd also has carotenoids, flavonoids, lycopene, β-carotene and phenolic compounds such as chlorogenic acid, ferulic acid, coumaric acid, caffeic acid, etc. [3]. The whole plant, including roots, leaves, fruits and also seeds, possesses potent pharmacological activity. The roots are used for the treatment of boils, headache, bronchitis, etc. [2]. The fruit extract exhibits potent gastroprotective activity in Wistar rats [4], and various parts of *T. cucumerina* show antipyretic, antidiabetic, immunomodulatory, antibacterial activity and cytotoxicity [5]. *Luffa acutangula*, also known as ridge gourd, contains several bioactive phytomolecules, such as luffangulin, luffaculin, saponin glycosides, terpenoids, gallic acid, chlorogenic acid, *p*-coumaric acid, ferulic acid, protocatechuic acid, acutoside C, acutoside D, unsaturated aliphatic alcohols, carboxylic acids, fatty acids and their esters [6–8]. *Luffa acutangula* fruit possesses in vitro carbonic anhydrase inhibitory activity [6] and also shows free radical scavenging and immunomodulatory activity [9]. Ridge gourd also exhibited hepatoprotective activity [10], antidiabetic, antimicrobial, anti-inflammatory activity and cytotoxicity [11]. *Sechium edule,* also known as chayote squash, is a climbing perennial vine mainly found in the hilly areas of India. This plant consists of diverse bioactive phytochemical compounds including cucurbitacin B, cucurbitacin I, carotenoids, gallic acid, chlorogenic acid, vanillic acid, *p*-hydroxybenzoic acid, vitexin, luteolin 7-O-rutinoside, naringenin, sterols, amino acids, 3-octadecenoic acid, trilinolenin, α-linolenic acid [12]. The fruit extract of *S. edule* shows induction of apoptosis in leukemic cell line [13]; antiepileptic and CNS depressant activity. [14]. The fruits of *S. edule* exhibit cardioprotective activity [15], antioxidant and antidiabetic activity [16]. *Trichosanthes dioica,* commonly known as pointed gourd, contains several potent bioactive phytomolecules including trichosanthin, lectin, euphol, α-amyrin, β-amyrin, butyrospermol, lupeol, taraxerol, betulin, karounidiol, terpenoids [17], sterols, steroidal saponin, tannin, flavonoids and also phenolic compounds [18]. Various parts of this plant possess antihyperglycemic, antidiabetic [19], antihyperlipidemic [20], antibacterial [21] and also immunomodulatory activity [22].

High-performance thin-layer chromatography (HPTLC) is a useful, uniform and rapid analytical technique for the standardization and quality evaluation of phytoconstituents present in various parts of the plants [23]. In the current scenario, quality evaluation of herbs and herbal products with proper quality control parameters together with the qualitative as well as quantitative estimation of bioactive phytoconstituent is utmost essential for maintaining the quality of botanicals [24]. Several scientific studies on the standardization and marker analysis of several medicinal plants by using HPTLC−densitometry have been performed in our laboratory and reported [23, 24]. Different scientific literature are lacking the report on standardization of the selected medicinal food plants by HPTLC−densitometry analysis using chlorogenic acid as standard phytomarker. The main focus of this study is to develop a specific and accurate validated method for the quantification of chlorogenic acid present in the fruit extract of *L. acutangula, S. edule, T. cucumerina* and *T. dioica* by HPTLC−densitometry analysis method. The proposed HPTLC method has been validated by different validation parameters according to the International Conference on Harmonization (ICH) guidelines [25].

2 Experimental

2.1 Chemicals and reagents

Silica gel 60 F_{254} HPTLC plates were purchased from Merck (Mumbai, India). Standard chlorogenic acid (> 98% purity), gallic acid (95% purity) and quercetin were purchased from Sisco Research Laboratories Pvt. Ltd. (Mumbai, India). The micropipettes and microtips were procured from Eppendorf (Hamburg, Germany), and Accupipet were procured from Tarsons Products Pvt. Ltd. (Kolkata, India). A 100 µL Hamilton syringe (Bonaduz, Switzerland) was used for sample application in the HPTLC system. Sodium bicarbonate, sodium nitrite, aluminum chloride, sodium hydroxide, ethyl acetate, chloroform, formic acid, methanol were purchased from Finar Limited (Ahmedabad, India).

2.2 Instrumentation

Rotary vacuum evaporator and lyophiliser were procured from IKA (Mumbai, India) and Instrumentation India (Kolkata, India), respectively, and they were used in the process of extraction. Spectramax iD3 multimode microplate reader (Molecular Devices, San José, CA, USA) was used for the determination of the total phenolic and total flavonoid content for both sample and standard. Linomat V (Automatic sample applicator), Reprostar 3 (Photo-documentation chamber), TLC Scanner 3 (Densitometric scanner) and twin-trough development chamber (all from CAMAG, Muttenz, Switzerland) were used for HPTLC analysis. Syringe filters (NYL 0.45 µm) were employed for the filtration of the sample and standard solution.

2.3 Collection of plant materials

The fruits of the selected plants were collected from a local market, Kolkata and authenticated. Voucher specimens of *L. acutangula* (ridge gourd), *S. edule* (chayote squash), *T. cucumerina* (snake gourd) and *T. dioica* (pointed gourd) fruits were prepared with specimen numbers SNPS/JU/2019/1097, SNPS/JU/2019/1098, SNPS/JU/2019/1099 and SNPS/JU/2019/1100, respectively. The voucher specimens were retained in the School of Natural Product Studies, Jadavpur University, Kolkata, India, for further study.

2.4 Extraction of the plant materials

The fruits of *L. acutangula, S. edule, T. cucumerina* and *T. dioica* were cut into small pieces and shade dried. The dried material was subjected to grind into coarse powder followed by the extraction by cold maceration method with methanol and water (hydroalcohol). Then, the filtrates were collected and concentrated in a rotary vacuum evaporator (IKA) at a temperature of 40−45 °C. After this, the extracts were lyophilized and stored in air-tight containers for further use. The % (*w*/*w*) yields for the individual plants have been calculated and are presented in Table 1.

2.5 Determination of total phenolic content (TPC)

The total phenolic contents of the selected plant extracts were determined by using Folin−Ciocalteu's phenol reagent. This blue-colored complex shows a λmax of 765 nm. Aliquots of 18 µL (1 mg/mL) of the plant extracts were taken with 90 µL of 10% Folin−Ciocalteu's reagent with 90 µL of 7.5% sodium bicarbonate buffer and incubated for 45 min at 45 °C. Gallic acid standard and blank control with methanol were prepared using the same method and absorbance was measured at 765 nm [26].

2.6 Determination of the total flavonoid contents (TFC) of the plants

The total flavonoid content of the extract was estimated colorimetrically at 510 nm. The reaction involves nitration of the catechol moiety of a flavonoid at three or four unsubstituted or sterically unhindered positions. An aliquot of 100 µL of the plant extract was taken and mixed with 10 µL of sodium nitrite, 10 µL of aluminum chloride and 50 µL of sodium hydroxide. Rutin standard and a blank control with methanol were prepared by the same method [27].

Table 1 Percentage yield and chlorogenic acid contents (as estimated in the HPTLC analysis) of the plants under study

Extract	Yield (% *w*/*w*)	Chlorogenic acid content (% *w*/*w*)
LAHE	10.64	1.54
SEHE	12.70	1.50
TCHE	18.90	0.16
TDHE	09.00	1.16

2.7 Quantitative analysis by HPTLC

2.7.1 Preparation of standard and sample solutions

In case of the HPTLC analysis of the selected medicinal food plants, the standard CA (0.1 mg) was weighed in a microcentrifuge tube (Eppendorf) and dissolved in 1 mL methanol to prepare a 0.1 mg/mL solution. Aliquots of 10 mg of each of the plant extracts were weighed and dissolved in 1 mL methanol to prepare 10 mg/mL extract solutions. Each of the standard and extract solutions was vortexed until dissolved completely and filtered through a 0.45 µm syringe filter (Millipore, Burlington, MA, USA). The linearity of the response prepared standards was determined using a calibration curve established with five different volumes of the standard (CA, 1 mg/mL) in the range of 2−10 µL. The solutions for HPTLC analyses were vortexed, filtered and sonicated thoroughly until there was no suspended particle visible in the solutions [28].

2.7.2 Chromatographic conditions

Standard 1 mg/mL chlorogenic acid solution was aspirated into a 100 µL syringe (Hamilton). Then five different volumes (2, 4, 6, 8 and 10 µL) were applied band-wise in five different tracks using CAMAG Linomat V sample applicator on five separate 10 cm × 10 cm silica gel 60 F_{254} plates. After that, three different volumes (10, 12 and 14 µL) of the plant extract solutions (10 mg/mL) were applied in three separate tracks. The plates were dried under warm air (40−45 °C) and were developed in a CAMAG twin-trough chamber using the solvent system ethyl acetate−chloroform−formic acid (6:4:0.5, *V*/*V*). Compounds were detected and scanned at 254 nm wavelength.

2.7.3 Method validation

Validation of the HPTLC method was performed as recommended by the International Conference on Harmonization (ICH) guidelines [25] defining linearity, specificity, limits of quantification and detection, precision, accuracy and robustness.

2.7.3.1 Specificity The results which were obtained from standardization were checked in terms of specificity according to the ICH guidelines to minimize errors due to the

contamination of the sample. The specificity of the method was determined by analyzing the standard and test samples. The purity of peaks was checked using multivariate analysis by comparison of retention times and peak area of standard compound with extract and fractions [27].

2.7.3.2 Limit of detection (LOD) and limit of quantification (LOQ) The LOD and LOQ were calculated by the method based on standard deviation (σ) and the slope (*S*) of the calibration plot, using the formula LOD = 3:1 σ/S and LOQ = 10:1 σ/S [25], where σ = standard deviation of the response from a number of blank run and *S* = slope of calibration plot.

2.7.3.3 Accuracy Accuracy of the method was determined by percentage recovery of markers in the plant extract and fractions. The method was studied by performing standard addition technique, and it is expressed in terms of percentage relative standard deviations (%RSD) from mean recovery of the theoretical concentrations [28]. Prior to application on plate, the tests were spiked with three different known amounts of standard compounds in triplicates. Analyses were done under the ambient condition to calculate the overall average recovery. The mean amounts of the markers achieved were taken as real values to calculate the spike recoveries.

2.7.3.4 Precision The precision of the method was assessed by applying six replicates at three different concentrations for the reference compound, the extract and fractions. The values were represented as %RSD of intra-day and inter-day runs. The mean amount and RSD values were calculated. The intra-day precision of the assay was determined by analyzing three concentrations in 1 day. Also, the intra-day precision was determined over three successive days by analyzing the same concentrations. Applications were done in six replicates to determine the repeatability of the process.

2.7.3.5 Robustness The robustness of the proposed method was investigated by the analysis of samples under different experimental conditions. The test solutions were analyzed with variation of flow rate, mobile phase composition, detection wavelength and column temperature and using different columns of the same configuration to determine their influence on the retention time.

3 Result and discussion

The percentage yield of *L. acutangula* hydroalcoholic extract (LAHE), *S. edule* hydroalcoholic extract (SEHE), *T. cucumerina* hydroalcoholic extract (TCHE) and *T. dioica* hydroalcoholic extract (TDHE) are presented in

Table 2 Total phenolic content and total flavonoid content of the selected plants

Selected plants	Total phenolic content (GAE, mg/g)	Total flavonoid content (RE, mg/g)
Luffa acutangula	20.0	0.875
Sechium edule	9.2	4.050
Trichosanthes cucumerina	6.5	3.075
Trichosanthes dioica	8.2	4.867

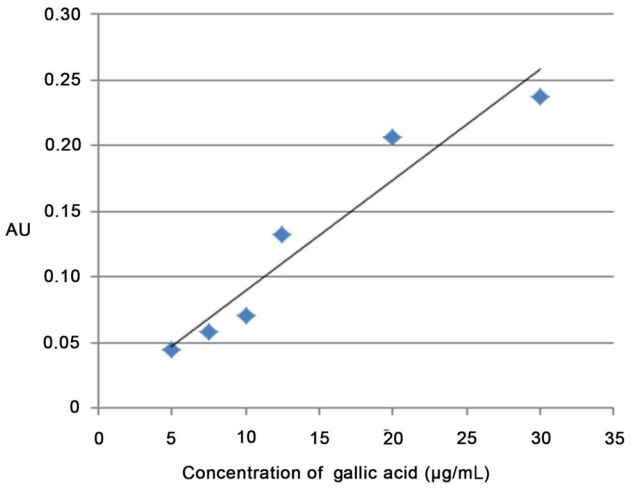

Fig. 1 Calibration curve of gallic acid

Table 1. Results of the total phenolics contents exhibited the presence of phenolic compound of *L. acutangula, S. edule, T. cucumerina* and *T. dioica* as 20.0 mg/g (*w*/*w*), 9.2 mg/g (*w*/*w*), 6.5 mg/g (*w*/*w*) and 8.2 mg/g (*w*/*w*), respectively (Table 2). The total flavonoid content assay suggested the presence of flavonoids of *L. acutangula, S. edule, T. cucumerina* and *T. dioica* as 0.875 mg/g (*w*/*w*), 4.050 mg/g (*w*/*w*), 3.075 mg/g (*w*/*w*) and 4.867 mg/g (*w*/*w*), respectively (Table 2). The calibration curves of gallic acid and quercetin are represented in graphical manner in Figs. 1 and 2, respectively. The standard curve of chlorogenic acid was constructed and is represented in Fig. 3a. The content of chlorogenic acid in selected medicinal food plants was determined by the HPTLC method, and the R_F value was found to be 0.55 ± 0.04 in all the samples. The contents of the chlorogenic acid in the hydroalcoholic extracts of the plants were determined using calibration curves by plotting the mean peak area (*y*-axis) against the concentrations (*x*-axis). Separate calibration curves of chlorogenic acid for the four plant extracts were plotted. The regression equations and correlation coefficients (*r*) are presented in Table 3. The HPTLC densitogram of the standard chlorogenic acid and all the extracts are shown in Fig. 3a–e. The plate photos

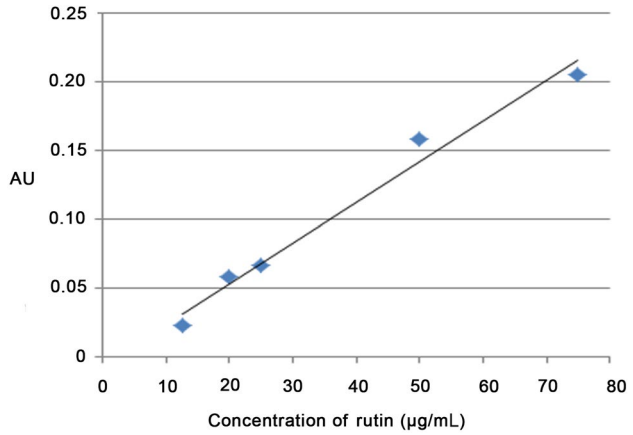

Fig. 2 Calibration curve of rutin

under 254 nm are presented in Fig. 4a–d. In the present study, it has been found that the chlorogenic acid content varies among different plants of the *Cucurbitaceae* family. The maximum content has been found in *L. acutangula* (1.54–1.74% *w*/*w*), and the minimum content has been found to be in *T. cucumerina* (0.16–0.52% *w*/*w*).

The linearity of the calibration plot was found to be 200–1000 ng/spot for all the selected plants. The correlation coefficient was found to be > 0.99 from the calibration curve. This data confirm the closeness of the data to the line of best fit. Intra-day and inter-day assay accuracy and precision for each phytomarker were determined at LQC (low quality control), MQC (medium quality control) and HQC (high quality control). Each of the experiments was repeated six times, and % recovery values were found in the range of 99.68–99.93%. The low %RSD values in the range of 0.076–2.63% indicated the high accuracy of method. The results of the recovery study are presented in Table 4. To determine the precision of the method, standard chlorogenic acid was used at two different levels: 200 µg and 400 µg. The average area and the R_F values for the same were determined in the case of both inter-day and intra-day precision. The results of the inter-day and intra-day precision study are presented in Table 5. The six repetitions for each type of determination yielded a %RSD 0.18–0.28% (intra-day) and 0.15–0.26% (inter-day) which is very low and is an indicator of the precision of the method. The R_F values exhibited RSD values in the ranges of 0.22–0.26% (intra-day) and 0.19–0.26% (inter-day). The LOD was found to vary from 623.20 ng per spot to 622.12 ng per spot and the LOQ was found to vary from 1950.12 ng per spot to 1951.32 ng per spot. The method also passed the robustness test. These results indicated that the method is quite robust, accurate, precise and specific for the HPTLC analysis of CA.

4 Conclusion

The medicinal food plants of the *Cucurbitaceae* family are highly used as effective components of regular diet by many people in India and have been reported for their therapeutic potential. These plants are regularly consumed as vegetables and possess large nutritional as well as ethno veterinary values. Chlorogenic acid content also varies in the different species of the *Cucurbitaceae* family. HPTLC fingerprints are implemented as a potential cost-effective, simple and highly selective tool that can ensure both quality and quantity of the constituents from medicinal plants. A simple and rapid HPTLC–densitometric method for the quantification and isolation of this compound in the extract of the plant has been developed and validated. This method may be useful for the development of quality control and marker analysis profile of these medicinal plants, which may be useful for ensuring the quality control of plants belonging to the *Cucurbitaceae* family as raw material for food and pharmaceutical preparation.

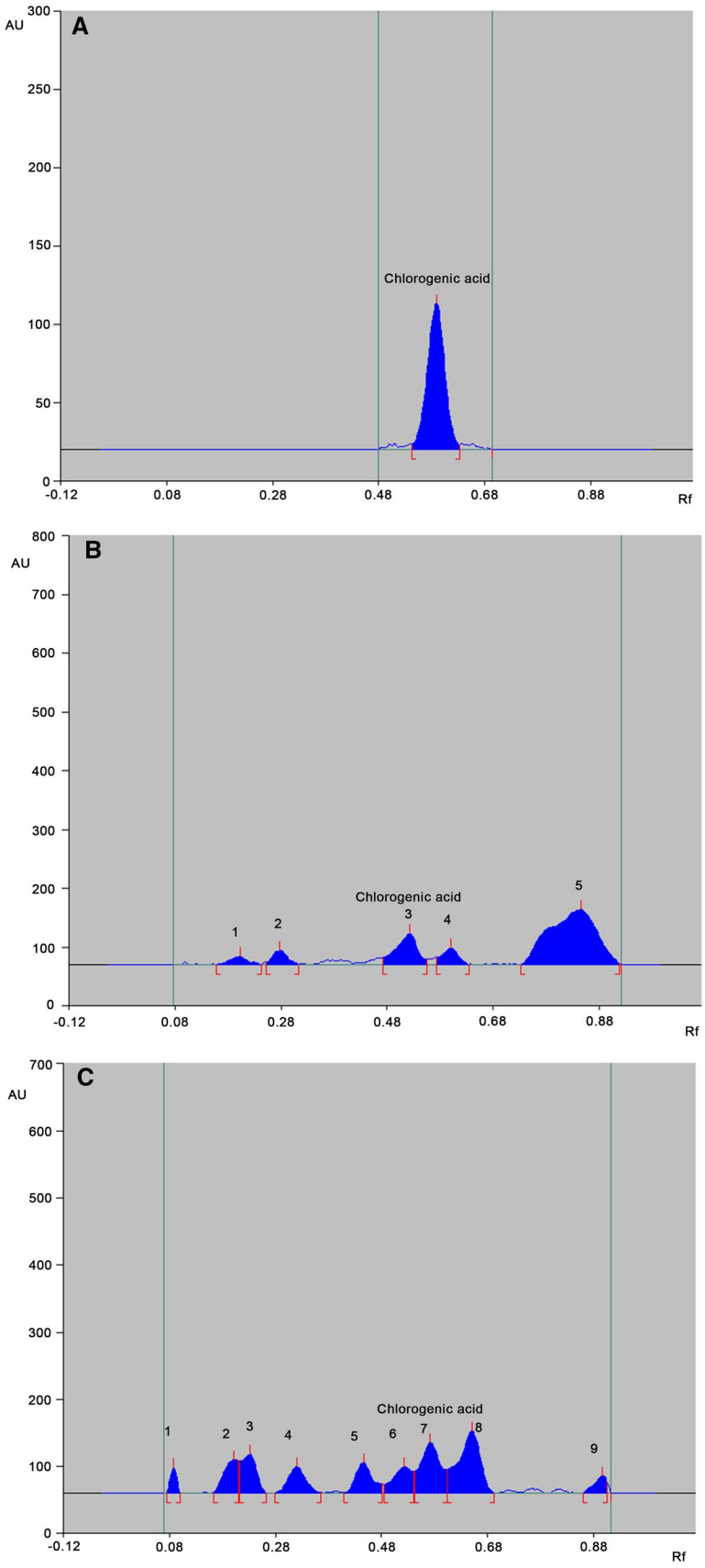

Fig. 3 HPTLC densitograms of chlorogenic acid and the plant extracts. **A** HPTLC chromatogram of standard chlorogenic acid. **B** HPTLC chromatogram of LAHE. **C** HPTLC chromatogram of SEHE. **D** HPTLC chromatogram of TCHE. **E** HPTLC chromatogram of TDHE

Fig. 3 (continued)

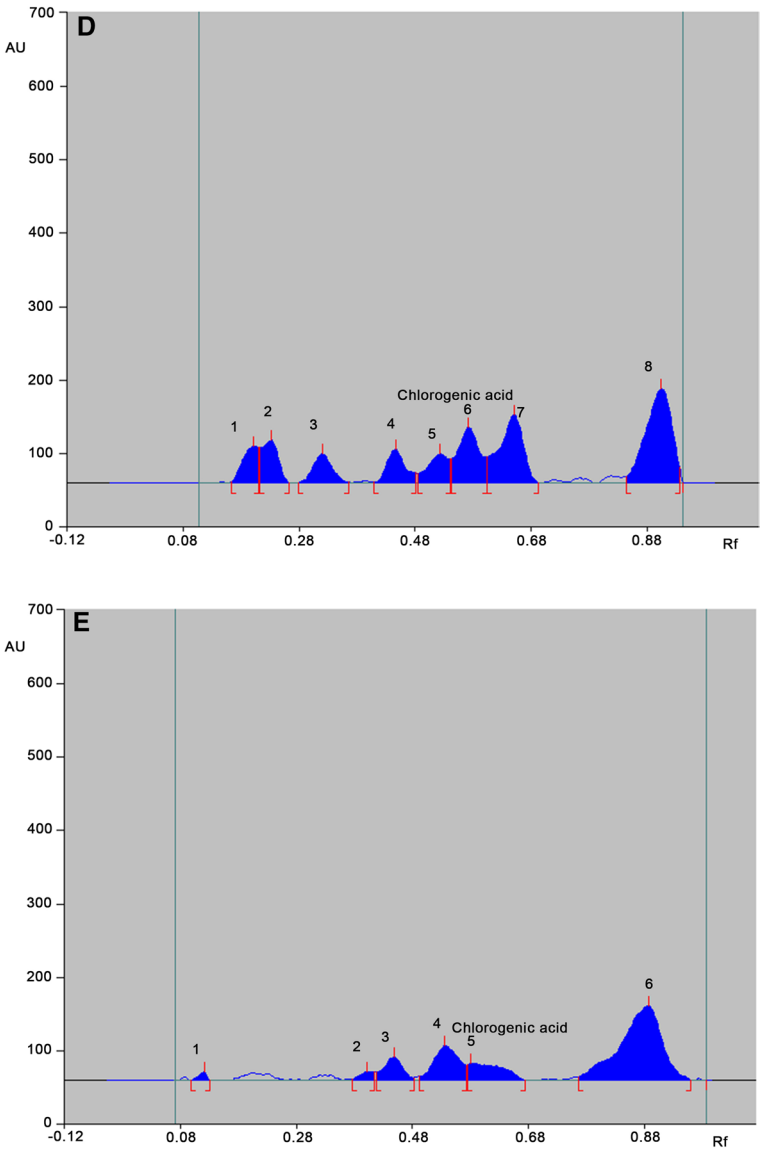

Table 3 Regression equations and correlation coefficient values (r) for each plant

Extract	Regression equation	Correlation coefficient (r)
LAHE	$y = 371.1 + 737.3x$	0.99609
SEHE	$y = 998.4 + 816.3x$	0.99583
TCHE	$y = 432.1 + 1691x$	0.99765
TDHE	$y = 887.9 + 1113x$	0.99739

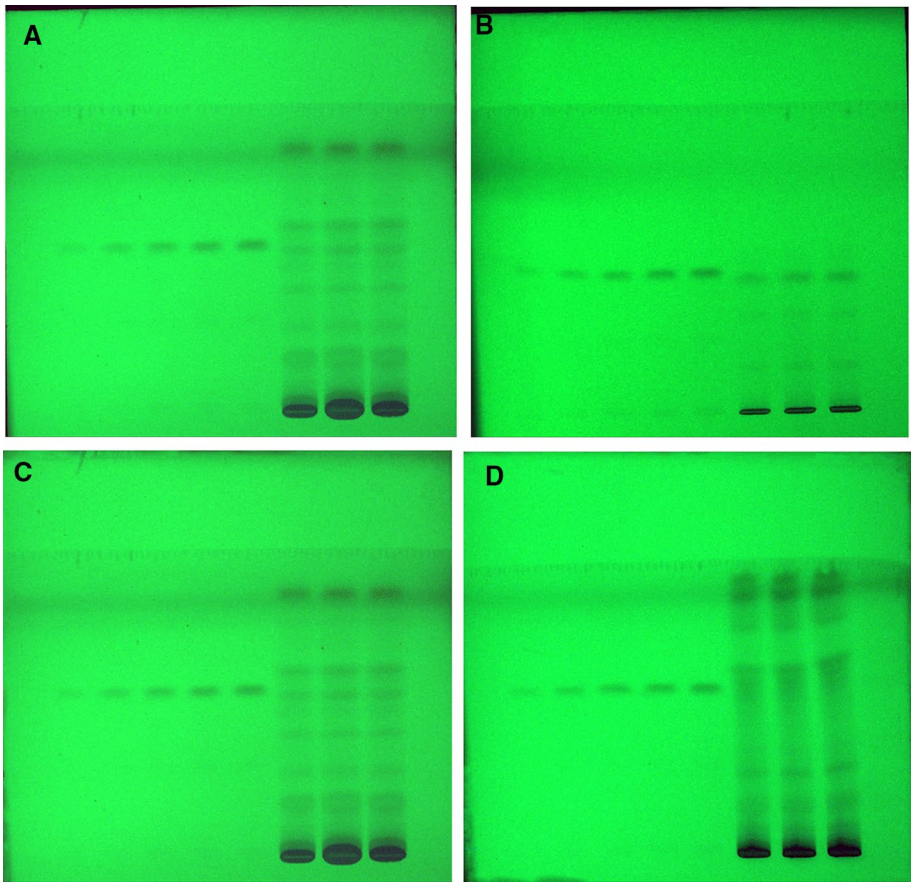

Fig. 4 Photo-documentation at 254 nm of the studied plant extracts. **A** Photo-documentation of LAHE. **B** Photo-documentation of SEHE. **C** Photo-documentation of TCHE. **D** Photo-documentation of TDHE

Table 4 Recovery studies of the selected plants

Biomarker	Amount added (ng)	Theoretically expected amount (ng/spot)	Experimentally obtained amount (ng/spot)	%RSD	Percentage recovery
LAHE					
Chlorogenic acid	0	1562.00	1560.83	0.08	99.93
	200	1762.00	1758.62	0.14	99.81
	400	1962.00	1959.67	0.12	99.88
	600	2162.00	2155.06	0.23	99.68
SEHE					
Chlorogenic acid	0	1500.00	1500.83	0.08	99.93
	200	1700.00	1700.62	0.14	99.81
	400	1900.00	1900.67	0.12	99.88
	600	2100.00	2100.06	0.23	99.68
TCHE					
Chlorogenic acid	0	160.00	160.83	0.08	99.93
	200	360.00	360.62	0.14	99.81
	400	560.00	560.67	0.12	99.88
	600	760.00	760.06	0.23	99.68
TDHE					
Chlorogenic acid	0	1100.00	1100.83	0.08	99.93
	200	1300.00	1300.62	0.14	99.81
	400	1500.00	1500.67	0.12	99.88
	600	1700.00	1700.06	0.23	99.68

Table 5 Intra-day and inter-day precision studies of the selected plants

	Response (area)		Response (area)	
	Mean	%RSD	Mean	%RSD
LAHE				
200	9494.69	0.28	9499.13	0.26
400	18,981.19	0.18	19,321.25	0.15
SEHE				
200	9466.68	0.25	9499.15	0.26
400	18,671.19	0.15	19,423.26	0.15
TCHE				
200	9398.69	0.24	9499.14	0.26
400	18,978.19	0.14	19,321.23	0.15
TDHE				
200	9467.69	0.27	9499.16	0.26
400	18,981.19	0.17	19,000.24	0.15

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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Chemo-profiling and exploring therapeutic potential of *Momordica dioica* Roxb. ex Willd. for managing metabolic related disorders: *In-vitro* studies, and docking based approach

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ABSTRACT

Ethnopharmacological relevance: Momordica dioica Roxb. ex Willd. (*M. dioica* Roxb.) a nutritious and therapeutic property rich crop of Cucurbitaceae plant family. In various folklore medicine including Ayurveda fruits are used to treat several metabolic related disorders i.e., hyperglycemia, hyperlipidemia, diabetes, obesity etc. Furthermore, traditionally it is used to treat fever, inflammation, ulcer, skin diseases, haemorrhoids, hypertension and also employed as cardioprotective, hepatoprotective, analgesic, diuretic.

Aim of the study: This study focuses to explore the therapeutic potential of *Momordica dioica* Roxb. ex Willd. through *in-vitro* and *in-silico* approach for managing hyperlipidemia, hyperglycemia and related metabolic disorders along with its phytochemical profiling for quality evaluation and validation of traditional claim.

Materials and methods: The present study was carried out on hydroalcohol extract of dried leaf and fruit of *Momordica dioica*. *In-vitro* antioxidant potential using DPPH and Nitric oxide scavenging assay along with *in-vitro* enzyme inhibitory potential against α-amylase, α-glucosidase, and pancreatic lipase enzymes was studied. The bioactive metabolites were identified from the most potent bioactive extract by analysis with LC-QTOF-MS and also studied their role to lessen the metabolic related disorder through *in-silico* approaches.

Results: The results confirmed that the fruit extract is more active to possess antioxidant and prominent enzyme inhibition potential compared to the leaf. Sixteen identified metabolites in *M. dioica* Roxb. fruits may be responsible for the therapeutic potential related to metabolic related disorder. The *in-silico* study of the identified phytomolecules against α-amylase, α-glucosidase and pancreatic lipase showed significant docking scores ranging from −9.8 to −5.5, −8.3 to −4.8 and −8.3 to −6 respectively.

Conclusion: The current study illustrated that *M. dioica* Roxb., a traditionally important plant is potential against metabolic related disorders. Phytocomponents present in the fruit extract may be responsible for antioxidant as well as the enzymes' inhibitory potential. Thus, fruits of *M. dioica* Roxb. will be useful as alternative therapeutics for treatment of hyperlipidemia, hyperglycemia and related metabolic disorders.

1. Introduction

Metabolic syndrome describes an assortment of risk factors for heart attacks, including diabetes, elevated fasting plasma glucose, abdominal obesity, hyperlipidemia etc. and it can be developed at any age (Ghosh, 2022; Booth et al., 2012). α-amylase, α-glucosidase and pancreatic lipase enzyme inhibitors can treat Diabetes mellitus (DM) type II and hyperlipidemia since it may lower blood glucose levels (post-prandial) after a meal and inhibits dietary triglyceride absorption in the intestine respectively (Gong et al., 2020; Chanda et al., 2019). Due to developments in the computational technique, the *in-silico* approach has been envisioned as a key instrument to boost the efficacy and efficiency of drug discovery research. Various quick, automated, and notably

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List of abbreviations

M. dioica	Momordica dioica Roxb. ex Wild
DM	Diabetes Mellitus
LC–QTOF–MS	Liquid chromatography–quadrupole time-of-flight mass spectrometry
TPC	Total phenolic content
TFC	Total flavonoid content
μL	Microlitre
STZ	Streptozotocin
mg	milligram
mL	millilitre
g	gram
DPPH	2,2-diphenyl-1-picrylhydrazyl
NO	Nitric oxide
MDL	M. dioica leaf extract
MDF	M. dioica fruit extract
GA	Gallic acid
QE	Quercetin
w/w	weight by weight
Na_2HPO_4	Disodium hydrogen phosphate
$NaHCO_3$	Sodium bicarbonate
S. cerevisiae	*Saccharomyces cerevisiae*
p-NPG	4-nitrophenyl-α-D-glucopyranoside
AG	α-glucosidase
AA	α-amylase
A. oryzae	Aspergillus oryzae
PL	Pancreatic Lipase
DNSA	Dinitrosalicylic acid
ACN	Acetonitrile
FC	Folin–Ciocalteu's Min- Minutes
°C	Degree centigrade
$NaNO_2$	Sodium nitrite
4MOU	4-methylumbelliferyl oleate
NaOH	Sodium hydroxide
NaCl	Sodium Chloride
$CaCl_2$	Calcium Chloride
$AlCl_3$	Aluminium chloride
U	Units
Q-TOF	Quadrupole Time-of-Flight
L. plantarum	Lactobacillus plantarum PDB Protein Data Bank
NF-κB	Nuclear factor kappa B
PPAR-γ	Peroxisome proliferator-activated receptor-gamma
HDL	High density lipoprotein
IRS-1	Insulin receptor substrate-1
ERK1/2	extracellular signal-regulated protein kinase
GPR40	G-protein coupled receptor 40
INS-1	Rat insulinoma
C/EBPα	CCAAT-enhancer binding protein α
PPARγ	Peroxisome proliferator-activated receptor γ
LCAT	Lecithin cholesterol acyl transferase
GLUT4	glucose transporter protein type-4
T. conophorum	Tetracarpidium conophorum

inexpensive bioinformatic technologies have been created. Tools like molecular docking systems can investigate macromolecule-ligand interactions and a potential method of validating *in-vitro* results with *in-silico* assessments (Paul et al., 2023).

A number of commercially accessible drugs are available as α-amylase (AA), α-glucosidase (AG) and pancreatic lipase (PL) enzyme inhibitors such as Acarbose inhibits both enzymes whereas Miglitol and Voglibose only inhibit AG enzyme and Orlistat is also a promising PL inhibitor. Plant-based medication should be capable of substituting the place of these synthetic pharmaceuticals due to their adverse effects on the gastrointestinal tract (Ghosh, 2022; Poovitha and Parani, 2016). Even though the Cucurbitaceae family is widely used for human consumption & nutrition, scientific validation of bioactive components is required (Mukherjee et al., 2022). *Momordica dioica* Roxb. ex Willd. (Spine gourd) is a member of the Momordiceae tribe of Cucurbitaceae family. In India, it is mainly cultivated in the Deccan plateau and Central India as it is mainly native to Indo-Malayan region. Globally it is distributed in South Africa, Bangladesh, China, Japan, Sri Lanka, Myanmar, Nepal, Pakistan, South East Asia, Polynesia and south America (Nagarani et al., 2014; Renner and Pandey, 2013). In Ayurveda, parts of *M. dioica* has been used to treat several ailments including *Madhumeha* (Diabetes), *Ashmari* (Urinary calculi), *Arsha* (Hemorrhoid), Svasa (Asthma), *Hikka* (Hiccup), Jvara (Fever), *Kamala* (Jaundice), *Kasa* (Respiratory Tract disorder), *Mutrakchakra* (Difficulty in Micturition), *Netraroga* (Eye diseases), *Sarpa Visa* (Venom infestation), *Raktarasa* (Bleeding piles), *Siraroga* (Disease related to head) etc (Anonymous, 2006). Traditionally fresh fruit juice of *M. dioica* used to treat hypertension as well as fruit cooked in small amount of oil used to reduce blood sugar level in diabetic patient (Kirtikar and Basu, 1999). Fresh juice in the morning in empty stomach can also be useful to treat diabetes; the juice is also a good remedy for inflammation and headache (Talukdar and Hossain, 2014). Decoction of various parts of *Momordica* species use to stabilize and lowering blood sugar level, body heat during parasitic infection by VhaVenda tribe of South Africa (Mokganya and Tshisikhawe, 2019).

M. dioica owns several phytochemicals such as momordicin, saponin glycosides, triterpenes of ursolic acid, hederagenin, momodicaursenol, 3β-o-benzoyl-11-oxo-ursolic acid, 3-o-benzoyl-6-oxo-ursolic acid, momodicaursenol. Methanolic fruit pulp extract of *M. dioica* possesses analgesic activity, nephroprotective activity against gentamicin (nephrotoxin), antiallergic activity against passive cutaneous anaphylaxis (Mukherjee et al., 2022; Talukdar and Hossain, 2014). Steroids and triterpenoids isolated from fruits of *M. dioica* found to possess antidiabetic potential in alloxan and STZ induced *in-vivo* models (Gupta et al., 2011; Reddy et al., 2005). Hydroalcohol extract of *M. dioica* fruit rind reported antidiabetic activity in STZ induced type-II DM rat models (Hassan et al., 2022). Insufficient scientific reports of *in-vitro* enzyme inhibition related to metabolic related disorders by *M. dioica* fruit have targeted our present study aimed to prospect inhibitory study of all the enzymes in *in-vitro* as well as molecular docking study along with antioxidant activity and identification of phytoconstituent through LC–QTOF–MS analysis.

2. Materials and methods

2.1. Chemicals and reagents

Methanol (analytical grade), Potassium sodium tartarate tetrahydrate, sodium nitroprusside, sulfanilic acid, napthylethylenediamine dihydrochloride, 4-methylumbelliferyl oleate, sodium citrate, NaCl, $CaCl_2$, NaH_2PO_4, Na_2HPO_4, $NaHCO_3$, $AlCl_3$, $NaNO_2$ were procured from Merck, Mumbai. From Sigma-Aldrich AG (from *S. cerevisiae*) type I (≥10 units/mg protein), LI (porcine pancreas Type II), *p*-NPG (purity ≥99%), AA from A. oryzae (30U/mg), DNSA, GA, QE, ACN, ammonium acetate, Acarbose (extrapure 95%), Starch (soluble extra pure), Orlistat was purchased.

2.2. Collection and extraction

In September 2021, the fruits and leaves were obtained from market

of Kolkata, West Bengal, India and further authenticated by a field botanist. The herbarium of *M. dioica* (voucher specimen number SNPS-JU/2019/1024) was developed and retained at the School of Natural Product Studies, Jadavpur University. Fruits and leaves were cleaned, dried in the shade, ground using a mechanical grinder and extracted (400 g) using the soxhlation process (45–50 °C, 48 h) with the hydro-alcoholic solvent system (Methanol: water- 70:30). The procedure was carried out three times. Then the two extracts were filtered and concentrated by using rotary evaporator. The filtrate was further lyophilized to obtain final yields [Leaf: 8.69 ± 0.43 % (w/w) and Fruit: 11.21 ± 0.8 % (w/w)].

2.3. Quantification of total phenolics and flavonoids

Quantification of total phenolics and flavonoid of dried leaves and fruit extracts of *M. dioica* were estimated as per the earlier mentioned methods (Chanda et al., 2020; Singha et al., 2020). 18 μL aliquots of 10 mg/mL sample was mixed with 10% Folin–Ciocalteu's (FC) reagent and 7.5% $NaHCO_3$. After proper incubation, absorbance at 765 nm by using Spectramax ID3 (Molecular Devices LLC, United States) was recorded. Gallic acid was used as standard (concentration: 5–30 μg/mL) and methanol was used as blank control. Total flavonoid content (TFC) was measured using Quercetin as standard compound. The plant samples were also prepared in methanol and mixed with 50 μL 1M NaOH and of 3% $NaNO_2$ and 15% $AlCl_3$. Total phenolic content (TPC) and TFC content was calculated as mg GA equivalent/g and mg QE equivalent/g of sample respectively.

2.4. In-vitro antioxidant potential

The antioxidant assay was estimated by using 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assay. In a 96-well microplate mixture of 100 μL sample and 0.2 mg/mL DPPH solution (100 μL) was mixed and kept in dark (10 min) at room temperature (rt). The absorbance was measured at 517 nm (Banerjee et al., 2023). Nitric oxide (NO) scavenging assay was measured spectrophotometrically with the use of Griess reagents. 50 μL sodium nitroprusside (0.2% w/v) was mixed individually with 50 μL of samples at various concentrations. Following 150 min incubation at 25 °C, 50 μL of sulfanilic acid was added to the reaction mixture, and it was let to stand for 5 min at room temperature. After that 50 μL 0.1% napthylethylenediamine dihydrochloride was added and incubated at room temperature for another 30 min. The absorbance was taken at 540 nm (Al Mahmud et al., 2017). For both test methods, the free radical scavenging activity was reported as an IC_{50} value (μg/mL). Ascorbic acid was used as a positive control in both the assays. The amount of DPPH and NO radical inhibited by the extract as well as ascorbic acid was calculated using the equation, as stated by Chanda et al. (2019).

$$\% \text{ Antioxidant activity} = [(\text{Asample} - \text{Ablank}) / \text{Asample}] \times 100$$

2.5. In-vitro α-amylase, α-glucosidase and pancreatic lipase enzyme inhibition assay

The AA enzyme inhibition assay of hydroalcoholic leaf and fruits extract of *Momordica dioica* Roxb. ex Willd. was performed using the DNSA method. The extract was dissolved in a phosphate buffer solution at a pH of 6.8 (concentrations ranging from 10 to 1000 μg/mL). 200 μL AA enzyme (2 U/mL) and the extract or standard inhibitor (Acarbose) was mixed and incubated. Further, 200 μL of starch solution (1%) was added to each tube and 200 μL DNSA reagent added after that. Following the addition boiled for in a water bath to conclude the reaction. The OD of diluted reaction mixture was measured at 540 nm to calculate % inhibition (Muritala et al., 2018). The AG enzyme inhibition assay was performed based on previous methods as demonstrated (Chanda et al., 2020). The sample, enzyme and substrate were dissolved in 50 mM phosphate buffer (pH 6.8). 10 μL sample (500–7000 μg/mL) and 20 μL of AG (0.5 U/mL) was mixed and incubated for 10 min at 37 °C. After that 20 μL p-NPG (5.0 mM) was added to the mixture. The OD was measured at 405 nm using spectrophotometer, acarbose was employed as positive control. The PL inhibition assay was performed in 96-well plate as per protocol of Chanda et al. with slight modifications (Chanda et al., 2019). 4-methylumbelliferyl oleate (4-MUO) was used as the substrate and Orlistat was used as the positive control. Sample and the standard were dissolved in Tris-HCl buffer (pH 8.0). The reaction mixtures underwent continued incubation and the reaction mixture was stopped by adding sodium citrate (0.1 M), absorbances were detected at 460 nm. Calculated inhibitory activities were represented as % of inhibition.

To investigate the inhibition kinetics of *M. dioica* fruit extract AA, AG and PL kinetics study were performed (Wang et al., 2019; Chanda et al., 2021). For AA and AG, the plant extract was taken in different concentrations ranging from 0 to 180 μM and the substrate potato starch and p-NPG respectively was taken in the range of 1.5–6.0 mM. For PL plant extract was taken in the range of 100–400 μM and substrate 4-MUO was taken in the range of 50–200 μM. Absorbance were calculated at every 1-min interval (0–30 min). Inhibitory mechanism (reversible or irreversible) at different sample concentrations was determined by plotting reaction velocity (ΔOD/min) vs concentrations of enzyme [E]. The reaction rates were measured to determine if AA, AG and PL enzyme inhibition occurred in a competitive, noncompetitive, uncompetitive or mixed manner. The Michaelis–Menten and Lineweaver-Burk plot were constructed using Sigma Plot 14 software and the values of K_m and V_{max} were generated.

Table 1
IC_{50} values (μg/mL) of MDL, MDF and standard by DPPH and NO free radical assay and α-amylase, α-glucosidase and pancreatic lipase enzyme inhibitory effects.

Extract	DPPH	NO	α-amylase	α-glucosidase	Pancreatic Lipase
MDL	155.7 ± 0.298 μg/mL	164.5 ± 0.264 μg/mL	139.6 ± 0.257 μg/mL	99.47 ± 0.402 μg/mL	133.8 ± 0.240 μg/mL
MDF	130.8 ± 0.344 μg/mL	141.2 ± 0.381 μg/mL	118.7 ± 0.183 μg/mL	89.23 ± 0.200 μg/mL	120.3 ± 0.310 μg/mL
Standard	113.8 ± 0.134 μg/mL	188.8 ± 0.122 μg/mL	97.23 ± 0.343 μg/mL	66.62 ± 0.147 μg/mL	90.45 ± 0.132 μg/mL

2.6. LC-QTOF-MS analysis

Based on the *in-vitro* antioxidant and enzyme inhibition assays, LC–QTOF–MS analysis was performed only for the *M. dioica* fruit extract. The LC-QTOF-MS analysis was conducted using an Agilent 1260 Infinity II LC System coupled with Accurate-Mass (Q-TOF) Spectrometer. To optimise the chromatographic method, alterations were made to ratio of mobile phase, injection volume and flow rate. 10 mM ammonium acetate in water (A) and acetonitrile (B) were used as the mobile phase. By using Agilent Zorbax Eclipse C18 column (50 mm × 2.1 mm, 1.7 μm) separation was done. The gradient elution was started with 100% A at 0–2 min, 95%–100% A at 2–5 min, 85%–95% A at 5–10 min, 70%–85% A at 10–15 min, 5%–70% A at 15–25 min, 5–100% A at 25–35 min. The flow rate and injection volume were set to 0.5 mL/min and 5 μL respectively. The mass spectrometer (Agilent 6530 Accurate Mass Q-TOF) in positive ion mode, was used for the mass spectrometry with skimmer voltage (60 V) and the fragment voltage (120 V). Data were gathered using a centroid mode, and an extended dynamic range was used to adjust the mass range to m/z 50–1000. To investigate the structure of the prospective biomarkers, MS/MS analysis was done.

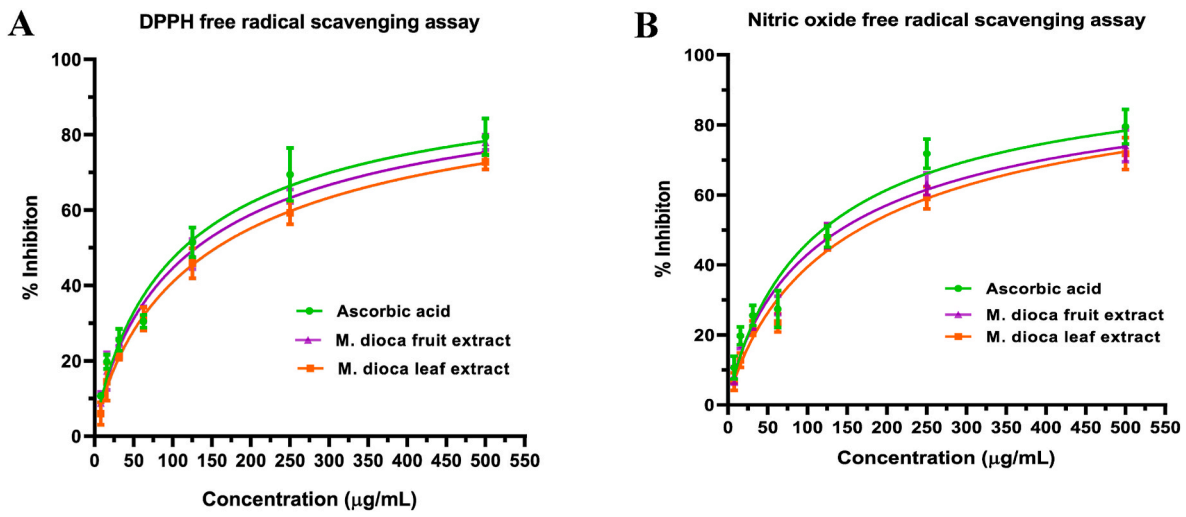

Fig. 1. Dose response curve of DPPH (A) and NO (B) free radical scavenging assay of *M. dioica* leaf and *M. dioica* fruit extract and Standard Ascorbic acid.

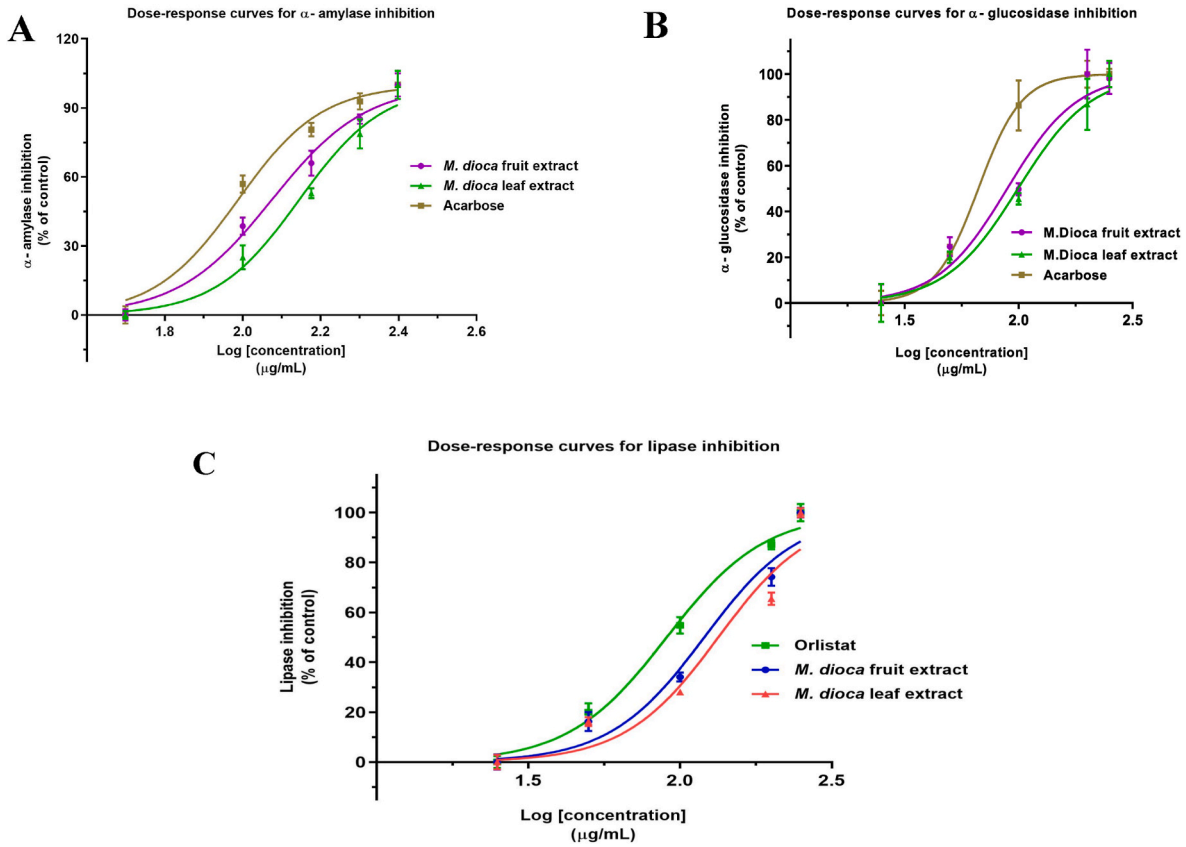

Fig. 2. Dose response curve of α-amylase (A), α-glucosidase (B) and pancreatic lipase (C) enzyme inhibitory effects of *M. dioica* leaf and *M. dioica* fruit extract and Standard Acarbose and Orlistat respectively.

Agilent MassHunter Qualitative Analysis v. 10.0 coupled with a customized database "" was used for data mining and identification of the phytoconstituent based on the Retention time, % Score, Observed mass and error ppm (Chanda et al., 2020).

2.7. Statistical analysis

All the experiments were performed in triplicate and results are shown as mean ± standard deviation. Graph pad prism 8.0.2 software (Boston, MA, USA) was used for statistical calculation. The significance difference and difference at P-value <0.05 were determined using Tukey's multiple comparison test. Pearson correlation coefficient was calculated to test correlation between the enzymes with their phenolic, flavonoid and antioxidant activity.

2.8. Molecular docking analysis

2.8.1. Protein preparation

The crystal structures (3D) were acquired with the integration of Protein Data Bank (https://www.rcsb.org) of AA (PDB ID: 5E0F), AG

Table 2
Correlation analysis between the α-Glucosidase, α-amylase, lipase, TPC, TFC and DPPH of different varieties of *M. dioica* leaf and *M.* dioica fruit with the enzyme activity their antioxidant property (IC_{50} value).

Pearson's correlation (r) coefficient (IC_{50} value of α-amylase inhibition potential)					
Sample	α-Glucosidase	Lipase	DPPH	GAE	QE
M. dioica leaf	0.99*	-0.46^{ns}	0.99*	0.99*	-0.6^{ns}
M. dioica fruit	1.00**	0.98^{ns}	0.99*	0.99*	0.99*
Pearson's correlation (r) coefficient (IC_{50} value of α-glucosidase inhibition potential)					
	α-amylase	Lipase	DPPH	GAE	QE
M. dioica leaf	0.99*	-0.42^{ns}	0.99*	0.99^{ns}	-0.57^{ns}
M. dioica fruit	1.00**	0.98^{ns}	0.99*	0.99^{ns}	0.99*
Pearson's correlation (r) coefficient (IC_{50} value of lipase inhibition potential)					
	α-Glucosidase	α-amylase	DPPH	GAE	QE
M. dioica leaf	-0.42^{ns}	-0.46^{ns}	-0.48^{ns}	-0.51^{ns}	0.98^{ns}
M. dioica fruit	0.98^{ns}	0.98^{ns}	0.98^{ns}	0.99^{ns}	0.99^{ns}

(PDB ID: 5NN8) and PL (PDB ID: 1LPB) with the resolutions 1.4, 2.45 and 2.46 Å. Inhibitors, unnecessary water molecules and all heteroatoms were filtered with DiscoveryStudio Visualizer (v21.1.020298), and the macromolecule preparation protocol was carried out using AutoDockTools 1.5.6. Gasteiger charges were introduced post merging the non-polar hydrogens, and docking evaluations were performed on the prepared complex structures (Aispuro-Pérez et al., 2020; Chen et al., 2020).

2.8.2. Ligand preparation

The.sdf files containing the 3D structures of 16 small molecules that were found by analysing LC-QTOF-MS data of *M. dioica* fruits were downloaded from https://pubchem.ncbi.nlm.nih.gov. Utilising the mmff94 method, the energy of these molecules was minimised. Additionally, hydrogen atoms were incorporated into the ligand molecules. Finally, determination of the most stable conformation of ligands, molecular docking research was performed using PyRx (Kumar et al., 2019).

2.8.3. Docking

The ligand-protein interactions were studied using molecular docking simulations. PyRx 0.8 performed the docking analysis using a grid-based methodology and AutoDock Vina performed the docking analysis using a rigid dock strategy algorithm. Every protein-ligand complex binding energy (Kcal/mol) was calculated using the docking results (Dallakyan and Olson, 2015). The docking score quantitatively indicates the binding affinity of the ligands, with lower values suggesting better interaction. The ligands interact with the receptor by many sorts of

Table 3
Compounds identified in *M. dioica* fruit extract by LC–QTOF–MS.

SI No.	RT (Min)	m/z (Estimated)	m/z (Expected)	Chemical Formula	Error PPM	Major MS-MS Fragments	Name of Compounds	Class of Compound
1.	1.9	450.3495	450.3497	$C_{31}H_{46}O_2$	−0.44	331, 315, 281, 257	Phytonadione	Vitamin
2.	15.25	634.4440	634.4444	$C_{37}H_{62}O_8$	−0.63	622, 576, 480	Balsaminoside A	Triterpene
3.	19.29	414.3867	414.3861	$C_{29}H_{50}O$	1.44	379, 341, 287, 255, 213	β-sitosterol	Phytosterol
4.	19.4	470.3398	470.3396	$C_{30}H_{46}O_4$	0.42	456	Gypsogenin	Pentacyclic triterpenoid
5.	22.9	278.2244	278.2245	$C_{18}H_{30}O_2$	−0.35	261, 243, 205, 149, 123	Linolenic acid	Fatty acids
6.	23.1	244.0888	244.0881	$C_{10}H_{16}N_2O_3S$	2.86	209, 199, 184, 166	Biotin	Heterobicyclic compound
7.	24.3	219.1108	219.1106	$C_9H_{17}NO_5$	0.91	146, 116, 99	Pantothenic acid	Vitamin
8.	25.1	412.3704	412.3705	$C_{29}H_{48}O$	−0.24	395, 315, 257, 199, 83	Stigmasterol	Phytosterol
9.	25.2	256.2400	256.2402	$C_{16}H_{32}O_2$	−0.78	183, 149, 118, 75	Palmitic acid	Fatty acid
10.	25.6	284.2710	284.2715	$C_{18}H_{36}O_2$	−1.75	275, 257, 241, 153, 125	Stearic Acid	Fatty acid
11.	26.6	282.2551	282.2558	$C_{18}H_{34}O_2$	−2.48	265, 205, 135, 107, 83	oleic acid	Fatty acid
12.	26.79	280.2405	280.2402	$C_{18}H_{32}O_2$	1.07	278, 254	9,12-Octadecadenoic acid	Fatty acid
13.	27.2	574.4236	574.4233	$C_{35}H_{58}O_6$	0.52	487, 356, 288	*α*-spinasterol-3-O-*β*-glucoside	Phytosterol
14.	28.95	310.2877	310.2871	$C_{20}H_{38}O_2$	1.93	290, 275, 227	Eicosenoic acid	Fatty Acid
15.	29.3	594.1588	594.1584	$C_{27}H_{30}O_{15}$	0.67	422, 304, 288, 256	Kaemperol	Flavonoid
16.	29.7	384.3395	384.3392	$C_{27}H_{44}O$	0.78	367, 273, 259	Cholecalciferol	Vitamin

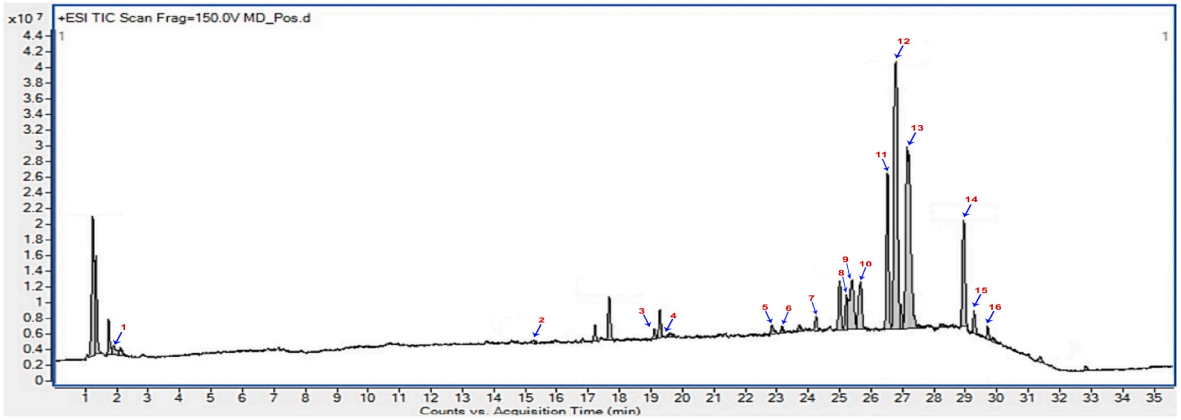

Fig. 3. Positive mode LC-MS Chromatogram of *M. dioica* Fruit Extract.

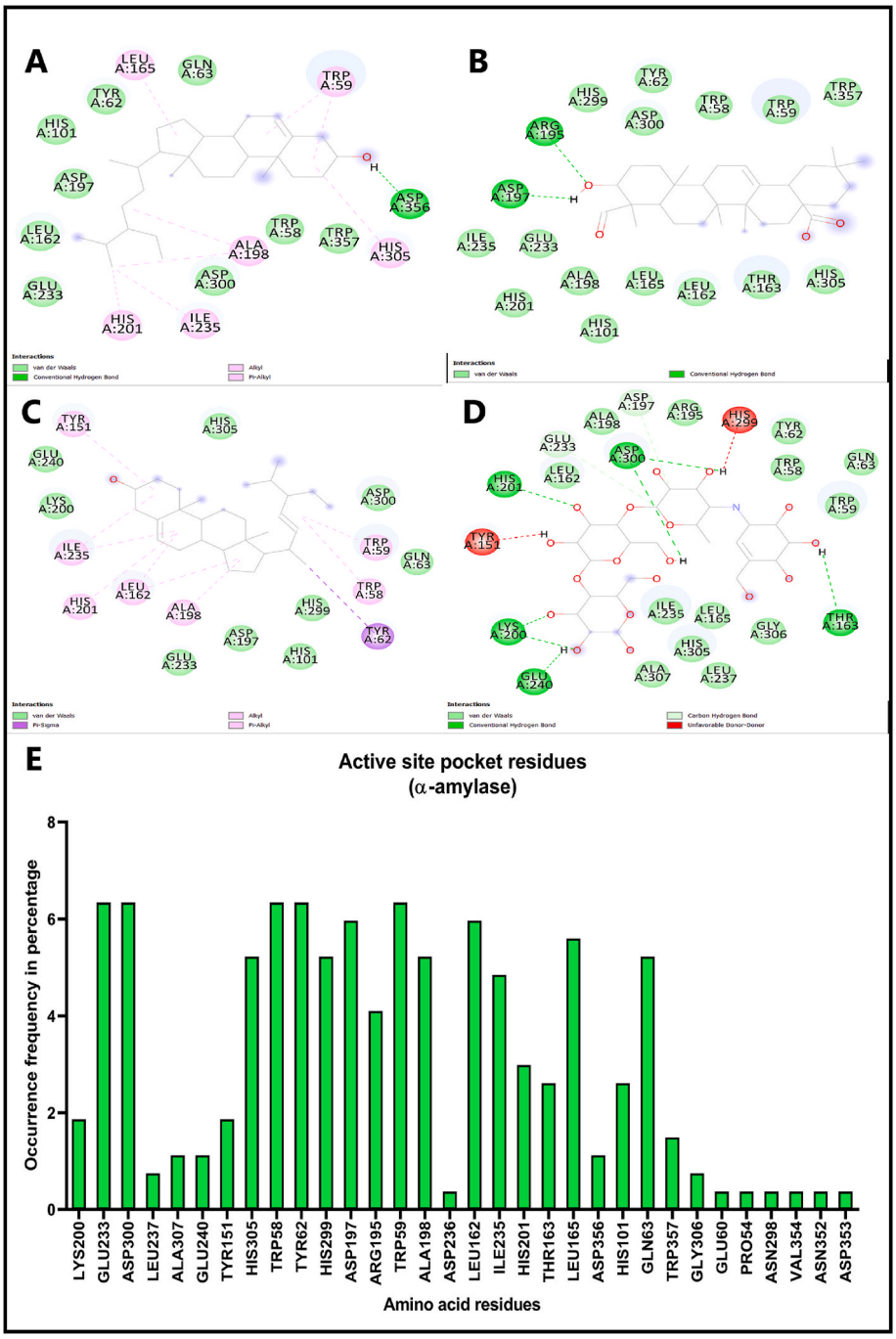

Fig. 4. 2D depiction showing various interactions of the most stable phyto-compounds with α-amylase (PDB ID: 5E0F): A: β-sitosterol; B: Gypsogenin; C: Stigmasterol; D: Acarbose; The amino acid residues that interacted with the docked ligands and their frequency of occurrence in percentage in case of α-amylase.

bonding interactions (Hasan et al., 2022). According to Agu et al., such interactions may contribute an understanding regarding the mechanism of action of the compounds and their probable therapeutic efficacies (Agu et al., 2023). Using Discovery Studio Visualizer, the interaction features of protein-ligand complex were examined. Once the binding site was identified, the hydrogen bond interactions with amino acids and the Pi-Alkyl, Pi-Pi, and Pi-Sigma interactions were computed.

3. Results & discussions

3.1. Quantification of total phenolic and flavonoid

Polyphenolic compounds are extensively found in the plant kingdom and can be found in foods like fruits, beverages made from plants, husk seeds, and peels; recognised for their potential biological activity both *in-vivo* and *in-vitro*. These compounds could be healthy-giving, aiding the several manifestations of metabolic disorder in particular (Ghosh, 2022). The TPC of the leaves and fruit extracts of *M. dioica* was reported in terms of Gallic acid equivalent (GAE) and TFC was reported in terms of quercetin equivalent (QE). The phenolic content of both the extracts estimated were 22.83 ± 1.32 (MDL) and 25.93 ± 1.01 (MDF) mg of GA/g. The flavonoid contents were estimated to be 20.13 ± 2.08 (MDL) and 26.37 ± 0.99 (MDF) mg of QE/g respectively. In comparison between the extracts, fruit extract of *M. dioica* contains more amount of phenolics and flavonoid content in terms of GAE and QE. In other studies, several phenolic acid and flavonoid contents was reported

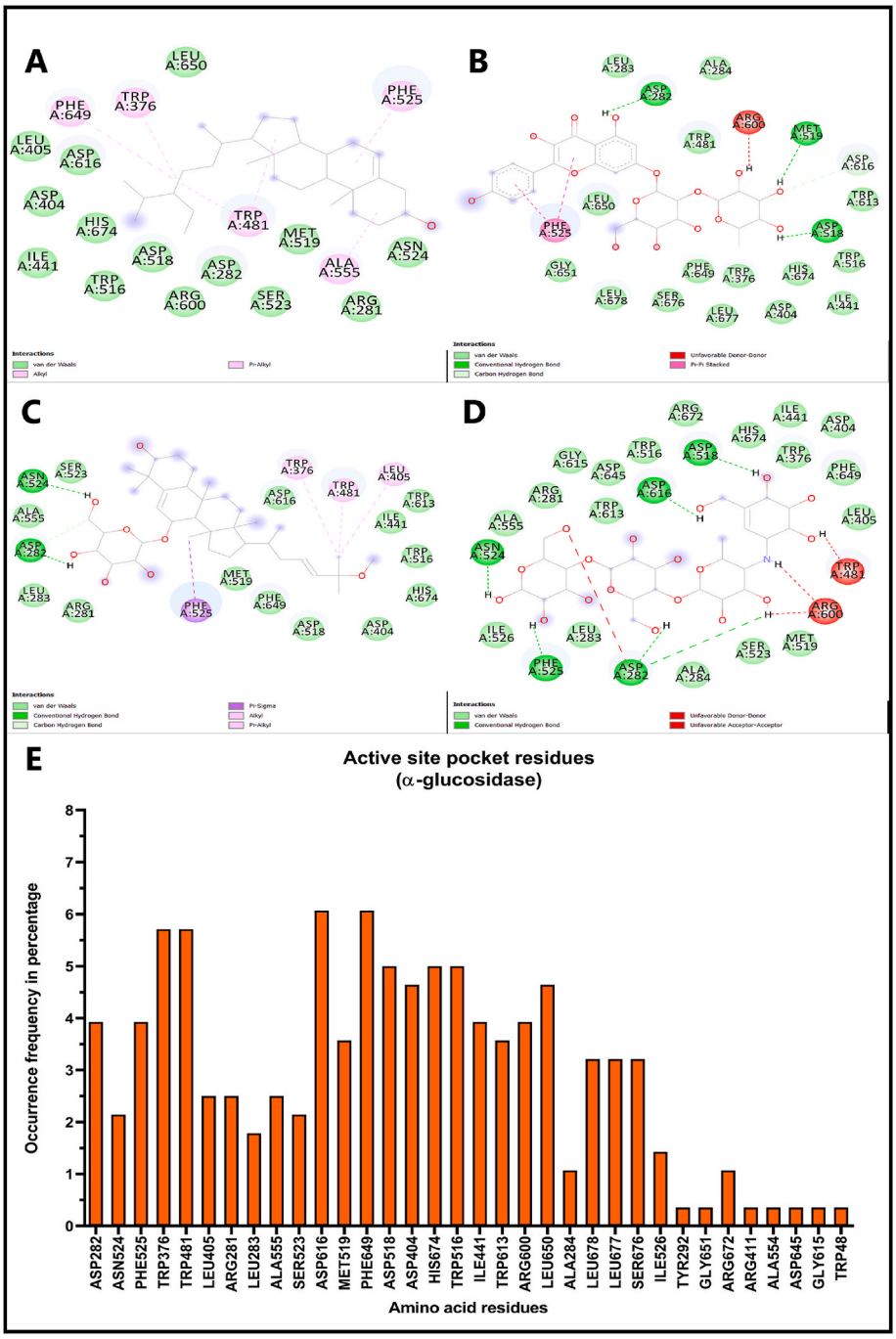

Fig. 5. 2D depiction showing various interactions of the most stable phyto-compounds with α-glucosidase (PDB ID: 5NN8): A: β-sitosterol; B: Kaempferol 7-O-neohesperidoside; C: Balsaminoside-A; D: Acarbose; E: The amino acid residues that interacted with the docked ligands and their frequency of occurrence in percentage in case of α-glucosidase.

including caffeic acid, ferulic acid, vanillic acid, quercetin, rutin, kaempferol etc. in various parts of *M. dioica* (Talukdar and Hossain, 2014).

3.2. DPPH free radical and NO free radical scavenging activity

The free radical scavenging activity of *M. dioica* leaf and fruits extracts on the DPPH free radical and NO free radical was compared with the standard ascorbic acid. The outcome of the studies was expressed as IC_{50} value presented in Table 1. Fig. 1 (A and B) represents the dose response curves of the leaf and fruits extract of *M. dioica*. In both *in-vitro* antioxidant studies the extracts showed activities comparable to the Positive control, Ascorbic acid. In comparison to leaf extract, fruit extract demonstrated significant concentration-dependent scavenging activity in both DPPH free radical and NO free radical. This indicates the plant extracts possess significant antioxidant and free radical scavenging property may be due to presence of polyphenolic compounds. One of the ways that *M. dioica* fruit extract functions as a traditional medicine is possibly through its capacity to scavenge free radicals. Consuming fruits of *M. dioica* can help avoid degenerative disorders brought on by oxidative stress (Chanda et al., 2020). Aqueous extract of *M. dioica* fruit having antioxidant potential in liver, kidney, pancreas, and serum by increasing the levels of hydroperoxide, thiobarbituric acid reactive substances and decreasing non-enzymatic antioxidants levels in diabetic

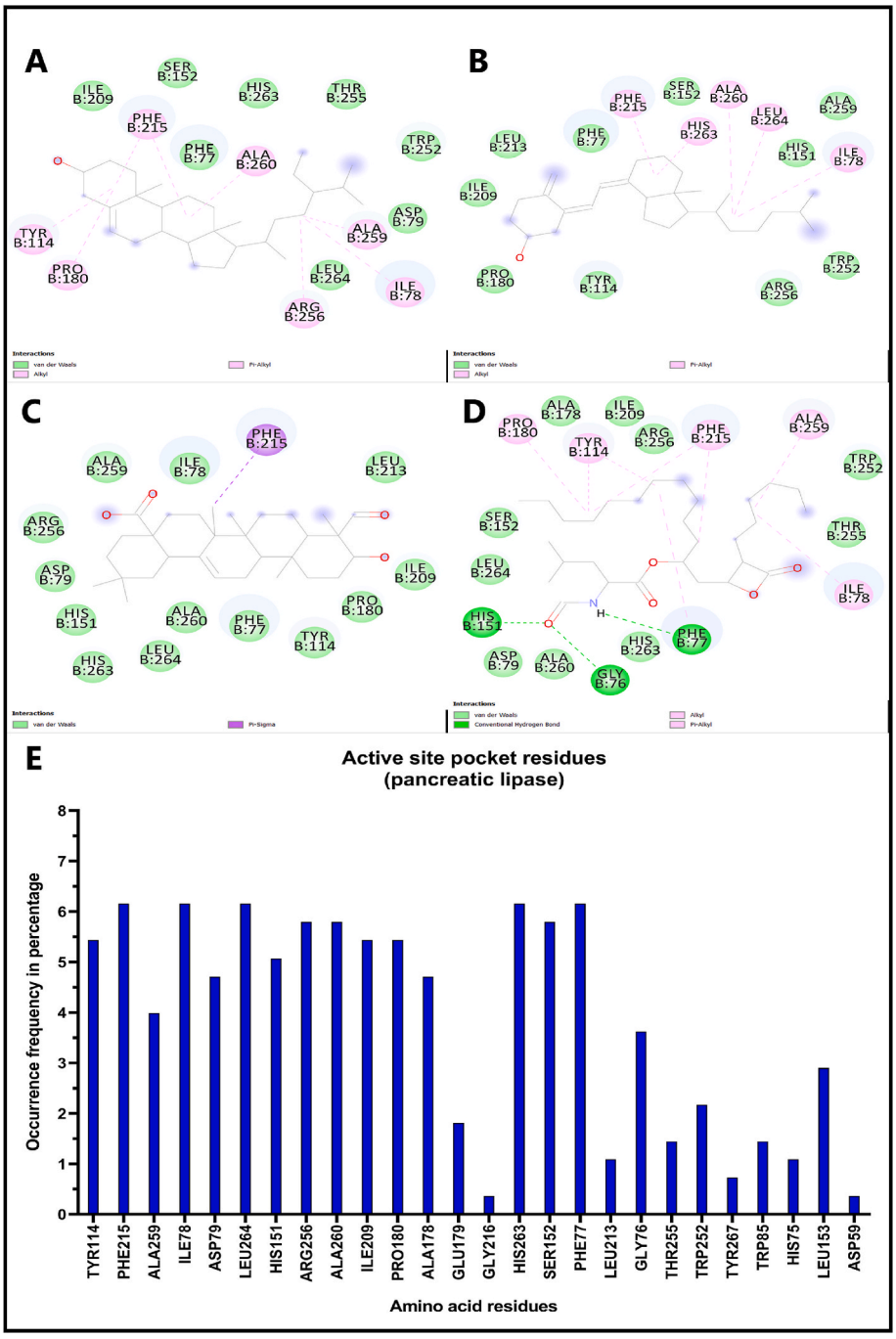

Fig. 6. 2D depiction showing various interactions of the most stable phyto-compounds with pancreatic lipase (PDB ID: 1PLB): A: β-sitosterol; B: Cholecalciferol; C: Gypsogenin; D: Orlistat; E: The amino acid residues that interacted with the docked ligands and their frequency of occurrence in percentage in case of α-glucosidase.

experimental animals (Sharma and Singh, 2014).

3.3. *α-amylase, α-glucosidase, and pancreatic lipase in-vitro enzyme inhibition assay*

Hyperglycemia, hyperlipidemia, obesity etc. are metabolic disorders associated with lifestyle related disorder, can be treated by inhibiting the enzymes AA, AG and PL which are involved in the metabolism and absorption of carbohydrates, fats, triglycerides etc. (Chanda et al., 2019; Cardullo et al., 2020). AA inhibitors can act by blocking or reducing carbohydrate intake, limiting the digestibility and absorption of carbohydrate in the gastrointestinal tract. AG inhibitors inhibit the absorption of carbohydrates and also inhibit enzymes (glucoamylase, sucrase, maltase) that alter complex non-absorbable carbohydrates into simple absorbable carbohydrates. Clinically, both the enzyme inhibitor can be used to prevent metabolic disorders such as diabetes, hyperglycemia, hyperlipemia, and obesity (Gong et al., 2020; Seetaloo et al., 2019). Inhibiting PL is the most reliable way to stop the excessive production of free fatty acids and limit the absorption of triacylglycerol in the intestine resulting antihyperlipidemic activity and exerting potential cure of metabolic disorder (Wang et al., 2013). As presented in Table 1, hydroalcoholic extracts of *M. dioica* (leaves and fruits), acarbose and orlistat has produced an inhibitory effect on AA, AG, PL enzyme *in-vitro* respectively. The IC_{50} values for AA of MDL, MDF and acarbose were 139.6 ± 0.257 μg/mL, 118.7 ± 0.183 μg/mL and 97.23 ± 0.343 μg/mL respectively. The IC_{50} values for AG (MDL: 99.47 ± 0.402 μg/mL; MDF:

Table 4
Details of molecular docking studies of the phytoconstituents on α-amylase (PDB ID: 5E0F) and α-glucosidase (PDB ID: 5NN8) which include Docking score, Conventional hydrogen bonds, Hydrophobic interactions, and active site pocket residues.

Phytocomponents	α-Amylase activity			
	Docking Score	Conventional –H Bond	Hydrophobic interactions	Active site pocket residues
Balsaminoside-A	−9.3	LYS200, GLU233(2), ASP300	–	LYS200, GLU233, ASP300, LEU237, ALA307, GLU240, TYR151, HIS305, TRP58, TYR62, HIS299, ASP197, ARG195, TRP59, ALA198, ASP236, LEU162, ILE235, HIS201, THR163, LEU165
β-sitosterol	−9.8	ASP356	HIS201, ILE235, ALA198(2), HIS305, TRP59(2), LEU165	ASP356, HIS201, ILE235, ALA198, HIS305, TRP59, LEU165, GLU233, LEU162, ASP197, HIS101, TYR62, GLN63, ASP300, TRP58, TRP357
Biotin	−6.4	ASP300	TYR62, ALA198	ASP300, TYR62, ALA198, LEU165, GLN63, HIS101, TRP59, LEU162, ARG195, GLU233, ASP197, ILE235, HIS299, TRP58
Cholecalciferol	−8.4	ASP300, ALA307	TRP59	GLN63, LEU165, TYR62, ILE235, ASP300, ALA307, GLY306, HIS305, GLU233, TRP58, ALA198, LEU162, TRP59, HIS101
Eicosenoic acid	−5.6	—	TYR62, LEU162, ALA198, LEU165, TRP59(2)	TYR62, LEU162, ALA198, LEU165, TRP59, HIS101, ILE235, THR163, GLU233, GLN63, ASP300, TRP58, HIS299, ASP197
Gypsogenin	−9.8	ASP197, ARG195	—	ASP197, ARG195, HIS299, TYR62, ASP300, TRP58, TRP59, TRP357, ILE235, GLU233, HIS201, ALA198, HIS101, LEU165, LEU162, THR163, HIS305
Kaempferol 7-O nonhesperidoside	−9.2	ASP197(2)	TRP59(2)	ASP197, HIS305, TRP59, TRP357, ASP356, TRP58, ASP300, ARG195, GLU233, ALA198, LYS200, ILE235, HIS201, TYR151, LEU162, HIS101, TYR62, LEU165, GLN63, GLU60, PRO54
Linoleic acid	−5.9	ASP300, GLU233	HIS305, TRP59(2), HIS299, TYR62(2)	ASP300, GLU233, HIS305, TRP59, HIS299, TYR62, TRP58, GLN63, LEU165, ILE235, ASP197, ARG195, ASN298
Oleic acid	−5.7	ARG195, ASP300	TRP59(4), TRP58, LEU162, HIS305, LEU165	ARG195, ASP300, TRP59, TRP58, LEU162, HIS305, LEU165, GLN63, TYR62, ASP197, HIS299, GLU233
Palmitic acid	−5.5	ASP197, GLU233	TYR62, TRP58, TRP59, LEU165, LEU162	ASP197, GLU233, TYR62, TRP58, TRP59, LEU165, LEU162, GLN63, HIS305, HIS299, ASP300, ALA198, ILE235, THR163, ARG195
Pantothenic acid	−5.6	GLU233	——	GLU233, ASP300, HIS299, ARG195, TYR62, TRP58, TRP59, ASP197, ILE235, LEU162, ALA198, HIS201
Phytonadione	−7.8	GLN63	TRP58, TRP59(2), TYR62(2), LEU162, HIS201	GLN63, TRP58, TRP59, TYR62, LEU162, HIS201, TYR151, LEU165, LYS200, THR163, ILE235, HIS305, ASP300, GLU233, ASP197, ALA198, HIS299
α-spinasterol-3-O-β-glucoside	−9.6	VAL354	TYR62, ALA198, LEU162, TRP59, LEU165	VAL354, TYR62, ALA198, LEU162, TRP59, LEU165, GLU233, ASP197, HIS299, ASP300, ARG195, THR163, GLN63, HIS305, ASN352, ASP356, ASP353, TRP357, TRP58
Stearic acid	−5.6	ASP300	LEU165, LEU162, TRP59(2), HIS305	ASP300, LEU165, LEU162, TRP59, TRP58, HIS305, GLN63, TYR62, GLU233, ARG195, ASP197, HIS299
Stigmasterol	−9.8	–	TYR62, LEU162(2), ALA198, HIS201, ILE235(2), TYR151, TRP58, TRP59	TYR62, LEU162, ALA198, HIS201, ILE235, TYR151, TRP58, TRP59, GLU240, LYS200, HIS305, ASP300, GLN63, HIS299, HIS101, ASP197, GLU233
(9E, 12E)-octadeca-9,12 dienoic acid	−5.8	–	TRP59(3), HIS305, TRP58(2), LEU165, TYR62	TRP59, HIS305, TRP58, LEU165, TYR62, ASP300, GLN63, LEU162, ALA198, GLU233, ASP197, HIS299
Acarbose	−8.4	LYS200(2), GLU240, HIS201, ASP300(2), THR163	—	LYS200, GLU240, HIS201, ASP300, THR163, GLY306, LEU237, HIS305, LEU165, ILE235, ALA307, TYR151, LEU162, GLU233, ALA198, ASP197, ARG195, HIS299, TYR62, TRP58, GLN63, TRP59

89.23 ± 0.200 μg/mL; Acarbose: 66.62 ± 0.147 μg/mL) and PL (MDL: 133.8 ± 0.240 μg/mL; MDF: 120.3 ± 0.310 μg/mL; Orlistat: 90.45 ± 0.132 μg/mL) enzyme inhibition and their respective standard inhibitor acarbose and orlistat expressed in Table 1. The dose response curve of AA, AG and PL enzyme inhibition activity of both the extract in Fig. 2. The inhibitory activity (IC_{50} value) of fruit extract was the maximum of the two extracts. Fruit extract was compared to the standard inhibitor acarbose and orlistat but no prominent difference was found and it was discovered that the inhibition was dose-dependent. Pearson correlation coefficient analysis showed positive correlation between AA and AG enzymes of fruit and leaf and their respective phenolic, flavonoid content and antioxidant potential. The fruits showed higher significant correlation than the leaves. PL showed negative correlation with both the AA and AG enzymes but showed positive correlation with phenolic, flavonoid content and antioxidant potential (Table 2). Few flavonoids including isorhamnetin, esculetin etc. were possesses to have prominent *in-vitro* AG inhibitory effects.

In our study, AA, AG and PL enzyme kinetics inhibition assay by *M. dioica* fruit extract showed reversible type of inhibition. The AG inhibition kinetics gave V_{max} and K_m as 25.0 mM/min and 2.1 mM, respectively, AA inhibition kinetics gave V_{max} and K_m as 22.7 mM/min, and 2.8 mM, respectively and PL inhibition kinetics gave V_{max} and K_m as 261.6 mM/min, and 1.9 mM, respectively (Supplementary F-1.). It was observed that both of the values of apparent V_{max} and K_m were changed with an increasing concentration of the inhibitor indicating mixed type of inhibition for all the three enzymes. The secondary plot was used to calculate Ki and αKi as 112.1 and 1 μg/mL for AG, 74.1 and 1.5 μg/mL for AA and 48.3 and 4.8 for PL respectively (Supplementary F-1). The Lineweaver–Burk plot of all the three enzymes showed different Ki values which indicates that both enzyme (Ki and αKi values) as well as enzyme-substrate complex (αKi) bind with the inhibitor confirming the hypothesis of mixed type of inhibition (Chanda et al., 2019). Moreover, a higher Ki value indicates that the inhibitor binds to the enzyme more than the enzyme substrate complex.

3.4. Identification of phytocompounds by LC–QTOF–MS analysis

M. dioica fruit extract was further studied by LC–QTOF–MS analysis due to the presence of higher amount of polyphenolic compound compared to the leaf extract and also the fruit extract demonstrated significant antioxidant and inhibitory effects against AA, AG and PL enzymes depending on the dose. Based on measurements of precise

Table 5
Details of molecular docking studies of the phytoconstituents on α-glucosidase (PDB ID: 5NN8) which include Docking score, Conventional hydrogen bonds, Hydrophobic interactions, and active site pocket residues.

Phytocomponents	α-Glucosidase activity			
	Docking Score	Conventional –H Bond	Hydrophobic interactions	Active site pocket residues
Balsaminoside-A	−8.1	ASP282, ASN524	PHE525, TRP376, TRP481, LEU405	ASP282, ASN524, PHE525, TRP376, TRP481, LEU405, ARG281, LEU283, ALA555, SER523, ASP616, MET519, PHE649, ASP518, ASP404, HIS674, TRP516, ILE441, TRP613
β-sitosterol	−8.3	–	TRP481(2), ALA555, TRP376, PHE649, PHE525	TRP481, ALA555, TRP376, PHE649, PHE525, ASN524, ARG281, SER523, MET519, ASP282, ARG600, ASP518, TRP516, ILE441, HIS674, ASP404, ASP616, LEU405, LEU650
Biotin	−6.1	ASP616, ARG600	PHE649, TRP376	ASP616, ARG600, PHE649, TRP376, ASP282, TRP613, ASP518, TRP516, ILE441, ASP404, HIS674, LEU405, TRP481
Cholecalciferol	−7.6	–	PHE525, TRP481, PHE649	PHE525, TRP481, PHE649, ALA284, LEU650, ASP616, ASP404, HIS674, ILE441, TRP516, TRP613, ASP518, TRP376, ARG600, MET519, LEU283, ASP282, SER523
Eicosenoic acid	−5	TRP481, ASP404	TRP481, PHE649(2), LEU678, LEU677, TRP376	TRP481, ASP404, PHE649, LEU678, LEU677, TRP376, PHE525, SER676, LEU650, ASP616, TRP516, HIS674, ARG600, ILE441, LEU405, ASP518
Gypsogenin	−7.6	ASP282, ASP616, ARG600	—	ASP282, ASP616, ARG600, ILE526, PHE525, ASN524, SER523, MET519, TRP481, TYR292, PHE649, LEU650, ARG281, ALA555
Kaempferol 7-O nonhesperidoside	−8.2	MET519, ASP518, ASP282	PHE525(2)	MET519, ASP518, ASP282, PHE525, GLY651, LEU650, LEU678, SER676, PHE649, TRP376, LEU677, HIS674, ASP404, ILE441, TRP516, TRP613, ASP616, ARG600, TRP481, ALA284, LEU283
Linoleic acid	−5.7	ASP404, HIS674	PHE649(2), TRP376(4), TRP481(3), LEU677, LEU678	ASP404, HIS674, PHE649, TRP376, TRP481, LEU677, LEU678, LEU650, ASP616, ILE441, TRP516, ARG672, TRP613, ASP518, SER676
Oleic acid	−5.3	HIS674, ASP518	PHE649(2), LEU650, TRP481(2), LEU677, LEU678, TRP376(2)	HIS674, ASP518, PHE649, LEU650, TRP481, LEU677, LEU678, TRP376, ARG411, TRP516, ASP404, ILE441, ASP616, SER6761
Palmitic acid	−4.8	ASP404	TRP376(3), PHE649, TRP481, LEU678, LEU677	ASP404, TRP376, PHE649, TRP481, LEU678, LEU677, SER676, ASP518, LEU650, ASP616, TRP613, TRP516, HIS674
Pantothenic acid	−4.9	ASP616(3), ARG600	——	ASP616, ARG600, MET519, ASP282, TRP481, PHE649, TRP376, TRP516, TRP613, HIS674, ASP518, PHE525
Phytonadione	−6.8	–	PHE525(2), ALA555, PHE649(2), LEU678, HIS674, TRP516, TRP376 (2), TRP481	PHE525, ALA555, PHE649, LEU678, HIS674, TRP516, TRP376, TRP481, LEU405, LEU650, ASP404, SER676, ASP616, ASP518, LEU677, ASN524, ARG281, MET519, ASP282
α-spinasterol-3-O-β-glucoside	−7.4	ARG281(2), PHE525	PHE525(2), ALA555, TRP481	ARG281, PHE525, ALA555, TRP481, SER523, MET519, ASP282, ASN524, ILE526, ALA554, LEU650, ASP616, PHE649, TRP376
Stearic acid	−5.2	ARG600	PHE649(2), TRP481(2), LEU678, LEU677(2), TRP376(4)	ARG600, PHE649, TRP481, LEU678, LEU677, TRP376, HIS674, ASP616, TRP516, TRP613, ASP518, ASP404, SER676, LEU650, ILE441
Stigmasterol	−7.7	–	ALA555, TRP376, TRP481, PHE649	ALA555, TRP376, TRP481, PHE649, LEU650, SER676, LEU678, LEU677, PHE525, ASP282, ARG600, MET519, ARG281, ASP616
(9E, 12E)-octadeca-9,12 dienoic acid	−5.5	—	LEU678, LEU677, TRP376(4), TRP481(3), PHE649	LEU678, LEU677, TRP376, TRP48, PHE649, LEU405, ASP404, ILE441, ASP518, ARG600, TRP613, TRP516, HIS674, ASP616, ARG672, LEU650, SER676
Acarbose	−7.2	ASN524, ASP616, ASP518, ASP282(2), PHE525	—	ASN524, ASP616, ASP518, ASP282, PHE525, ILE526, LEU283, ALA284, SER523, MET519, ARG600, TRP481, LEU405, PHE649, TRP376, HIS674, ILE441, ASP404, TRP516, ASP645, TRP613, GLY615, ARG281, ALA555, ARG672

molecular mass, retention times, and isotope peak pattern, LC-QTOF-MS analysis primarily employed for identification of the phytomolecules contained in *M. dioica* fruit extract (Kar et al., 2021). Table 3. Tentatively 16 compounds along with their corresponding retention times, chemical formulas, ppm errors, major MS-MS fragments, and measured masses (both predicted and estimated m/z). LC-MS Chromatogram of *M. dioica* fruit extract is shown in Fig. 3. From the study the presence of Phytonadione, Balsaminoside A, β-sitosterol, Gypsogenin, Linoleic acid, Biotin, Pantothenic acid, Stigmasterol, Palmitic acid, Stearic acid, Oleic acid, 9,12-Octadecadenoic acid, α-spinasterol 3-O-β-glucoside, Eicosenoic acid, Kampferol, Cholecalciferol. Synergistically many phytomolecules especially poly phenolic compounds exhibit strong activity against AG enzyme in a noncompetitive type of inhibition (Sheng et al., 2014). Polyphenolic compound Ellagic acid having most AA and AG inhibitory potential followed by quercetin, azelaic acid, gallic acid (Wu et al., 2021). Isoferulic acid-3-glucoronide, chrysophanol-8-O-glucoside, oleic acid and stearic acid having potent antihyperglycemic activity by inhibiting AA and AG by reducing glucose intake and possesses glucose modulation (Mokhele et al., 2020). Bioactivity guided LC-MS/MS approach have been attempted in many studies including fractions of hydroalcoholic extract of *Lagenaria siceraria* exhibited potent carbonic anhydrase enzyme inhibition (Chanda et al., 2021); Ethyl acetate fraction of *Momordica charantia* fruit also exhibited strong inhibitory activity against PL enzyme due to the presence of wide range of bioactive compounds identified by UPLC-QTOF-MS mostly polyphenolic components (Chanda et al., 2019).

Palmitic acid and Steraic acid having antidiabetic properties through preventing glucose uptake via activation of Akt and ERK1/2 in skeletal muscle cells and activate GPR40 and benefited glucose homeostasis respectively (Syed et al., 2018). Aqueous and ethanolic extracts from have been shown to have AA and PL inhibitory properties *in-vitro*. Presence of loganic acid, cornuside in *Cornus alba* and Cornelian cherry potentially inhibit PL enzyme, whereas Iridoids and pelargonidin 3-O-galactoside in cherry fruit extracts inhibit AA and quercetin, kaempferol derivatives presence in the Cornus fruit extract also responsible for hypolipidedemic and antihyperlipidemic activity

Table 6
Details of molecular docking studies of the phytoconstituents on pancreatic lipase (PDB ID: 1LPB) which include Docking score, Conventional hydrogen bonds, Hydrophobic interactions, and active site pocket residues.

Phytocomponents	Pancreatic lipase activity			
	Docking Score	Conventional –H Bond	Hydrophobic interactions	Active site pocket residues
Balsaminoside-A	−6.2	TYR114, PHE215	ALA259, ILE78	TYR114, PHE215, ALA259, ILE78, ASP79, LEU264, HIS151, ARG256, ALA260, ILE209, PRO180, ALA178, GLU179, GLY216, HIS263, SER152, PHE77, LEU213, GLY76
β-sitosterol	−9.5	–	TYR114, PRO180, PHE215(2), ALA260, ALA259, ILE78, ARG256	TYR114, PRO180, PHE215, ALA260, ALA259, ILE78, ARG256, ILE209, SER152, HIS263, THR255, PHE77, TRP252, ASP79, LEU264
Biotin	−6.9	HIS263, SER152, GLY76, HIS151, ASP79	ILE78(2), LEU264, PHE215, PHE77	HIS263, SER152, GLY76, ASP79, HIS151, ILE78, LEU264, PHE215, PHE77, ALA178, TYR267, TRP85, ARG256
Cholecalciferol	−9.2	–	PHE215, HIS263, ALA260, LEU264, ILE78	PHE215, HIS263, ALA260, LEU264, ILE78, TYR114, PRO180, ILE209, LEU213, PHE77, SER152, HIS151, ALA259, TRP252, ARG256
Eicosenoic acid	−6.5	HIS263, SER152, ASP79	TYR114(2), PRO180, ALA178(2), PHE215(3), ALA259, LEU264, ILE78, ALA260(2), HIS263	HIS263, SER152, ASP79, TYR114, PRO180, ALA178, PHE215, ALA259, LEU264, ILE78, ALA260, ILE209, GLU179, PHE77, GLY76, TRP85, HIS75, ARG256, HIS151
Gypsogenin	−9.2	—	PHE215	PHE215, ILE78, ALA259, ARG256, ASP79, HIS151, HIS263, LEU264, ALA260, PHE77, TYR114, PRO180, ILE209, LEU213
Kaempferol 7-O nonhesperidoside	−8.6	ARG256, GLU179	ILE78, PHE77, PHE215, ALA178, PRO180	ARG256, GLU179, ILE78, PHE77, PHE215, ALA178, TRP252, ALA259, ALA260, TYR267, LEU264, TRP85, ASP79, HIS263, HIS151, GLY76, ILE209, TYR114, SER152, LEU153, PRO180
Linoleic acid	−6.9	HIS151, PHE77, GLY76	ILE78, ALA260, PHE215(3), PRO180, ALA178(2), TYR114(2), HIS263, PHE77	HIS151, PHE77, GLY76, ILE78, ALA260, PHE215, PRO180, ALA178, TYR114, SER152, HIS263, ASP79, LEU153, ILE209, LEU264
Oleic acid	−6.4	HIS151, GLY76, HIS263, PHE77	PRO180(2), ALA178, TYR114(2), PHE215(4), ALA260(2), LEU264, HIS263	HIS151, GLY76, HIS263, PHE77, PRO180, ALA178, TYR114, PHE215, ALA260, LEU264, ILE209, GLU179, HIS75, ARG256, ASP79, ALA259, ILE78, SER152, LEU153
Palmitic acid	−6	ASP79	PHE215(4), TYR114(2), PRO180, ALA178, HIS263, PHE77, ILE78	ASP79, PHE215, TYR114, PRO180, ALA178, HIS263, PHE77, ILE78, GLU179, ILE209, LEU264, HIS151, ARG256, ALA260, SER152, LEU153
Pantothenic acid	−6.1	HIS263, SER152, ASP79	PHE215	HIS263, SER152, ASP79, PHE215, ARG256, PHE77, LEU264, HIS151, GLY76, ALA178, ILE78, ALA260
Phytonadione	−8.6	ARG256	PHE77, TYR114(2), PRO180(2), ALA178, ALA260, ALA259, LEU264, HIS263	ARG256, PHE77, TYR114, PRO180, ALA178, ALA260, ALA259, LEU264, HIS263, ILE209, PHE215, SER152, LEU153, ASP59, HIS151, GLY76, ILE78
α-spinasterol-3-O-β-glucoside	−7.9	ASP79	HIS263, TYR114, PHE77, PHE215, ALA260(2), ALA259, ILE78	ASP79, HIS263, TYR114, PHE77, PHE215, ALA260, ALA259, ILE78, ILE209, PRO180, ALA178, SER152, LEU264, TRP252, ARG256, THR255, LEU153
Stearic acid	−6.3	ARG256	TYR114(2), PRO180(2), ALA178(2), HIS263, PHE215(3), ALA260	ARG256, TYR114, PRO180, ALA178, HIS263, PHE215, ALA260, ILE209, LEU153, PHE77, ILE78, SER152, HIS151, LEU264, ASP79
Stigmasterol	−9.7	–	PHE215(2), PRO180, ILE78, ALA260, TYR114	PHE215, PRO180, ILE78, ALA260, ILE209, PHE77, SER152, HIS263, TRP252, THR255, ARG256, LEU264, ALA259, TYR114
(9E, 12E)-octadeca-9,12 dienoic acid	−6.6	HIS151, GLY76, ASP79, PHE77	TYR114(2), LEU264, ALA260(2), HIS263, PHE215(4), ALA178, PRO180 (2), PHE77	HIS151, GLY76, ASP79, TYR114, LEU264, ALA260, HIS263, PHE215, ALA178, PRO180, HIS75, TRP85, ARG256, ILE78, SER152, LEU153, ILE209, PHE77
Orlistat	−6.5	HIS151, GLY76, PHE77	PRO180, TYR114(2), PHE215(2), ALA259, ILE78, PHE77	HIS151, GLY76, PHE77, PRO180, TYR114, PHE215, ALA259, ILE78, ASP79, ALA260, HIS263, THR255, TRP252, ARG256, ILE209, ALA178, SER152, LEU264

(Świerczewska et al., 2019). Saponins such as gypsogenin isolated from various plant extract exhibited antihyperlipidemic activity by inhibiting *in-vitro* PL enzyme and possess a potential treatment of obesity (Marrelli et al., 2016). Presence of 9,12-Octadecadenoic acid in the seed oil extract of *T. conophorum* exhibited antihyperlipidemic activity on high fat diet induced experimental animals by lowering lipid profile, creatine kinase, lactate dehydrogenase enzyme level, enhancing HDL level (Oriakhi and Uadia, 2020).

3.5. Molecular docking study

Models of the molecular docking interactions between the three most promising compounds, including a comparison to AA, AG and PL are shown in Figs. 4–6. Tables 4–6 represents the details of molecular docking studies of the identified phytoconstituents on AA (PDB ID: 5E0F), AG (PDB ID: 5NN8) and PL (PDB ID: 1LPB) respectively which include Docking score, Conventional hydrogen bonds, Hydrophobic interactions, and active site pocket residues. PyRx 0.8 was used to place the protein-ligand complex. The 2D docking poses of the other identified phytomolecules are shown in supplementary (F 2–4). A study based on *in-silico* and *in-vivo* study of β-sitosterol possesses antidiabetic activity in diabetic rats through glycemic dominance and induction of insulin receptor and GLUT4 activation in the adipose tissue of experimental animals (Ponnulakshmi et al., 2019).

3.5.1. Molecular docking study against α-amylase

The favorable docking scores of phytocompounds against AA (PDB ID: 5E0F) ranged from −9.8 to −5.5 was presented in Fig. 4 (A-D) and Table 4. The most stable were β-sitosterol, Gypsogenin, and Stigmasterol, followed by α-spinasterol-3 glucoside, while the least stable among the 16 compounds was Palmitic acid. Other than Eicosenoic acid, Stigmasterol, and (9E, 12E)-octadeca-9,12 dienoic acid, all phytoconstituents interact with various amino acid residues in the active site. Additionally, the ligands also displayed a wide variety of hydrophobic

interactions, viz. alkyl and pi interactions, including pi-pi stacked, pi-sigma, and pi-alkyl interactions, in addition to the conventional hydrogen bonds. Within the active site, the residues LYS200, GLU233, ASP300, LEU237, and ALA307 are the critical amino acids for interacting with the ligand molecules (Fig. 4E). As reported earlier by Sobhy et al., β-sitosterol and Stigmasterol possess promising inhibitory activity against AA and AG enzymes (Sobhy et al., 2019). In another study, Gypsogenin also markedly inhibited AA compared with Acarbose (Yao et al., 2010) and Kaempferol 7-O-glucoside present in *Careya arborea* leaves showed AA inhibition (Kamble et al., 2022). These findings complement the docking simulation and suggest that these phytocompounds may collectively inhibit *in-vitro* AA activity.

3.5.2. Molecular docking study against α-glucosidase

The docking analysis of phytocompounds against AG (PDB ID: 5NN8) showed satisfactory scores (−8.3 to −4.8) (Fig. 5 (A-D), Table 5). The most stable was β-sitosterol, followed by Kaempferol-7-O-neohesperidoside, while Palmitic acid was the least stable among the 16 compounds. Apart from β-sitosterol, Cholecalciferol, Phytonadione, Stigmasterol and (9E, 12E)-octadeca-9,12 dienoic acid, it was observed that other compounds formed several hydrogen bonds with numerous amino acid residues present in the active site of the protein. Other hydrophobic interactions, such as alkyl and pi interactions, including pi-pi stacking, pi-sigma, and pi-alkyl interactions, exist in addition to the conventional hydrogen bonds. The crucial amino acids (in active sites) are ASP616, PHE649, TRP376, and TRP481, as shown in Fig. 5E β-sitosterol and the aglycone component of Kaempferol 7-O-neohesperidoside showed potential inhibition of AG enzymes (Sobhy et al., 2019; Zhang et al., 2022). 9-hydroxyhexadecanoic acid or palmitic acid derived from brown sea algae possesses strong AA and AG activity according to the docking score (Paramasivam et al., 2023). The above results support the docking simulation; in other words, these phytocompounds might be responsible for inhibiting AG activity *in-vitro*.

3.5.3. Molecular docking study against pancreatic lipase

Molecular docking analysis of the identified phytocompounds against PL (PDB ID: 1LPB) showed significant docking scores (−8.3 to −6). The details have been represented in Fig. 6 (A-D) and Table 6. Stigmasterol, followed by β-sitosterol and Cholecalciferol was found more stable whereas Palmitic acid was the least stable among the 16 compounds. Phytocompounds other than β-sitosterol, Cholecalciferol, Gypsogenin, and Stigmasterol interacted with different amino acids through multiple H-bonds in the active site. The crucial amino acids were found to be PHE215, ILE78, LEU264, HIS263, and PHE77 residues (Fig. 6E). In several literatures, Stigmasterol and β-sitosterol showed good potential inhibition of PL (Ezzat et al., 2022; Ibrahim et al., 2020). In other words, it may be concluded that *in-vitro* inhibition of PL is responsible due to these phytocompounds.

4. Conclusion

The present study explores the dose dependent inhibition potential of *Momordica dioica* Roxb. ex. Willd. leaf and fruit extract against AA, AG and PL enzyme. *M. dioica* Roxb. fruit extract showed better in-vitro antioxidant and enzyme inhibitory activities related to metabolic disorders. The presence of polyphenolics in the *M. dioica* Roxb. fruit extracts may be responsible for *in-vitro* antioxidant as well as all the enzyme inhibitory activities. This observation further validated with molecular docking study to indicate the effect of phytoconstituent related with the desired activities. This study can be effective approach for quality evaluation and therapeutic validation of traditional claim, which may step forward to develop safe and efficacious healthcare alternatives product. *In-vivo* and *in-vitro* studies of the *M. dioica* Roxb. fruits can be done to explore further mechanism. This study further promotes the evidence-based development of functional food components and also support supply food chain.

CRediT authorship contribution statement

Seha Singha: Writing – original draft, Software, Methodology, Formal analysis, Data curation. **Barun Das Gupta:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis. **Arnab Sarkar:** Writing – review & editing, Software, Investigation, Formal analysis. **Sandipan Jana:** Writing – review & editing, Software, Formal analysis, Data curation. **Pardeep K. Bharadwaj:** Writing – review & editing, Resources, Methodology. **Nanaocha Sharma:** Validation, Resources, Methodology. **Pallab K. Haldar:** Writing – review & editing, Validation, Software, Investigation. **Pulok Kumar Mukherjee:** Writing – review & editing, Validation, Funding acquisition, Data curation. **Amit Kar:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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Therapeutic importance of Cucurbitaceae: A medicinally important family

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ABSTRACT

Ethnopharmacological relevance: Medicinal plants of Cucurbitaceae family consist of several edible fruits and vegetables consumed worldwide since ancient times. The plants of this family have played an essential role in the ethnopharmacological as well as traditional medicinal system globally and their evidence is well established in several traditional literatures. Various plant parts have been used to treat several human ailments viz. *Pandu* (anemia), *Pliharoga* (splenomegaly), *Sopha* (inflammation), *Gulma* (tumor growth), *Adhmana* (indigestion. acidity), *Garavisa* (poisoning) etc.

Aim of the review: This review article aims to systematically document and bridge scientific evidences with the ethnopharmacological, ethnoveterinary and folklore claims along with the therapeutic efficacy with mechanism of action found in different literature, books, and scientific articles belonging to the Cucurbitaceae family.

Materials and methods: To construct the manuscript a comprehensive literature review was done based on the information collected from Ayurvedic Pharmacopoeia of India; books, research articles and databases such as ScienceDirect, Wiley Online Library, SciFinder, Scopus, Springer, Google Scholar, Web of Science, ACS Publications and PubMed.

Results: The plants of Cucurbitaceae family are rich in phytochemicals like terpenoids, glycosides, alkaloids, saponins, tannins, steroids, etc., responsible for the therapeutic effect. Various parts of these plants such as leaves, stems, flowers, fruits, seeds, roots etc. exhibit a plethora of pharmacological activity viz. hypolipidemic, antihyperglycemic, anticancer, antimicrobial, analgesic, anti-inflammatory, anti-stress and immunomodulatory activities. Also, *in-vitro* and *in-vivo* reports suggest strong inhibitory potential against α-glucosidase, α-amylase, lipase, carbonic anhydrase enzyme along with antioxidant, anti-inflammatory, antidiabetic, anti-tumor, antifungal, etc. Furthermore many reports suggest these plants are beneficial for nutritional, economical and ethnoveterinary uses.

Conclusions: The current review enlightens the therapeutic potential of the gourd family, comprising of the geographical origins, morphology, phytochemistry, ethnopharmacology, ethnoveterinary, nutritional importance, therapeutic benefits, safety, efficacy and related aspects. The phytochemical and pharmacological potential indicated will popularize this family as a potential source of novel therapeutic agents and functional foods. This study will help to validate the therapeutic claims of several ethnomedicinal uses of this plant family. Furthermore the Cucurbitaceae family needs to be evaluated based on the combine approaches of chemoprofiling and bioexploration to develop the concept of food as medicine for the development of new generation therapeutics leading to the human wellness.

1. Introduction

Cucurbitaceae is the largest family of vegetable and fruit crops, which includes approximately 125 genera and 960 species. Vegetables of the Cucurbitaceae family are part of ancient medicine and culinary traditions. It is mentioned in Ayurveda and folk medicine in India for their therapeutic importance and may be considered as the potential source for the development of safe and effective therapeutics (Mukherjee, 2019). The plants of this

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family are further classified into two major subfamilies, Cucurbitoideae and Zanonioideae based on their morphological, cytological and floral characteristics. Most of the edible varieties originated from the subfamily Cucurbitoideae which can be further classified into 15 tribes and related genera. Specifically, there are four tribes *viz.* Benincaseae, Cucurbiteae, Momordiceae, Sicyoeae which mainly produces edible food plants in the Indian subcontinent (Renner and Pandey, 2013). The members of Cucurbiteae tribe produce economically valuable fruits which include crops like squashes (*Cucurbita* spp.), gourds (*Cucurbita* spp.), luffas (*Luffa* spp.), and melons (*Cucumis melo*). A detailed list of different tribal classification and the genera of the Cucurbitaceae family food plants has been shown in Fig. 1. Several therapeutic benefits have been reported of these food plants of Cucurbitaceae family including *Benincasa hispida* (Wax Gourd), *Benincasa fistulosa* (Apple Gourd), *Coccinia grandis* (Ivy Gourd), *Lagenaria siceraria* (Bottle gourd), *Cucumis melo* (Musk melon), *Cucumis sativus* (Cucumber), *Cucurbita maxima* (Pumpkin), *Cucurbita pepo* (Field pumpkin) *Citrullus lanatus* (Watermelon), *Luffa acutangula* (Ridge gourd), *Luffa cylindrica* (Sponge gourd) *Trichosanthes dioica* (Pointed gourd), *Trichosanthes cucumerina* (Snake gourd), *Momordica dioica* (Spine gourd), *Momordica charantia* (Bitter gourd) etc (Dhiman et al., 2012; Saboo et al., 2013; Avinash and Rai, 2017). The utilisations of different parts of Cucurbitaceae plants are observed for nutritional, medicinal, ethnoveterinary purposes. Apart from human consumption, the plants of this family are used as an essential component of poultry and aqua feed. In addition, some fruits of the Cucurbitaceae family offer potential application in the cosmetic industries (Ajuru and Nmom, 2017). With this background, this review will focus on various aspects of Cucurbitaceae family in relation to its phytochemical, pharmacological, therapeutic, nutritional and toxicological aspects in brief highlighting the importance of this plant family in exploring drugs from the botanicals in order to develop safe and efficacious new generation therapeutics.

2. Ethnopharmacological relevance of Cucurbitaceae family

Plants belonging to the Cucurbitaceae family are very popular for their consumption as food in both raw and cooked form, globally. The importance of these food plants in traditional medicine, including Ayurveda and other systems of medicine in India is well documented (Renner and Pandey, 2013). An ethnomedicinal survey indicates widespread therapeutic use of cultivated Cucurbitaceae family plants in the treatment of various ailments among tribal medicinal practitioners from India and Bangladesh (Rahmatullah et al., 2012). Plants like *Momordica charantia* (Bitter gourd), *Lagenaria siceraria* (Bottle gourd), *Citrullus lanatus* (Watermelon), *Coccinia grandis* (Ivy gourd), *Cucumis melo* (Musk melon), *Momordica cochinchinensis* (Gac), *Trichosanthes kirilowii* (Chinese cucumber) and others are useful in the treatment of diabetes and edema, whereas *Citrullus lanatus* (Watermelon), *Benincasa hispida* (Wax gourd), *Coccinia grandis* (Ivy gourd) and *Lagenaria siceraria* (Bottle gourd) were reported to be used in cardiac problems. *Luffa cylindrica* (Sponge gourd), *Momordica charantia* (Bitter gourd), *Momordica cochinchinensis* (Gac), *Trichosanthes dioica* (Pointed gourd), *Trichosanthes kirilowii* (Chinese cucumber) were observed to be beneficial for the patients of cancer and tumor (Shrivastava and Roy, 2013).

Citrullus colocynthis (Bitter apple) known as *'Indravaruni'* (Sanskrit) and also Rakhal (Bengali) having several therapeutic uses such as *'Krimiroga'* (worm infestation), *'Kamala'* (jaundice), *'Svasa'* (asthma), *'Kasa'* (cough), *'Kustha'* (skin related disorder), *'Gulma'* (tumor growth), *'Udararoga'* (intestinal disorder) as depicted in Ayurvedic text (Anonymous, 2001). The important formulation of this plant is 'Jvaraghni Gutika' (II). *Luffa acutangula* (Ridge gourd) is known as 'Kosataki' in Sanskrit and Zinga in Bengali. The whole plant is used to treat many diseases like *'Kustha'* (skin related disorders), *'Pandu'* (anaemia), *'Pliharoga'* (splenomegaly), *'Sopha'* (inflammation), *'Gulma'* (tumor growth), *'Adhmana'* (indigestion. Acidity), *'Garavisa'* (poisoning), *'Arsa'* (piles) used in the traditional Ayurvedic formulation of Abhaya Lavana. *Lagenaria siceraria* (Bottle gourd) also known as 'Tumbini' (Sanskrit) or Lau (Bengali) is a constituent of 'Maha Visagarbha Taila' a polyherbal oil formulation used in joint inflammation. Fresh fruit parts of *Lagenaria siceraria* having therapeutic activity against *'Jvara'* (fever), *'Kasa'* (cough), *'Svasa'* (asthma), *'Visa Roga'* (poisoning), *'Sopha'* (inflammation), *'Vrana'* (ulcer), *'Sula'* (pain) (Anonymous, 2001). 'Kusmanda' (Sanskrit) or Chal kumra (Bengali) or *Benincasa hispida* (Wax gourd) is an important plant that is mentioned in Ayurveda. Dried fruits of *Benincasa hispida* is known for their healing properties against *'Mutraghata'* (urinary disorder), *'Prameha'* (metabolic disorder), *'Mutrakrcchra'* (dysuria), *'Asmari'* (kidney stones), *'Trishna'* (thirst), *'Manasa Vikara'*

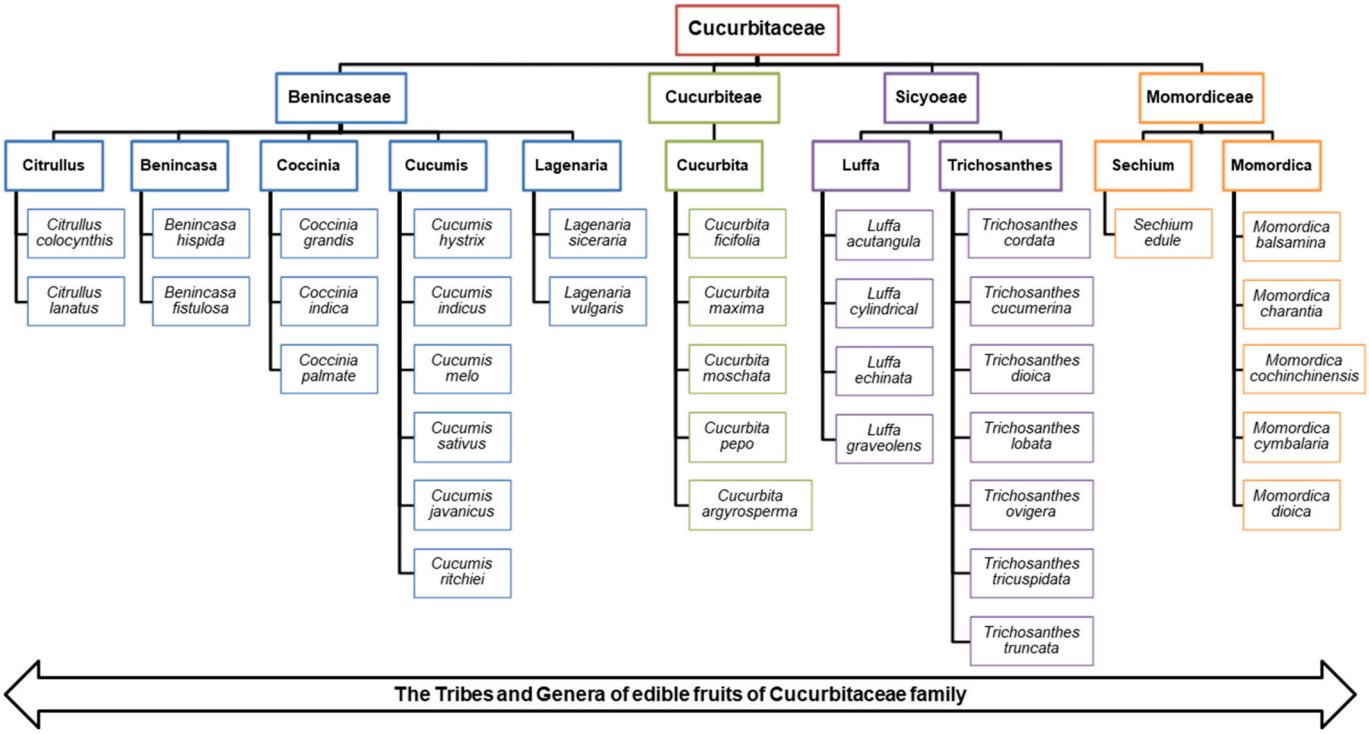


Fig. 1. Different tribal classification and genera of the Cucurbitaceae family food plants.

(mental disorder), *'Malabandha'* (constipation) and used in Ayurvedic formulations like 'Kusmandaka Rasayana', 'Dhatryadi Ghrita', 'Vastyamayanaka Ghrita' (Anonymous, 2004). Ayurvedic herb Karkasa consist of dried root of *Momordica dioica* (Spine gourd) and commonly known as Titkaankrol (Bengali) used in several Ayurvedic formulations like 'Hiraka Rasayana', 'Visanasaka Yoga' (Ayurvedic Prakasa), 'Kakadani Taila', 'Kalagnirudra Rasa', 'Sannipata Vidhvamsa', 'Candrarudra Rasa' with remedial potential to treat several diseases such as *'Visarpa'* (oro facial herpes), *'Sarpavisavikara'* (snake poisoning), *'Mutrakrcchra'* (dysuria), *'Sarpavisa'* (snake poison), *'Jvara'* (fever), *'Kasa'* (cough), *'Svasa'* (Asthma), *'Hikka'* (hiccough), *'Arsa'* (piles), *'Ksaya'* (debility), *'Raktarsa'* (bleeding piles), *'Madhumeha'* (diabetes), *'Netraroga'* (eye diseases), *'Siroroga'* (Neuro disorder), *'Kamala'* (jaundice), *'Asmari'* (Kidney stones). *Cucumis sativus* (Cucumber) is an annual trailing plant commonly known as 'Trapusam' (Sanskrit) or Shashaa (Bengali). Dried seeds are used against *'Mutraghata'* (obstructive uropathy), *'Mutrakrcchra'* (dysuria), *'Raktapitta'* (bleeding disorder), *'Daurbalya'* (general debility), *'Daha'* (burning sensation), *'Raktavikara'* (blood disorder), *'Anidra'* (Chronic insomnia), *'Sirah Sula'* (headache), *'Chardi'* (vomiting), *'Sitajvara'* (cold fever). 'Dadhika Ghrita' is an important formulation of *Cucumis sativus*. *Trichosanthes bracteata* (Bitter snake gourd) known as 'Visala' (Sanskrit) or Maakal (Bengali) and is used in formulations like 'Paniya kalyanka Ghrita' and 'Visaladi Churna'. Dried roots of this plant are used in various restorative activity like *'Jvara'* (fever), *'Amadosa'* (food poisoning), *'Prameha'* (metabolic disorder), *'Antarvrddhi'* (swelling in the abdominal region), *'Kustha'* (skin related disorder), *'Stanapida'* (breast congestion in galactopoiesis), *'Kamala'* (jaundice), *'Silpada'* (elephantiasis), *'Vrddhi'* (hyper functional), *'Plihodara'* (splenomegaly), *'Svasa'* (asthma), *'Kasa'* (cough), *'Gulma'* (tumor growth), *'Granthi'* (cystic swelling), *'Vrana'* (ulcer), *'Mudhagarbha'* (obstructed labour) (Anonymous, 2006). The leaves and stems of *Coccinia grandis* (Ivy gourd) or 'Bimbi' (Sanskrit) or Tela kuccha (Bengali) used to treat and cure *'Aruci'* (Tastelessness), *'Prameha'* (Metabolic disorder), *'Raktapitta'* (Blood disorder), *'Pravaahikaa'* (Dysentry). 'Bimbighratam', 'Bimbi Guna', 'Bimbi Phala Guna', 'Bimbigunah', 'Bimbi Gunam', 'Bimbi Saka', 'Bimbi Puspam', 'Bimbi Phalam', 'Tundi Ghrt', 'Kunadaru guna', 'Tiktatundi Guna', 'Bimbaghritam', 'Bimbi (Pancanga)', 'Bimbiphalam' are important Ayurvedic formulations of this plant. In Ayurvedic medicine Sukanasa consisted of the rhizomes of *Corallocarpus epigaeus* (Red fruit creeper) also known as 'Mirchiakand' is used in the treatment of *'Amavata'* (rheumatism), *'Aruci'* (tastelessness), *'Atisara'* (diarrhoea), *'Daha'* (burning sensation), *'Hikka'* (hiccup), *'Jirna Antrasotha'* (chronic intestinal pain), *'Jirnajvara'* (chronic fever), *'Jvara'* (fever), *'Kasa'* (cough), *'Krimi rogue'* (worm infestation), *'Pravahika'* (dysentery), *'Sarpa visa'* (snake poison), *'Sotha'* (inflammation), *'Svasa'* (asthma), *'Vatakapha Jvara'* (fever due to Vata Kapha), *'Visphotaka'* (blister eruption), *'Vrana'* (ulcer), *'Yoni roga'* (disease of female genital tract). 'Kasmaryadi Ghrita' is one of the important formulations of Sukanasa (Anonymous, 2008).

Traditionally *Momordica dioica* (Spine gourd) is used for various ailments. Fresh fruit juice is used in hypertension, unripe fruits in diabetes, tender fruits for acne and pimples and other uses include diuretic, laxative, hepatoprotective, anti-inflammatory, anti-pyretic, anti-asthmatic (Talukdar and Hossain, 2014). The fruits of *Momordica charantia* (Bitter gourd) used as an anthelmintic, stomachic, antibilious, laxative, antirheumatic and antidiabetic. Traditionally the juice of fresh leaves was consumed by children for its mild purgative effect. The leaf juice with a little bit of salt was also used to treat migraine as a nasal drop. A root decoction of *Momordica charantia* possesses abortifacient effect. Due to the abundant calcium and phosphorous content of *Sechium edule* (Chayote), it is consumed widely in many countries including India. The fruits have a cooling and stomachic effect whereas the leaf juice is used to treat depression. Traditionally leaf juice of *Luffa cylindrica* (Sponge gourd) is used to treat conjunctivitis, fruits are used to cure Jaundice, Roots are a potent laxative and the seed oil is used in various skin disorder (Khulakpam et al., 2015). *Cucumis sativus* (Cucumber) is traditionally used to cure constipation and improve digestion and act as demulcent; seeds also have a cooling, diuretic and anthelmintic activity. The fruits (whole) of *Cucumis melo* (Musk melon) is traditionally used to treat chronic eczema and exhibits laxative, galactagogue, diuretic and diaphoretic effect. In Chinese medicinal system the seeds of *Trichosanthes kirilowii* (Chinese cucumber) are widely utilized as an anti-inflammatory agent and an expectorant (Saboo et al., 2013). *Benincasa fistulosa* (Apple gourd) is commonly known as Tinda and traditionally used as a pesticide and fodder (Gautam et al., 2011). The rind of *Benincasa hispida* (Wax gourd) fruit is known to be a potent diuretic and the ashes of the rind as analgesic (Pagare et al., 2011). Leaves and stems of *Trichosanthes dioica* (Pointed gourd) are hypocholesterolemic, hypoglycemic, hypophospholipemic. Fruits are used as mouth freshener and for the treatment of fever, wounds and boils. Various parts of *Coccinia grandis* (Ivy gourd) possess therapeutic effect to treat various human ailment. The leaves are used to cure several skin diseases, roots are used against osteoarthritis whereas the fruits are potent antidiabetic, anti-anaphylactic and antihistaminic and used to treat leprosy, fever, asthma, bronchitis and jaundice. *Benincasa hispida* (Wax gourd) is traditionally used to treat diabetes and obesity for its high nutritive and low calorific value. The fruit juice is useful to heal peptic ulcer, haemoptysis, respiratory trouble, internal haemorrhage discharges. It also mitigates the periodic attacks of hysteria, convulsion and other nervous diseases. *Citrullus lanatus* (Watermelon) acts as a natural diuretic which helps to clean kidney and bladder and is recommended during menstruation and pregnancy. Fatigue, typhoid, malnutrition, scanty urination, asthma attack, indigestion, hypercholesteromia, arthritis etc. can also be treated by the fruits of *Citrullus lanatus* (Khulakpam et al., 2015). The seeds of *Telfairia occidentalis* (Fluted gourd) are used in traditional medicine to cure anaemia, convulsion, cardiovascular diseases, liver attack and also stimulate breast milk secretion in women after delivery (Teugwa et al., 2013). In folklore medicine, the seeds of *Cucurbita maxima* (Pumpkin) are used to enhance the functioning of the urinary bladder and inhibit the formation of kidney stones (Smith, 1997). It is also used traditionally in Indian and Chinese medicine as an anti-inflammatory, antiviral, antidiabetic and antioxidant; the seeds have also been used as a vermifuge (Perez-Gutierrez, 2016). In India, Iberian Peninsula, Argentina, *Cucurbita maxima* seeds orally consumed for the management of digestive disorders (Salehi et al., 2019). *Bryonia dioica* (White Bryony) is used as a potent cathartic, cytotoxic, diaphoretic, expectorant, hydrogogue, irritant, pectoral, purgative and also as a vermifuge. Various inflammatory conditions, bronchial complaints, asthma, intestinal ulcers, hypertension and arthritis have been traditionally treated by *Bryonia dioica*. *Cucurbita ficifoila* (Black seed squash) is mainly employed for the treatment of haemorrhoids, fever and also diabetes type 2 (Saboo et al., 2013). In Thai traditional medicine *Trichosanthes tricuspidata* (Bitter snake gourd) is extensively used as a laxative, anthelmintic and also in the treatment of migraine (Dhiman et al., 2012). In countries such as China, India, Argentina, Brazil, and Mexico *Cucurbita pepo* (Field pumpkin) is traditionally used to treat patients internally and externally for worms and parasites. In Africa, seeds of *Cucurbita pepo* are used to treat tapeworm, bladder and kidney disorders. *Citrullus colocynthis* (Bitter apple) was used as hair growth promoter, purgative, analgesic, and abortifacient agent. In Native America, it is used as a traditional medicine to heal urinary ailments and intestinal worms and the seeds are used to treat high blood pressure and prevent kidney stones. The leaves of *Citrullus colocynthis* is used as cholagogue and roots are useful in breast inflammation, uterine and arthritic pain. The traditional healers in India prescribe *Trichosanthes cucumerina* (Snake gourd) for the treatment of headaches, abdominal tumors, fever, diarrhoea, and several skin allergies (Rolnik and Olas, 2020). Ethnopharmacological and ethnomedicinal uses of medicinal food plants of the Cucurbitaceae family have been shown in Fig. 2.

3. Geographical distribution and morphological properties

The Cucurbitaceae family is predominantly tropical, having 90% of the species in three main areas; Africa and Madagascar, Central, and South America and Southeast Asia (Saboo et al., 2013; Avinash and Rai, 2017). According to United Nations' Food and Agriculture Organization (FAO) *Citrullus lanatus* (Watermelon) is the most cultivated plant with an average

area of 2.5 million ha and between 1996 and 1998 annual production of *Citrullus lanatus* fruits was 46.6 million tons. China, Turkey, Iran and Ukraine are major cultivators of Cucurbitaceae family plants. Argentina produces *Cucurbita pepo* (Field pumpkin) and *Cucurbita maxima* (Pumpkin) and the United States also produces Cucurbitaceae food plants like *Cucumis sativus* (Cucumber), *Cucumis melo* (Musk melon) and *Citrullus lanatus* (Watermelon). Brazil majorly produces *Cucurbita pepo* (Field pumpkin), *Citrullus lanatus* (Watermelon) and *Cucumis melo* (Musk melon), whose total production in 1995 was 535 million fruits harvested from an area of 206,000 ha (Bisognin, 2002). In India, the highest number of species of Cucurbitaceae family plants is mostly found in the east, northeast India (Assam, Manipur, Mizoram, Tripura, Sikkim, West Bengal) and peninsular India (Kerala, Karnataka, Tamil Nadu, Andhra Pradesh) state and Bangladesh. Western regions of the Himalayas such as Jammu, Kashmir and Himachal Pradesh have the lowest number of Cucurbitaceae plant species. From the photographic perspective, species of this plant family vary from Africa to India such as *Corallocarpus epigaeus* (Red fruit creeper), *Corallocarpus schimperi*, *Cucumis prophetarum* (Wild gourd), *Blastania cerasiformis* (Cherry bur cucumber), *Corallocarpus conocarpus*, *Coccinia grandis* (Ivy gourd), *Luffa echinata* (Bitter sponge gourd), *Momordica cymbalaria, Dactyliandra welwitschii* and *Zehneria thwaitesii*. In both India and Africa Diplocyclos and Kedrostis genus are widely found. Medicinally important plants of Cucurbitaceae family are originated in India and other parts of Western Asia, due to the wide range of climatic zones. In India, the plants are mostly cultivated in Uttar Pradesh, Uttarakhand, Madhya Pradesh, West Bengal, Gujrat, Bihar, Karnataka, Tamil Nadu and Maharashtra (Renner and Pandey, 2013). There are some species found in the foothills of the Himalayas, which cannot be cultivated due to their extreme bitterness as well as dormancy in seeds and delayed maturity. Although there are some varieties found in China and the Middle East. The plants of the Cucurbitaceae family grow vigorously in warm temperature, which helps in faster germination of seeds and loamy soil mostly enriched with nutrients and moisture. The well-irrigated, fertilized land with a lot of vertical and horizontal growing rooms and well-drained soil containing clay, sand is required for the development of most plants belonging to this family and should avoid a potential frost encounter during its cultivation. The growth of some climber plants of the Cucurbitaceae family can be controlled by using some fence, rallies or other vertical structure (McCreight, 2017). The geographical distribution of Cucurbitaceae family plants has been described in Table 1 (Renner and Pandey, 2013; Mitra et al., 2012).

In Egypt, the cultivated varieties and landraces of Cucurbitaceae family plants exhibit different morphological characters include vegetative, floral, fruit and seed characters types distributed among 27 taxa, belonging to three genera, six species, and five subspecies where a significant correlation can be observed between seed size and growth parameters whereas seed volume and leaf size showed the best correlation to identify different varieties (Rabei et al., 2013). Plants of the Cucurbitaceae family are mostly annual or perennial. The roots are taproot, branches get thickened due to storage of food and water. The stem is herbaceous, climbing by means of tendrils, rooting at nodes, with angular decumbent vines. Leaves are alternate, broad, usually simple, but often deeply lobed or divided and palmately veined, reticulate, petiole long and hollow. Tendrils may be simple or branched, arising in the Axil or opposite to the leaf at the node. Leaves and stems contain plenty of juicy sap (Dhiman et al., 2012). The floral characters of the species exhibit a range of inflorescence, white or yellow flowers often solitary, large and showy or sometimes in racemes or cymes or in panides, monoecious (e.g., *Luffa* spp., *Cucumis* spp.) or dioecious (e.g., *Trichosanthes* spp.). The male flowers are usually produced in a much larger number, campanulate in nature. The calyx is divided into five sepals and fused, pointed, petaloid, campanulate, estimation is embarked. The corolla consists of five petals, fused in *Momordica* spp. Only at the base, in *Cucurbita* spp. throughout and campanulate, or free in *Luffa* spp.; often deeply five-lobed, velvet, imbricate, inserted on calyx tube when the free form of corolla may be campanulate or rotate. The androecium consists of five stamens, sometimes three, free or combined to form a central column inserted on the calyx tube. The female flowers are fewer than the male flowers. The fruits are soft, fleshy, and generally indehiscent and sometimes of enormous size (Saboo et al., 2013). Fig. 3 represents some fruits of selected medicinal food plants of the Cucurbitaceae family.

4. Phytochemical profile

The plants belonging to the Cucurbitaceae family have immense medicinal importance owing to the presence of various phytoconstituents like tannins, glycosides, carbohydrates, resins, saponins, carotenoids and phytosterols and most importantly triterpenoid cucurbitacins. The details of primary and secondary metabolites have

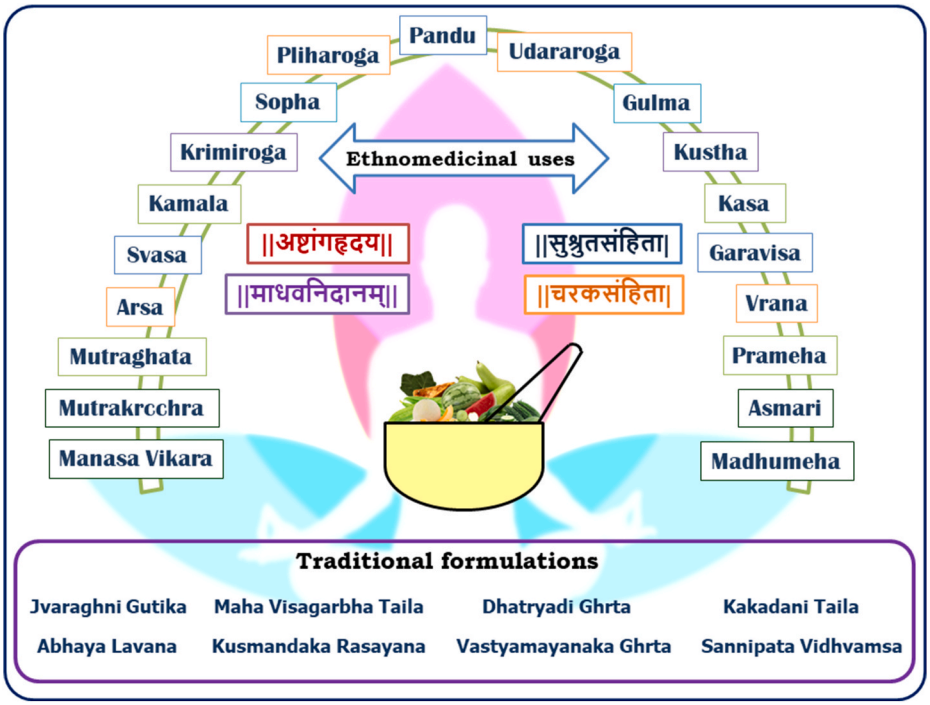


Fig. 2. Ethnopharmacological relevance of Cucurbitaceae medicinal food plants.

Table 1
Geographical distribution of plants from Cucurbitaceae family (Renner and Pandey, 2013; Mitra et al., 2012).

Sl No.	Binomial name	Distribution in India	Distribution outside India
1.	*Actinostemma tenerum*	Arunachal Pradesh, Assam, Bihar, Meghalaya, Mizoram, Uttar Pradesh, West Bengal	Bangladesh, Vietnam, Laos, Cambodia, Russia, China, Taiwan, Korea, Japan
2.	*Benincasa fistulosa* (Apple Gourd)	Punjab, Rajasthan, Uttar Pradesh	Tropical Africa
3.	*Benincasa hispida* (Wax gourd)	Cultivated in tropical and subtropical regions of India	Pakistan, Malayasia, Eastern Australia, Polynesia, China, Japan
4.	*Blastania cerasiformis*	Wild on wastelands in Gujarat	Old World tropics from Mauritania and Senegal east to Pakistan and in Estern Africa south to Transvaal
5.	*Blastania garcinii*	Andhra Pradesh, Delhi, Gujarat, Haryana, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Odisha, Punjab, Rajasthan, Tamil Nadu, Uttar Pradesh	Sri Lanka
6.	*Bryonia aspera*	Jammu (Upper Chenab Valley), Himachal Pradesh	Turkey, Iran, Georgia, Armenia, Azerbaijan, Turkmenistan, Norther Afghanistan, Pakistan
7.	*Bryonia monoica*	Probably near the Pakistani border	Kazakhstan, Uzbekistan, Kirgizstan, Turkmenistan, Afghanistan, Iran, Pakistan
8.	*Citrullus colocynthis* (Bitter apple)	Andhra Pradesh, Assam, Bihar, Jharkhand, Delhi, Goa, Gujarat, Karnataka, Kerala, Maharashtra, Odisha, Punjab, Rajasthan, Tamil Nadu, Uttar Pradesh	Afghanistan, Myanmar, Pakistan, Sri Lanka, west to the Sahara (Lybia) and Sahel region
9.	*Citrullus lanatus* (Water melon)	Andaman and Nicobar Islands, Assam, Bihar, Jharkhand, Delhi, Gujarat, Karnataka, Madhya Pradesh, Maharashtra, Punjab, Rajasthan, Tamil Nadu, Tripura, Uttar Pradesh, Uttarakhand, West Bengal	Nepal, Pakistan, native to tropical Africa
10.	*Coccinia grandis* (Ivy Gourd)	Andaman and Nicobar Islands, Andhra Pradesh, Assam, Bihar, Jharkhand, Goa, Gujarat, Himachal Pradesh, Karnataka, Kerala, Lakshadweep, Madhya Pradesh, Maharashtra, Manipur, Odisha, Rajasthan, Tamil Nadu, Tripura, Uttarakhand, West Bengal	Africa, China, Japan, Malesia, Myanmar, Pakistan, Sri Lanka
11.	*Corallocarpus conocarpus*	Gujarat, Karnataka, Maharashtra, Rajasthan, Tamil Nadu	Pakistan, Central Africa
12.	*Corallocarpus epigaeus* (Red fruit creeper)	Andhra Pradesh, Assam, Bihar, Gujarat, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Punjab, Rajasthan, Tamil Nadu, Uttar Pradesh, West Bengal	Baluchistan, Pakistan, Sri Lanka, tropical East Africa, Sudan
13.	*Corallocarpus schimperi*	Unclear	Pakistan and tropical East Africa and Arabia
14.	*Cucumis hystrix* (wild cucumber)	Arunachal Pradesh, Assam, Meghalaya, Mizoram	Myanmar, North and West Thailand, South West China
15.	*Cucumis indicus*	Kerala, Maharashtra	….
16.	*Cucumis javanicus*	Assam	Java, China, Thailand
17.	*Cucumis leiospermus*	Andhra Pradesh, Assam, Karnataka, Madhya Pradesh, Maharashtra, Manipur, Rajasthan, Sikkim, Tamil Nadu	Sri Lanka
18.	*Cucumis maderaspatanus* (Madras pea pumpkin)	Andhra Pradesh, Arunachal Pradesh, Bihar, Delhi, Gujarat, Himachal Pradesh, Karnataka, Madhya Pradesh, Maharashtra, Mizoram, Rajasthan, Tamil Nadu, Tripura	Bhutan, China, Myanmar, Nepal, Pakistan, Sri Lanka
19.	*Cucumis melo* (Musk melon)	Andhra Pradesh, Assam, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Manipur, Rajasthan, Tamil Nadu, Tripura, Uttar Pradesh	Widely cultivated
20.	*Cucumis prophetarum*	Andhra Pradesh, Goa, Gujarat, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Punjab, Rajasthan, Tamil Nadu	Pakistan to North Africa
21.	*Cucumis ritchiei*	Karnataka, Kerala, Maharashtra, Punjab, Tamil Nadu	… …
22.	*Cucumis sativus* (Cucumber)	Northern India (Ganges region)	Bhutan, China, Myanmar, Nepal, Thailand
23.	*Cucumis setosus*	Gujarat, Karnataka, Madhya Pradesh, Maharashtra, Rajasthan	Unclear
24.	*Cucumis silentvalleyi*	Kerala	Unclear
25.	*Cucurbita argyrosperma* (Cushaw pumpkin)	Cultivated throughout the India	Native to Mesoamerica, widely cultivated
26.	*Cucurbita ficifolia* (Black seed pumpkin)	Meghalaya	Native to Mesoamerica or northern South America, widely cultivated
27.	*Cucurbita maxima* (Pumpkin)	Andhra Pradesh, Arunachal Pradesh, Assam, Bihar, Goa, Gujarat, Himachal Pradesh, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Rajasthan, Tamil Nadu, Tripura, Uttar Pradesh	Native to Central America
28.	*Cucurbita moschata* (Crookneck pumpkin)	Arunachal Pradesh, Assam, Bihar, Delhi, Goa, Himachal Pradesh, Kerala, Madhya Pradesh, Maharashtra, Manipur, Mizoram, Rajasthan, Sikkim, Tamil Nadu, Tripura, Uttar Pradesh	Native to Central or South America
29.	*Cucurbita pepo* (Field pumpkin)	Arunachal Pradesh, Assam, Bihar, Delhi, Goa, Himachal Pradesh, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Manipur, Mizoram, Punjab, Tamil Nadu, Tripura, Uttar Pradesh	Native to Central or South America
30.	*Cyclanthera pedata* (Kaywa)	Cultivated in northern India	Native to South America, cultivated in Bhuthan
31.	*Dactyliandra welwitschii*	Gujarat, Haryana, Rajasthan	Southwest Africa (Namibia, Angola), coastal West Pakistan (Karachi)
32.	*Diplocyclos palmatus*	Andhra Pradesh, Arunachal Pradesh, Bihar, Jharkhand, Goa, Gujarat, Himachal Pradesh, Jammu and Kashmir, Karnataka, Kerala, Madhya Pradesh, Chhattisgarh, Maharashtra, Manipur, Rajasthan, Tamil Nadu, Tripura, Uttar Pradesh	Bhutan, China, Nepal, Pakistan, Th ailand, South Japan, Sri Lanka, Philippines, Indonesia, Peninsular Malaysia, Papua New Guinea, North East Australia
33.	*Gomphogyne cissiformis*	Arunachal Pradesh, Himachal Pradesh, Mizoram, Sikkim, Uttar Pradesh, West Bengal	Nepal, Bhutan, China (Yunnan)
34.	*Gynostemma pentaphyllum* (Jiaogulan)		Bangladesh, Bhutan, China, Myanmar, Sri Lanka

(*continued on next page*)

Table 1 (*continued*)

Sl No.	Binomial name	Distribution in India	Distribution outside India
		Cultivated in Arunachal Pradesh, Assam, Himachal Pradesh, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim, Tamil Nadu, Tripura, Uttar Pradesh, West Bengal	
35.	*Hemsleya macrocarpa*	Assam, Arunachal Pradesh, Manipur, Nagaland	China (Yunnan)
36.	*Herpetospermum darjeelingense*	Arunachal Pradesh, Sikkim, West Bengal	Bhutan, Nepal, China (Xizang)
37.	*Herpetospermum pedunculosum* (Himalayan bitter gourd)	Arunachal Pradesh, Assam, Himachal Pradesh, Manipur, Meghalaya, Nagaland, Sikkim, Uttar Pradesh, West Bengal	Bhutan, Nepal, China
38.	*Herpetospermum tonglense*	Assam, Manipur, Sikkim, West Bengal, Eastern Himalyan ranges	China, Nepal, Myanmar
39.	*Hodgsonia heteroclite* (Chinese lard seed)	Arunchal Pradesh, Assam, Meghalaya, Sikkim, Tripura, West Bengal	Bangladesh, Bhutan, Cambodia, Laos, Myanmar, Thailand, Vietnam
40.	*Indofevillea khasiana*	Arunachal Pradesh, Assam, Meghalaya	Bhutan, Tibet
41.	*Kedrostis courtallensis*	Andhra Pradesh, Karnataka, Kerala, Maharashtra, Tamil Nadu	Myanmar, Sri Lanka
42.	*Kedrostis foetidissima*	Andhra Pradesh, Gujarat, Karnataka, Madhya Pradesh, Maharashtra, Tamil Nadu	West African species cultivated in Bangladesh, Myanmar, Pakistan, Sri Lanka
43.	*Lagenaria siceraria* (Bottle gourd)	Native and cultivated throughout India	Native of tropical Africa
44.	*Luffa cylindrica* (Sponge gourd)	Native and cultivated throughout India	widely cultivated in Egypt and Sudan
45.	*Luffa acutangula* (Ridge gourd)	Native and cultivated throughout India	Cultivated worldwide
46.	*Luffa echinata* (Bitter sponge gourd)	Assam, Bihar, Gujarat, Himachal Pradesh, Madhya Pradesh, Maharashtra, Tamil Nadu, Uttar Pradesh, Uttarakhand, West Bengal	Wild from Egypt to Niger
47.	*Luffa graveolens*	Bihar, Maharashtra, Sikkim, Uttar Pradesh	Nepal
48.	*Momordica balsamina* (Balsam apple)	Cultivated in Gujarat, Haryana, Rajasthan	Native in the dry savannas of Southern most Africa and the northern margin of the tropical belt, Naturalized in parts of tropical Asia, the Americas and most of the Pacific islands
49.	*Momordica charantia* (Bitter gourd)	Western and Eastern Ghats, Chhattisgarh (Bastar), Jharkhand and all over Central and South India	Native in tropical and subtropical Africa, naturalized in parts of tropical Asia
50.	*Momordica cochinchinensis* (Spiny bitter cucumber)	Andaman and Nicobar Islands, Arunachal Pradesh, Assam, Bihar, Karnataka, Manipur, Nagaland, Rajasthan, Tamil Nadu, Tripura, Uttar Pradesh, West Bengal	Native in the West to New Guinea, Australia in the Southeast
51.	*Momordica cymbalaria*	Andhra Pradesh, Karnataka, Madhya Pradesh, Maharashtra, Tamil Nadu	North and East Africa
52.	*Momordica denudata*	Gujarat, Maharashtra, Karnataka, Kerala	Sri Lanka
53.	*Momordica dioica* (Spine gourd)	Deccan plateau and Central India	Bangladesh, China, Myanmar, Nepal, Pakistan
54.	*Momordica sahyadrica*	Kerala	Unclear
55.	*Momordica subangulata*	Karnataka, Kerala, Maharashtra, Meghalaya, Sikkim, Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim, Tripura, West Bengal	China, Bangladesh, Indonesia (Java, Sumatra), Laos, Peninsular Malaysia, Myanmar, Thailand, Vietnam
56.	*Neoalsomitra clavigera*	Arunachal Pradesh, Assam, Haryana, Himachal Pradesh, Jammu and Kashmir, Manipur, Meghalaya, Sikkim, Punjab, Uttar Pradesh, West Bengal	Bangladesh, Bhutan, Myanmar, South China (Yunnan, Hainan), Vietnam, Laos, Cambodia, North Sumatra, Philippines, east to north east Australia (Queensland), Pacific (Solomon Island and east to Fiji)
57.	*Schizopepon bicirrhosus*	Northeast India	China, Myanmar
58.	*Schizopepon longipes*	Northeast India	China, Myanmar
59.	*Schizopepon macranthus*	Northeast India	China (West Sichuan and North West Yunnan).
60.	*Sicyos edulis* (Squash)	Cultivated throughout India	Native to Mexico, cultivated throughout the tropics
61.	*Siraitia sikkimensis*	Sikkim, West Bengal	China (South Yunnan)
62.	*Solena amplexicaulis* (Creeping Cucumber)	Tamil Nadu, Karnataka, Kerala	Unclear
63.	*Solena heterophylla*	Widely distributed all over India	North East Afghanistan, Indonesia (Java), Peninsular Malaysia, Myanmar, Nepal, Thailand, Vietnam
64.	*Solena umbellata*	Goa, Karnataka, Kerala, Tamil Nadu	Sri Lanka
65.	*Thladiantha hookeri*	Assam, Manipur, Meghalaya, Nagaland	China (Yunnan), Bhutan, Laos, Myanmar, Thailand, Vietnam
66.	*Thladiantha cordifolia* (Himalayan tuber gourd)	Andhra Pradesh, Arunachal Pradesh, Assam, Manipur, Meghalaya, Nagaland, Sikkim, Tamil Nadu, Tripura, West Bengal	Nepal, China (Guangdong, Guangxi, Sichuan, Yunnan), Indonesia (Java, Sumatra), Laos, Peninsular Malaysia, Myanmar, Thailand, Vietnam
67.	*Trichosanthes anaimalaiensis*	Andaman and Nicobar Islands, Andhra Pradesh, Arunachal Pradesh, Karnataka, Kerala, Maharashtra, Tamil Nadu, Tripura	Unclear
68.	*Trichosanthes bracteata*	Peninsular India, Khasia Hills, Dehra Doon, West Bengal	China (Guizhou), Nepal
69.	*Trichosanthes cordata*	Andhra Pradesh, Arunachal Pradesh, Assam, Bihar, Chhattisgarh, Jharkhand, Manipur, Meghalaya, Mizoram, Nagaland, Rajasthan, Sikkim, Tamil Nadu, Tripura, Uttar Pradesh, Uttarakhand, West Bengal	Bangladesh, Bhutan, China, Myanmar, Nepal
70.	*Trichosanthes costata*	Andaman and Nicobar Islands, Andhra Pradesh, Arunachal Pradesh, Assam, Bihar, Manipur, Meghalaya, Sikkim, Tripura, Uttar Pradesh, West Bengal	China, Java, Myanmar, Sri Lanka, Vietnam
71.	*Trichosanthes cucumerina* (Snake gourd)	Native and cultivated throughout India	Sri Lanka, tropical China through Malesia, West, North and North East Australia
72.	*Trichosanthes cucumeroides*	Meghalaya, Sikkim, Uttar Pradesh, West Bengal	China (Guangxi, South East Xizang)
73.	*Trichosanthes dioica* (Pointed gourd)	Arunachal Pradesh, Assam, Bihar, Delhi, Himachal Pradesh, Jammu and Kashmir, Meghalaya, Punjab, Rajasthan, Uttar Pradesh, West Bengal	Bangladesh, Myanmar, Nepal, Pakistan, Sri Lanka

(*continued on next page*)

Table 1 (*continued*)

Sl No.	Binomial name	Distribution in India	Distribution outside India
74.	*Trichosanthes dunniana*	Meghalaya	China, Myanmar, Thailand
75.	*Trichosanthes kerrii*	Nagaland, Mongsemdi Naga hills	China (South West Yunnan), Laos, North Thailand, North Vietnam
76.	*Trichosanthes khasiana*	Meghalaya	Endemic
77.	*Trichosanthes lepiniana*	Union Territory	Unclear
78.	*Trichosanthes lobata* (Bitter snake gourd)	Andhra Pradesh, Karnataka, Kerala, Puducherry, Tamil Nadu, Uttar Pradesh, West Bengal	China
79.	*Trichosanthes nervifolia*	Andhra Pradesh, Arunachal Pradesh, Assam, Bihar, Goa, Karnataka, Kerala, Madhya Pradesh, Manipur, Meghalaya, Mizoram, Nagaland, Rajasthan, Sikkim, Tamil Nadu, Tripura, Uttar Pradesh, West Bengal	Sri Lanka
80.	*Trichosanthes ovigera*	Andaman and Nicobar Islands, Arunachal Pradesh, Assam, Meghalaya, Sikkim, Tripura, Uttar Pradesh, West Bengal	Australia, Bangladesh, China, Japan, Java, Myanmar, Nepal
81.	*Trichosanthes rubriflos*	Unknown	China, Myanmar, Cambodia, Thailand
82.	*Trichosanthes quinquangulata*	Andaman Islands	South China, Myanmar, Thailand, Vietnam, Cambodia, Laos, Peninsular Malaysia, Singapore, Indonesia (Sumatra, Borneo, Java, Moluccas), New Guinea (West Papua and Papua New Guinea), Philippines
83.	*Trichosanthes scabra*	Unclear	China, Cambodia, Indonesia, Laos, Peninsular Malaysia, Myanmar, Philippines, Sri Lanka, Thailand, Vietnam
84.	*Trichosanthes tricuspidata* (Bitter snake gourd)	West Bengal	Myanmar, Thailand, Vietnam
85.	*Trichosanthes truncata*	Andhra Pradesh, Arunachal Pradesh, Assam, Meghalaya, Sikkim, West Bengal	Bangladesh, Bhutan, China, Thailand, Vietnam.
86.	*Trichosanthes tubiflora*	Kerala	Sri Lanka
87.	*Trichosanthes villosula*	Andhra Pradesh, Assam, Karnataka, Kerala, Nagaland, Tamil Nadu, West Bengal	Bangladesh, China
88.	*Trichosanthes wallichiana*	Arunachal Pradesh, Assam, Bihar, Himachal Pradesh, Manipur, Meghalaya, Nagaland, Sikkim, Tripura, West Bengal	Nepal, China (Guangdong, Guangxi, Guizhou, Xizang, Yunnan)
89.	*Zanonia indica*	Andaman and Nicobar Islands, Assam, Goa, Karnataka, Kerala, Maharashtra, Meghalaya, Sikkim, Tamil Nadu, West Bengal	Sri Lanka, South China, Indochina, through Malesia east to New Guinea
90.	*Zehneria bodinieri*	Karnataka, Kerala, Tamil Nadu	China, Myanmar, Sri Lanka, Sumatra, Malaysia Sabah, Thailand, Vietnam, Cambodia, Laos, Vietnam, Peninsular Malaysia, Philippines (Palawan)
91.	*Zehneria hookeriana*	South India, Tamil Nadu	… ….
92.	*Zehneria japonica*	Arunachal Pradesh, Assam, Bihar, Himachal Pradesh, Meghalaya, Nagaland, Punjab, Sikkim, Uttar Pradesh, Uttarakhand, West Bengal	Thailand, China, Japan; Indonesia (Java, Sumatra)
93.	*Zehneria maysorensis*	Andhra Pradesh, Karnataka, Kerala, Maharashtra, Meghalaya, Tamil Nadu	… …
94.	*Zehneria thwaitesii*	Kerala	Africa, Madagascar, Sri Lanka
95.	*Biswarea tonglensis*	West Bengal, Sikkim	….
96.	*Edgeria darjeelinensis*	North east India	….
97.	*Gymnopetalum cochinchinense*	West Bengal, North east India	….
98.	*Gynostemma pedatum*	West Bengal, North east India, Sikkim	….
99.	*Hodgsonia heteroclitiata*	North east India	….
100.	*Luffa hermophrodita*	West Bengal	….
101.	*Mukia maderaspatana*	West Bengal	….
102.	*Neoalsomitra clavigera*	West Bengal, Sikkim	….
103.	*Schizopepon dioiceous*	Wild in the hilly tract of Himalayan range	….
104.	*Sechium edule* (chayote)	West Bengal	….
105.	*Solena amplexlcaulis*	Tropical region	….
106.	*Zehnaria indica*	Arunachal Pradesh, Assam, Bihar, Himachal Pradesh, Sikkim, Meghalaya, Nagaland, West Bengal	….

been discussed in the subsequent sections. Different metabolites present in the food plants of the Cucurbitaceae family has been represented in Fig. 4. Major phytoconstituents found in some edible Cucurbitaceae family listed in Table 2.

4.1. Primary metabolites

4.1.1. Proteins

Plants of the Cucurbitaceae family are composed of several amino acids and proteins with diverse pharmacological activities which mostly offer promising activity against some specific pathogen related to fungal infection. On the basis of their nature and biological activity, the antifungal proteins are classified as pathogen-related (PR) proteins, ribosomal inactivating proteins (RIP), vicilin like proteins and others (Yadav et al., 2013). PR proteins are classified into different sub-families (PR-1 to PR-5) depending on their amino acid sequences and enzymatic or biological activity (Brederode et al., 1991). The role of RIPs involves arresting the synthesis of foreign proteins by inactivating fungal ribosomes by N-glycosidase activity (Yadav et al., 2013). The two major RIPs, viz. hispin, α-momorcharin is reported to offer potent antifungal activity against various pathogenic fungal species. Another protein derivative charantin, viciline (isolated from *Momordica charantia* or Bitter gourd) showed antidiabetic and insulinomimetic activities (Ng and Parkash, 2002; Wang et al., 2012). Luffangulin, a novel ribosome inactivating peptide was also reported from *Luffa acutangula* (Ridge gourd) with some therapeutic benefits (Wang and Ng, 2002).

4.1.2. Vitamins

Most of the vegetables belonging to the family are a good source of α, β, γ-carotene, non-provitamin A carotenoids (*viz.* lutein, violaxanthin, and neoxanthin) (Hemmige et al., 2017). Specifically, *Cucurbita maxima* (Pumpkin) is a good source of Vitamin A (Ragasa and Lim, 2005) whereas small amounts of vitamin A is present in *Momordica dioica* (Spine gourd), *Cucumis Sativus* (Cucumber) and *Citrullus lanatus* (Watermelon) (Bawara et al., 2010; Abou-Zaid et al., 2001). Vitamin-B complex, thiamin, riboflavin, niacin, folate, Vitamin D, F, E is reported in several species of

Cucurbitaceae family plants such as *Lagenaria siceraria* (Bottle gourd), *Trichosanthes cucumerina* (Snake gourd) (Shah and Seth, 2010; Adebooye, 2008). In addition, vitamin C was found in *Trichosanthes cucumerina* (Snake gourd), *Coccinia indica* (Ivy gourd) fruit (Sachin and Trisa, 2018). Some other plants belonging to Cucurbitaceae family such as *Cucumis sativus* (Cucumber), *Cucurbita pepo* (Field pumpkin), *Cucurbita maxima* (Pumpkin), *Luffa acutangula* (Ridge gourd) also contain a trace amount of vitamin K and vitamin E (Avinash and Rai, 2017).

4.1.3. Minerals

Cucurbitaceae family plants are a good source of minerals. In specific, *Momordica charantia* (Bitter gourd) leaves are a source of calcium, magnesium, potassium, phosphorus, and iron (Kumar et al., 2010), whereas *Cucurbita pepo* L. (Field pumpkin), contains phosphorus, potassium, magnesium, calcium, iron, zinc etc. The seeds and fruit pulps of *Cucurbita pepo* have been reported to possess high contents of potassium and sodium (Hashash et al., 2017). The mineral content of the crops of this family is very beneficial for their nutritive and health benefits (Avinash and Rai, 2017).

4.1.4. Carbohydrates and dietary fibres

Cucurbitaceae crops are enriched with carbohydrates and dietary fibres (Avinash and Rai, 2017). *Cucurbita pepo* L. (Field pumpkin) (Hashash et al., 2017), *Cucurbita maxima* (Pumpkin) and *Citrullus lanatus* (Watermelon) seeds and fruits (Elkhedir and Mustafa, 2015) contain a significant amount (6–10%) of carbohydrates. It has been observed that the fruits of Cucurbitaceae family plants are an important source of dietary fibres with several therapeutic benefits against constipation, diabetes, obesity and some related disorders (Saboo et al., 2013).

4.1.5. Others

Seeds contain several fixed oils, monounsaturated, unsaturated and polyunsaturated fatty acids like linoleic and oleic acid, stearic acids, Myristic acids etc. *Cucurbita pepo* (Field pumpkin) seed yields 50% oil (mostly linoleic and oleic acid). The presence of fixed oil and free acids are also reported in *Momordica charantia* (Bitter gourd) seeds (Kumar et al., 2010). *Citrullus colocynthis* (Bitter apple) seed contains a significant amount of palmitic, stearic and linoleic acid (Dhakad et al., 2017). Trace of volatile oil is also present in *Benincasa hispida* (Wax gourd) fruits (Al-Snafi, 2013). In addition, some reducing sugar, resins, crude fibres, free acids, lycopene, carotenoids, lutein and zeaxanthin, stereos, tocopherol, are also found and used as nutritional components (Avinash and Rai, 2017).

4.2. Secondary metabolites

4.2.1. Triterpenoids

The presence of triterpenoid compounds, cucurbitacins are one of the major characteristics of the plants of Cucrbitaceae family. Cucurbitacin consists of a tetracyclic cucurbitane nucleus with a variety of oxygenation functionalities at different positions with diverse chemical categories. The cucurbitacins are present as non-glycosylated or glycosylated triterpenoids and divided into twelve categories, incorporating cucurbitacins A-T. The structural diversity of the cucurbitacin lies in various unsaturation as well as the presence of numerous keto-, hydroxy- and acetoxy-groups (Chen et al., 2005). The hydrophobic property of the cucurbitacin nucleus is a major regulating factor for their cytotoxic effects and it increases linearly with their hydrophobicity (Bartalis and Halaweish, 2005). In particular, cucurbitacin E and their glycosides are the most widely distributed chemical constituents in food plants of the Cucurbitaceae family (Dhiman et al., 2012). Cucurbitacin E has also been reported in many fruit extracts of *Lagenaria siceraria* (Bottle gourd), *Benincasa hispida* (Wax gourd), *Momordica charantia* (Bitter gourd), *Coccinia grandis* (Ivy gourd), *Cucurbita pepo* (Field pumpkin), *Luffa acutangula* (Ridge gourd) in a study determined through ReversePhase- High Performance Liquid Chromatography (RP-HPLC) (Chanda et al., 2019). Cucurbitacins have been reported to possess anti-inflammatory, anti-angiogenic, immunomodulatory, cytotoxic, cytostatic and hepatoprotective properties (Attard and Cuschieri, 2004;

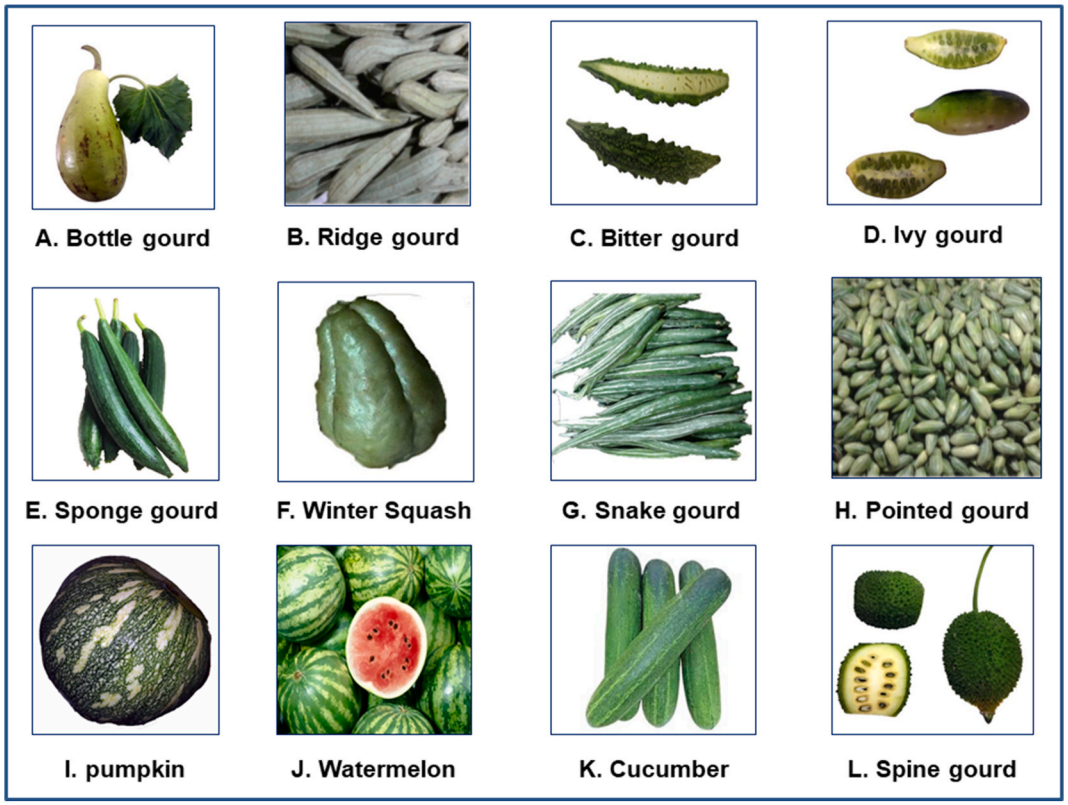


Fig. 3. Some selected medicinal food plants of Cucurbitaceae family.

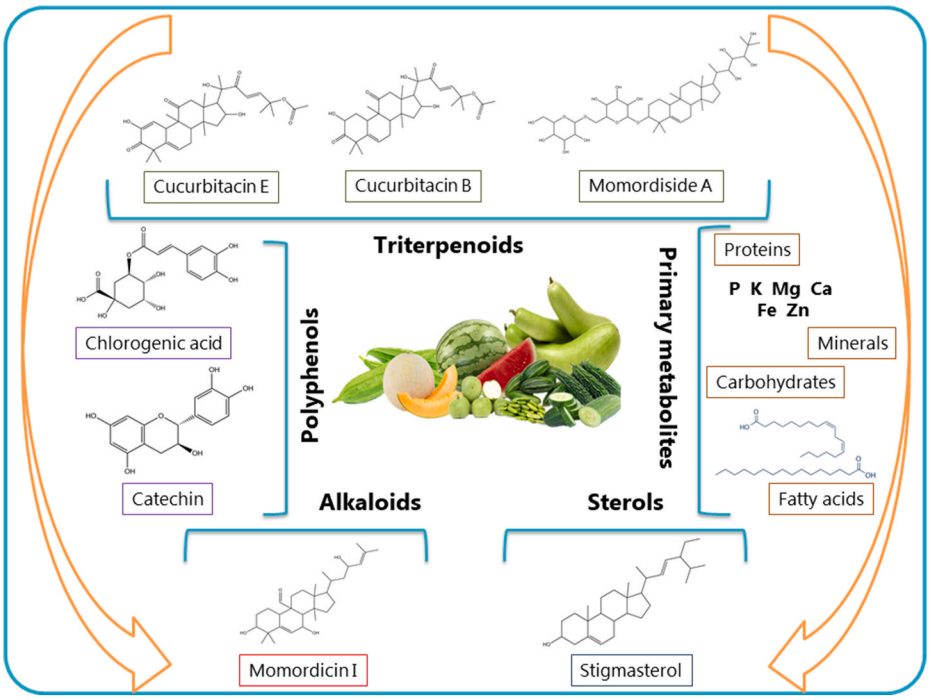
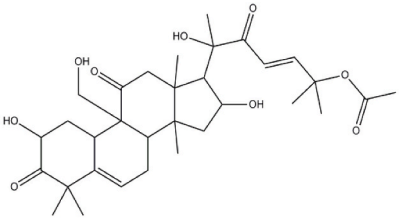


Fig. 4. Metabolites present in the food plants of Cucurbitaceae family.

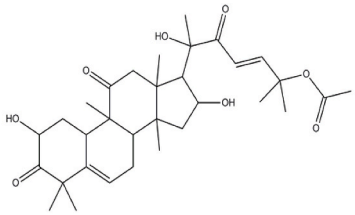
Shyam et al., 2010) both *in-vitro* and *in-vivo* model. Despite the potential therapeutic activity of Cucurbitacin E and cucurbitacin glycosides, their chronic exposure is undesirable due to their extremely bitter and disagreeable taste as well as their toxicological effects found in experimental animals (Rupachandra and Sarada, 2013). The different types of structurally diverse cucurbitacins are described as follows:

Cucurbitacin A (1) is a member of the class of compounds known as cucurbitacins. Cucurbitacins are with a variety of oxygenation functionalities at different positions. Cucurbitacin A is insoluble in water and a very weakly acidic compound. Cucurbitacin A is only found in *Cucumis* species (Chen et al., 2005).

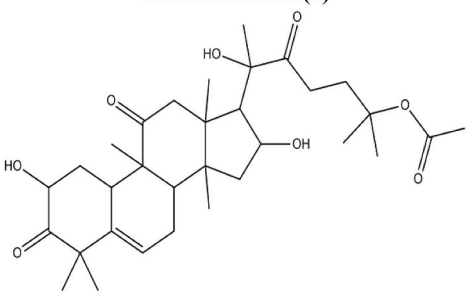
Cucurbitacin B (2) is widely present in Cucurbitaceae family plants. Most of the cucurbitacin B occurs as dihydrocucurbitacin (3), dihydroisocucurbitacin, isocucurbitacin B (4) and their glucosidic form. Cucurbitacin B, iso-cucurbitacin B are reported to possess potent antitumor activity. Cucurbitacin B has been shown to be effective against inflammation and chronic hepatitis (Miro, 1995). Cucurbitacin C (5) is only identified in *Cucumis* species. It is reported to have antimicrobial activity (Miro, 1995). Cucurbitacin D (6) is most ubiquitously present in Cucurbitaceae family and lacks the acetyl group at the 25-OH in its parent structure. Several congeners of cucurbitacin D *viz.* dihydrocucurbitacin D (7), deoxycucurbitacin D (8), epi-isocucurbitacin D have been isolated from plants, which have exhibited potential anti-proliferative and anti-tyrosinase activity (Chen et al., 2005).



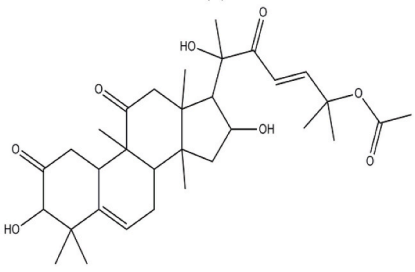
Cucurbitacin A (1)



Cucurbitacin B (2)



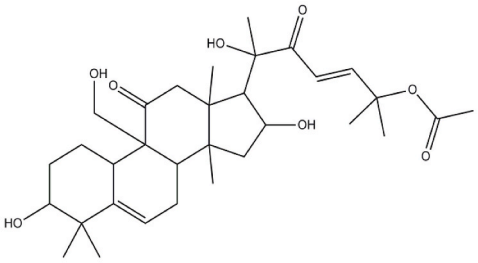
Dihydrocucurbitacin B (3)



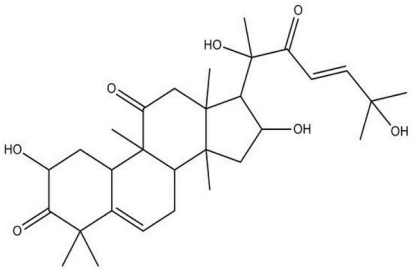
Isocucurbitacin B (4)

Table 2
The major phytoconstituents found in some edible Cucurbitaceae family.

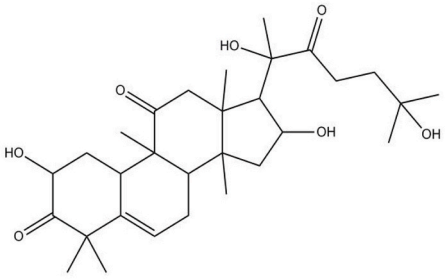
Scientific name	Common Name	Major phytoconstituents
Benincasa hispida	Wax Gourd	Pentacyclic triterpene, bryonolic acid, lupeol, beta sitosterol, cucurbitin, avenasterol, multiflorenol, isomultiflorenyl acetate, stigmasterol, stigmasterol 3-O-β-D-glucopyranoside, α-spinasterol 3-O-β-Dglucopyranoside, daucosterol, 2,5-dimethyl pyrazine (Han et al., 2013; Ghosh and Baghel, 2011)
Citrullus colocynthis	Bitter Apple	Flavone C Glycosides, ursolic acid, Cucurbitacin I 2-O-beta-D-glucopyranoside, cucurbitacin E 2-0-β-D-glucopyranoside, colocynthoside A, B, hexanocucurbitacin I 2- O-β-D glucopyranoside, khekadaengoside E, Cucurbitacin E 2-O-β-D-glucopyranoside, flavonoid glycosides (isosaponarin), isovitexin (Rajasree et al., 2016; Hussain et al., 2014; Miao et al., 2012)
Citrullus lanatus	Watermelon	Vitamin A, Lycopene, Cucurbitacin E, Flavonoids, vitamin C, thiamine, riboflavin, polyphenolic compounds, terpene, steroid, flavonoid, Glycoprotein-vicilin (Rajasree et al., 2016; Gupta et al., 2018)
Coccinia grandis	Ivy Gourd	Polyprenol 1, saponin, flavonoids, glycosides, taraxerone, taraxerol, 24R-24-ethylcholest-5-en-3β-ol glucoside, Cephalandrins A and B, β-sitosterol, stigma-7-en-3-one, cucurbitacin B, coccinoside (saponin), flavonoid glycoside, Lupeol, β-amyrin, β-sitosterol (Pekamwar et al., 2013)
Coccinia indica	Ivy Gourd	Steroids, terpenoids, saponins, flavonoids alkaloids, tannins, glycosides, phenol and mucilage compounds (PushpaRani et al., 2016)
Cucumis melo	Muskmelon	Phenolic glycosides, fatty acids, amino acid, gallic acid, ellagic acid; catechin, quercetin, vanillin, eugenol, vanillic acid, Luteolin-7-glycoside, Naringenin glycosides, Apigenin-7-glycoside, Oleuropein, m-coumaric acid, Phenylacetic acid, linoleic acid, tocopherols, oleic acid (Mallek-Ayadi et al., 2017, 2018; Rajasree et al., 2016)
Cucumis sativus	Cucumber	Glycoside, steroid, saponin and tannin, flavonoid, flavone glycosides (isovitexin, saponarin), acylated flavone C-glycosides, 9-beta-methyl-19-norlanosta-5- ene type glycosides, cucurbitacins, cucumegastigmanes I and II, cucumerin A and B, vitexin, orientin, isoscoparin 2″-O-(6‴-(E)-p-coumaroyl) glucoside, apigenin 7-O-(6″-O-p-coumaroylglucoside) (Mukherjee et al., 2013; Rajasree et al., 2016)
Cucurbita maxima	Pumpkin	Spinasterol, 24-ethyl-5α-cholesta-7, 22, 25-trien-3β-ol, flavonoids, polyphenolics, Cucurbitaxanthin, gibberellin and α-tocopherol, β-carotene, carotenoids, γ-amino butyric acid, 11 E-octadecatrienoic acid, polysaccharides, 13-hydroxy-9Z and phenolic glycosides, protocatechuic, caffeic, syringic, vanillic, p-coumaric and ferulic, oleic, linoleic, and palmitic acids (Muchirah et al., 2018; Rezig et al., 2012)
Cucurbita pepo	Field Pumpkin	Linoleic acid, oleic acid, Δ7 sterols (avenasterol, spinasterol), Δ5 sterol (sitosterol, stigmasterol), triterpenoids, sesquiterpenoids, squalene, tocopherols, hexanorcucurbitane glycosides, lutein, α-carotene, β-carotene violaxanthin, auroxanthin epimers, flavoxanthin, luteoxanthin, chrysanthemaxanthin, α-cryptoxanthin, β-cryptoxanthin, cucurbitacin I, J, L,K,M (Perez-Gutierrez, 2016; Rajasree et al., 2016)
Lagenaria siceraria	Bottle gourd	Cucurbitacins B, D, G and H, saponins, flavone-C-glycoside,polyphenol, campesterol, fucosterol, sitosterol, Lagenin (Prajapati et al., 2010), D: C-Friedooleanane-type triterpenoids (Chen et al., 2008), *p*-coumaric acid, Hespiridin, Ferulic acid (Chanda et al., 2020b)
Luffa acutangula	Ridge gourd	Luffangulin, luffaculin, saponin glycosides, gallic acid, p-coumaric acid, ferulic acid, protocatechuic acid, Acutoside C; Acutoside D, Unsaturated aliphatic alcohols, Carboxylic acids, fatty acids and their esters (Nagarajaiah and Prakash, 2015; Suryanti et al., 2015), chlorogenic acid (Singha et al., 2020)
Luffa cylindrica	Sponge Gourd	Lucyin A, lucyoside, maslinic acid; ginsenosides, luffin P1; luffin S, luffacylin, apigenin-7-O-D-glucuronidemethyl ester; luteolin-7- O–D-glucuronide, methyl ester, p-coumaric acid, chlorogenic acid, caffeic acid (Du et al., 2006; Lucy and Abidemi, 2012)
Luffa echinata	Bitter sponge gourd	Echinatin, saponin, Cucurbitacin B and E, beta-sitosterol, echinatol A and B, oleanolic acid (Dogar et al., 2018)
Momordica charantia	Bitter gourd	Momordicin I, momordicin IV, aglycone of Momordicoside, kuguacin, charantoside, vicine, goyaglycoside, quercetin-, kaempferol- and isorhamnetin-O-glycosides (Chen et al., 2005; Supraja et al., 2015)
Momordica cochinchinensis	Gac	Hydroxybenzoic acids, hydroxycinnamic, Gallic acid, p-hydroxybenzoic acid, Apigenin, oleic, palmitic, linoleic acids, carotenoids (Ishida et al., 2004), lycopene, Momordin, peptides MCoTI-II (Chuyen et al., 2015; Müller-Maatsch et al., 2017)
Momordica dioica	Spine gourd	Pleuchiol, momodicaursenol triterpenes of ursolic acid (Ali and Srivastava, 1998), hederagenin, oleanolic acid, α-spiranosterol, stearic acid, gypsogenin (Sadyojatha and Vaidya, 1996), steroidal tritrpenoids (Luo et al., 1998)
Trichosanthes cucumerina	Snake gourd	Palmitic, Stearic, Arachidic, Behenic, Lignoceric acid, Lutein, Zeaxanthine, Cucurbitacins, Coumaric acid, *p*-Coumaric acid; Caffeic acid (Adebooye, 2008), Chlorogenic acid (Singha et al., 2020)
Trichosanthes dioica	Pointed gourd	Trichosanthin, lectin; euphol, α-amyrin, β-amyrin, Butyrospermol, lupeol, taraxerol, betulin, and karounidiol; Cucurbitacin B, Cucurbitacin E, sterols, steroidal saponin, tannin, flavonoids (Khandakera et al., 2018), Chlorogenic acid (Singha et al., 2020)
Trichosanthes tricuspidata	Bitter snake-gourd	Cucurbitane glycosides (Cucurbitacin K 2-O-beta glucopyranoside), methyl palmitate, palmitic acid, suberic acid, bryonolic acid, cucurbitacin B, isocucurbitacin B, 3-epi-isocucurbitacin B, 23,24-dihydrocucurbitacin D, isocucurbitacin D, hexanorcucurbitane octanorcucurbitane glycosides (Duvey et al., 2012; Kanchanapoom et al., 2002)
Sechium edule	Chayote/Mirliton squash	Cucurbitacins B and D, cucurbitacin I, vitexin, luteolin 7-O-rutinoside, luteolin 7-O-glucoside, phloridzin, naringenin, phloretin, apigenin, carboxylic acids and esters, Chlorogenic, Vanillic, p- hydroxybenzoic, sterols, amino acids, 3-octadecenoic acid, trilinolenin, α-linolenic acid (Ragasa et al., 2014; Aguiñiga-Sánchez et al., 2015), Chlorogenic acid (Singha et al., 2020)



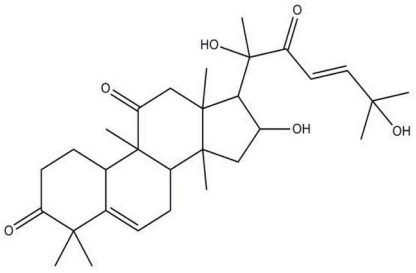
Cucurbitacin C (5)



Cucurbitacin D (6)

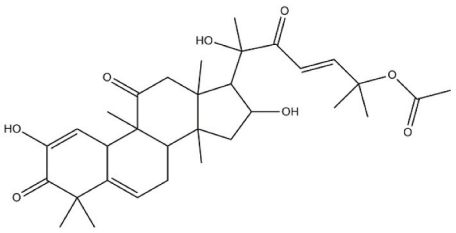


Dihydrocucurbitacin D (Cucurbitacin R) (7)

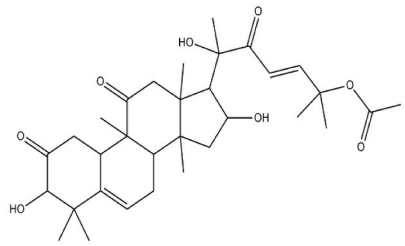


Deoxycucurbitacin D (8)

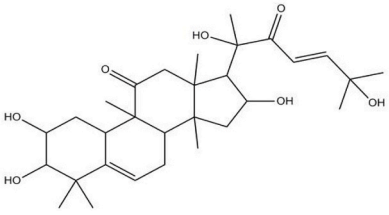
Cucurbitacin E (9), a highly oxygenated triterpene is considered to be the most important triterpenoid due to its wide range of therapeutic activity. It is the first cucurbitacin isolated from *Elaterium* usually present as monoglucoside (Miro, 1995). Cucurbitacin E is also present as dihydrocucurbitacin E, dihydroisocucurbitacin E, isocucurbitacin E (10) and their glucosidic form. Cucurbitacin E and its glycosides possess neuroprotective (Arel-Dubeau et al., 2014), anti-inflammatory, antipyretic, antitumor (Abdelkhalek et al., 2017), antiallergic (Yoshikawa et al., 2007), anthelmintic, purgative activity (Miro, 1995). Cucurbitacin E was also found in the ethyl acetate fraction of *Coccinia grandis* (Ivy gourd) fruit through Liquid Chromatography-Quadrupole Time-of-Flight- Mass spectrometry (LC-QTOF-MS) study (Chanda et al., 2020a).



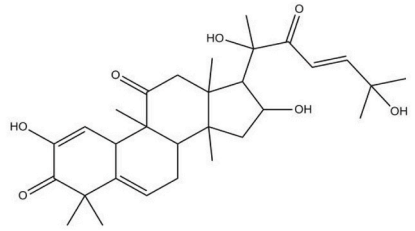
Cucurbitcin E (9)



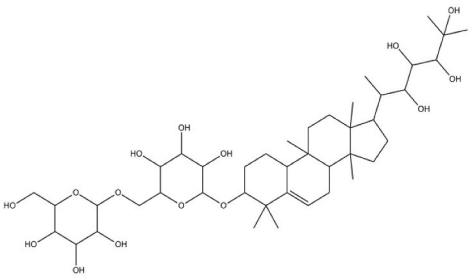
Isocucurbitacin E (10)



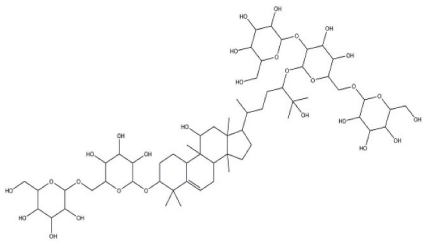
Cucurbitacin F (11)



Cucurbitacin I (12)



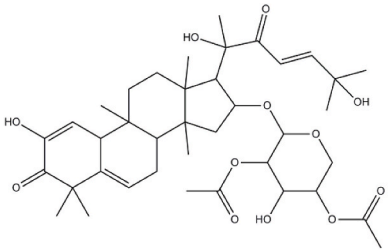
Momordiciside A (13)



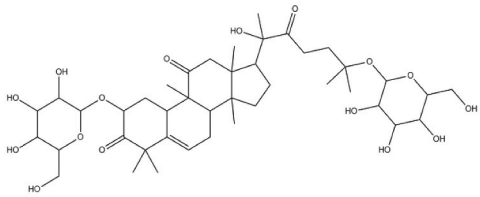
Morgoside V (14)

Cucurbitacin F (11) is well known in traditional Chinese medicines for their potential pharmacological benefits. It widely occurs as dihydrocucrbitacin F, hexanorcucurbitacin F, oxocucurbitacin F and their glycosidic form (Chen et al., 2005). Some isolated derivatives of cucurbitacin F, *viz.* 23, Dihydro and 15-oxo-cucurbitacin F (3), and 15-oxo-23, 24-dihydro cucurbitacin F have been reported to possess anti-HIV activity (Konoshima et al., 1994).

Other Cucurbitacins including J (17) K, O (18), P (19) Q (20), R, S (21), T and their glycosides have been reported but they are not therapeutically important. Apart from that, there are a few specific cucurbitacins and cucurbitane glycosides, which are present in the genus *Momordica*, viz. Momordicosides A–E (13), momordicines, neomorgoside etc. (Miro, 1995). Some sweet cucurbitane glycosides, viz. mogrosides (14), oxomogroside, and their glycosides are reported from



Spinosides A (15)



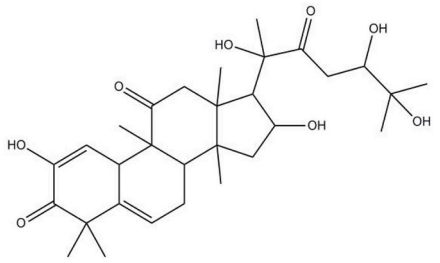
Brydioside A (16)

Cucurbitacin I (12) exist as its convener *viz.* Hexanorcucurbitacin, 23, 24-dihydrocucurbitacin I (Cucurbitacin L), Khekadaengosides D, Deoxycucrbitacin glycoside (Spinosides A) (15) with potential antitumor activity. Several glycosides of Cucurbitacin L (brydioside A (16) and bryoamaride etc.) are also isolated from Cucurbitaceae family plants.

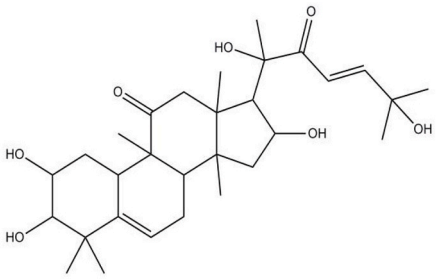
Cucurbitaceae family plants (Chen et al., 2005).

4.2.2. *Polyphenols*

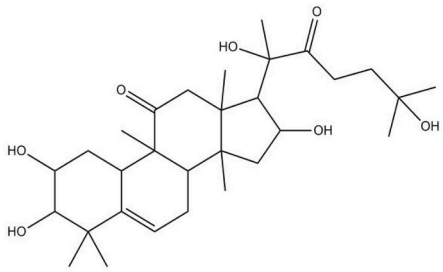
Polyphenols are a class of natural compounds, possessing antioxidant activity. Phenolic compounds, including simple phenols (eg.



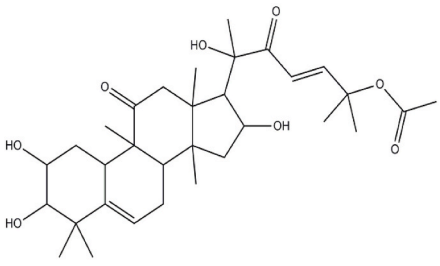
Cucurbitacin J (17)



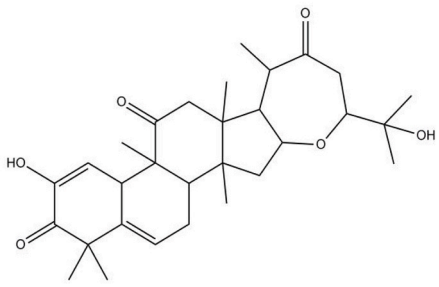
Cucurbitacin O (18)



Cucurbitacin P (19)

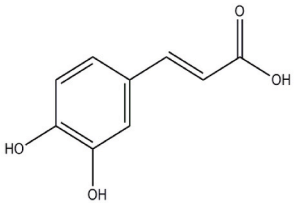


Cucurbitacin Q (20)

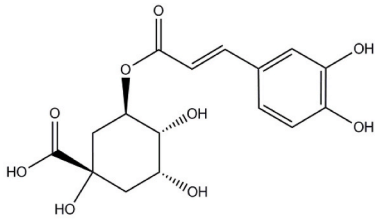


Cucurbitacin S (21)

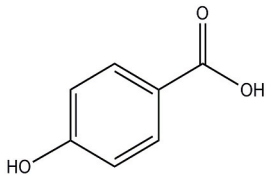
hydroquinone), phenolic acids, hydroxycinnamic acid derivatives and flavonoids are bioactive substances occurring widely in Cucurbitaceae family plants (Oksana et al., 2012). It has been observed that several hydroxycinnamic acids (22) such as chlorogenic acid (23), *p*-Coumaric acid (24), Gallic acid, *p*-Hydroxybenzoic acid, vanillic acid (25), Sinapic acid, Ferulic acid etc and their esters and glycosides are present in Cucurbitaceae family plants. The occurrence of flavonoid compounds viz. Catechins (26), proanthocyanins, anthocyanidins, flavanones (27), isoflavones (28), dihydroflavonols, flavonols, flavan-3-ols (29) and their glycosides are also found in these medicinal food plants of the Cucurbitaceae family. Chemoprofiling of certain Cucurbitaceae food plants like *Luffa acutangula* (Ridge gourd), *Sechium edule* (Chayote), *Trichosanthes dioica* (Pointed gourd) and *Trichosanthes cucumerina* (Snake gourd) showed the presence of chlorogenic acid (Singha et al., 2020). In a Liquid Chromatography-Quadrupole Time-of-Flight- Mass spectrometry (LC-QTOF-MS) study it was found out that in the ethyl acetate fraction of *Luffa acutangula* (L.) Roxb. (Ridge gourd) fruit, having many phenolic compounds such as Gentisic acid, Dihydroferulic acid, Coumaric acid, Chrysin, Tetramethyl scutellarein, Mellein etc., (Chanda et al., 2018a). LC-QTOF-MS analysis of the ethyl acetate fraction of *Coccinia grandis* (Ivy gourd) fruits identified hydroxybenzoic acids, hydroxycinnamic acid, flavonol, isoflavonoid, flavanone etc., (Chanda et al., 2020a). Liquid Chromatography-Mass Spectrometry (LC-MS/MS) metabolite profiling of *Lagenaria siceraria* (Bottle gourd) revealed the presence of phenolic acids like Ferulic acid, *p*-Coumaric acid and Hesperidin (Chanda et al., 2020b). Several phenolic compounds such as Feruloyl quinic acid, Vanillic acid, Gentisic acid, Phenylacetic acid, Esculetin, Scopoletin, Benzoic acid, Coumaric acid etc were identified from ethyl acetate fraction of *Momordica charantia* (Bitter gourd) fruit through LC-QTOF-MS study (Chanda et al., 2018b).



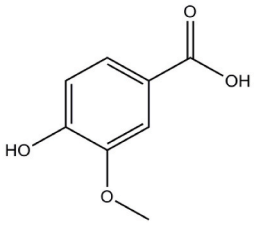
Caffeic acid (22)



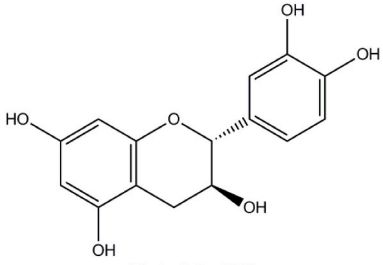
Chlorogenic acid (23)



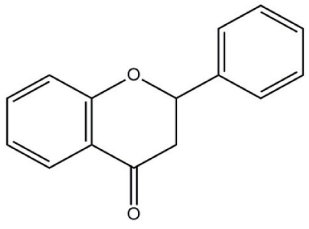
***p*-Coumaric acid (24)**



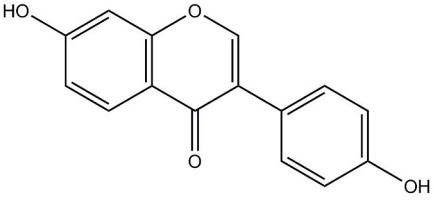
Vanillic acid (25)



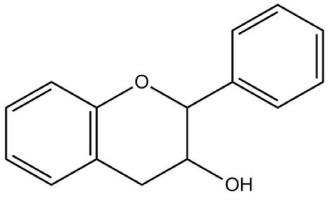
Catechin (26)



Flavanone (27)



Isoflavone (28)



Flavan-3-oil (29)

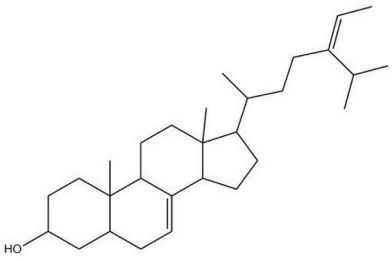
4.2.3. Sterols

Most of the sterol and its derivatives are found in the seed part of the fruits belong to Cucurbitaceae family, mostly in *Benincasa cerifera*, *Cucumis sativus* (Cucumber), *Cucurbita maxima* (Pumpkin), *Cucurbita pepo* (Field pumpkin). Sterols mainly occur as 24-ethyl-Δ(7) and Δ (7,22) -sterol as major components, whereas a small amount of saturated and Δ(5) -and Δ(8) -sterols are present in seeds. The major sterol compounds reported in the Cucurbitaceae family plants are avenasterol (30), sitosterol glucoside (31), spinasterol (32), stigmasterol (33), β-sitosterol, fucosterol, campesterol etc.

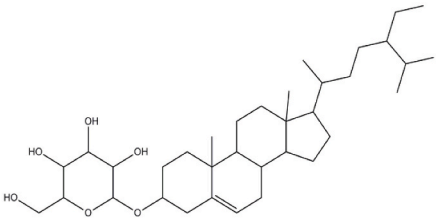
A steroidal glycoside, quarantine was isolated from *Momordica charantia* (Bitter gourd) which exists as an equal mixture of stigmasterol glucoside and β-sitosterol glucoside (Desai and Tatke, 2015). In addition, *Cucurbita pepo* (Field pumpkin) (Badr et al., 2011), *Citrullus vulgaris* (Watermelon) seeds (Hannah and Krishnakumari, 2015) contain a trace amount of cholesterol.

4.2.4. Alkaloids

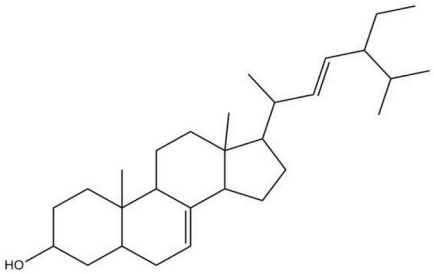
Among the plants of the Cucurbitaceae family, the genus *Momordica* contains a substantial amount of alkaloids. The alkaloid, momordicine



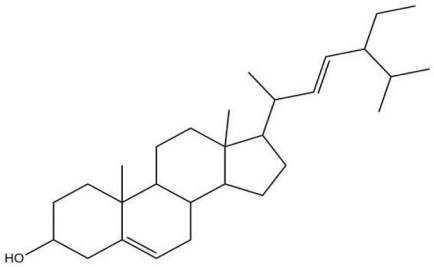
Avenasterol (30)



Sitosterol glucoside (31)



Spinasterol (32)



Stigmasterol (33)

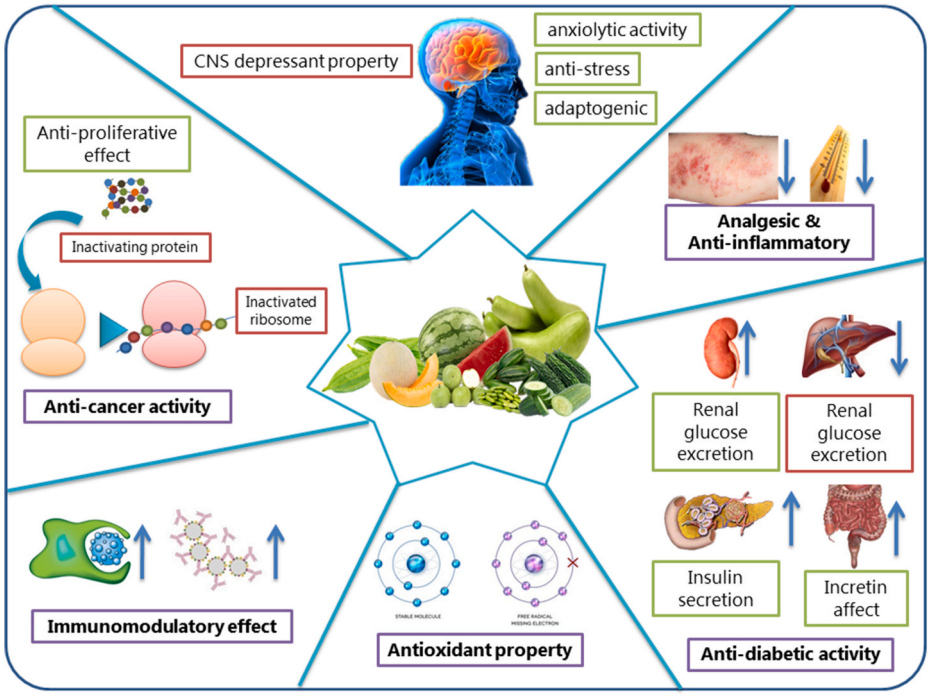


Fig. 5. Different pharmacological and therapeutic potential of Cucurbitaceae food plants.

Table 3
The pharmacological aspects of few edible Cucurbitaceae plants.

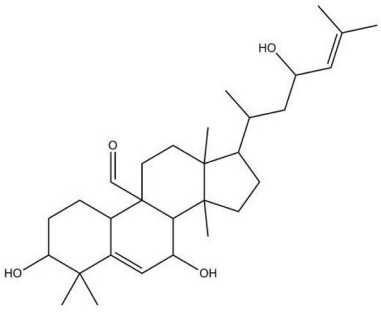
Scientific name (Common name)	Plant part	Pharmacological activity	
Benincasa hispida (Wax Gourd)	Fruits	Anti-angiogenic	Attard and Cuschieri (2004)
		Antibacterial, Antifungal	Natarajan et al. (2003)
		Hypoglycemic	Chakraborty et al. (2018)
	Seeds	Antinociceptive, Antipyretic	Qadrie et al. (2009)
Citrullus colocynthis (Bitter Apple)	Seeds	Antidiabetic, Antilipidemic	Hussain et al. (2014)
	Leaves, Stem, Fruits, Seeds	Antibacterial, Anticandidal	Marzouk et al. (2009)
	Fruits	Analgesic, Anti-inflammatory	Pashmforosh et al. (2018)
		anticancer, cytotoxic	Rezai et al. (2017)
Citrullus lanatus (Watermelon)	Fruits	Anti-urolithiatic, Diuretic	Siddiqui et al. (2018)
	Seeds	Antimicrobial	Adunola et al. (2015)
		Hepatoprotective, Cardioprotective, Analgesic, Anti-inflammatory	Gupta et al. (2018)
Coccinia grandis (Ivy Gourd)	Leaves	Anti-dyslipidemic	(Singh et al., 2007)
		Antiproteolytic, Leishmanicidal	(Das et al., 2015)
	Fruits	Mast cell stabilizing, Antianaphylactic, Antihistaminic	(Taur and Patil, 2011)
		Antioxidant, Antibacterial and cell proliferative	(Sakharkar and chouhan, 2017)
		Antidiabetic, Insulinotrophic	(Meenatchi et al., 2017)
		α-glucosidase inhibitory effect	Chanda et al. (2020a)
	Aerial parts	Analgesic	(Hossain et al., 2014)
Coccinia indica (Ivy Gourd)	Aerial parts	Antihyperglycemic, Hypolipidemic	(Balaraman et al., 2010),
		Antifungal	(Shaheen et al., 2018)
Cucumis melo (Muskmelon)	Aerial parts	Promote skin hydration, antioxidant, anti-inflammatory	(Dhiman et al., 2012),
	Peel and seeds	Antiproliferative	(Rolim et al., 2018)
Cucumis sativus (Cucumber)	Fruits	Hypolipidemic	(Sudheesh and Vijayalakshmi 1999)
		Antiwrinkle, Antiaging,	(Nema et al., 2011),
		Antiurolithiatic	(Pethakar et al., 2017)
		Antimicrobial	(Sotiroudis et al., 2010)
	Exudates of the fruit	Anti-parasitic	(Mukherjee et al., 2013)
	Peels	Antimicrobial, Antidiabetic	(Dixit and Kar, 2010),
Cucurbita maxima (Pumpkin)	Seeds	Antidiabetic, Antihyperlipidemic	(Sharma et al., 2013)
		CNS stimulant	(Doke et al., 2011)
		Immunosuppressive	(Rajasree et al., 2016)
	Fruits	Antitumor, Antihypertensive, Anti-inflammatory, Antibacterial, Antihypercholestramia	
	Fruit pulp	Diuretic	(Saravanan and Manokaran, 2012)
	Aerial parts	Anticancer	(Saha et al., 2011)
Cucurbita pepo (Field Pumpkin)	Fruits	Diuretic, Antiandrogenic, Immunological, Anti-inflammatory, Hepatoprotective, Anti-ulcer, Antileprotic	(Rajasree et al., 2016)
		Antibacterial, Antioxidant, Antitumor, Hypoglycemic (anti- diabetic)	
	Seeds	Hypolipidemic	(Adnan et al., 2017)
Lagenaria siceraria (Bottle gourd)	Seeds	Antimicrobial	(Dash and Ghosh, 2018)
	Fruits	Lipase inhibitory activity	(Maqsood et al., 2017)
	Leaf	Antitumor	(Prajapati et al., 2010)
	Fruits	Diuretic, Immunoprotective, Hepatoprotective	
		Cardio-protective, Antiproliferative, CNS depressant, Antihyperglycemic	(Rajasree et al., 2016)
		Antihyperlipidemic, Anticancer, Immunomodulatory	
		Diuretic	(Chanda et al., 2020b)
Luffa acutangula (Ridge gourd)	Fruits	Hepatoprotective	(Jadhav et al., 2010)
		Anti-Diabetic, Antimicrobial Property, Cytotoxic Activity, Antibacterial, Immunomodulatory	(Manikandaselvi et al., 2016)
		Diuretic	(Chanda et al., 2018a)
Luffa cylindrica (Sponge Gourd)	Seeds	Anti-Ischemic, Anti-Hyperlipidemic, Immunomodulatory	(Khajuria et al., 2007)
	Whole plant	Antimicrobial	(Indumathy et al., 2011)
Luffa echinata (Bitter sponge gourd)	Fruits	Antihepatotoxic	(Dogar et al., 2018)
		Hepatoprotective	(Ahmed et al., 2001)
	Seeds	Analgesic, Anti-Inflammatory	(Modi and Kumar, 2014)
Momordica charantia (Bitter gourd)	Fruits	Antidiabetic, Antihyperlipidemic, Hepatoprotective, Antiviral Potential, Wound Healing Activity, Anti-Inflammatory, Analgesic (Jia et al., 2017)	
	Peel	Anticarcinogenic,	(Grover and Yadav, 2004)
Momordica cochinchinensis (Gac)	Seeds	Antioxidant, Antimicrobial, Antiproliferative	(Yu et al., 2017)
	Fruits	Anticancer, Provitamin A Activity	(Chuyen et al., 2015)
		Trypsin Inhibitor	(Felizmenio-Quimio et al., 2001)
Momordica dioica (Spine gourd)	Fruits	Hepatoprotective, antihepatotoxic, antidiabetic, antibacterial	(Pingle et al., 2018)
		Immunostimulant	(Venkateshwarlu et al., 2017)
Trichosanthes cucumerina (Snake gourd)	Fruits	Anti-Diabetic	(Devi, 2017)
		Antibacterial	(Reddy and Jose, 2013)
		Cytotoxic, Anti-Cancer,	(Kongtun et al., 2009)
		Gastroprotective	(Arawwala et al., 2010)

(*continued on next page*)

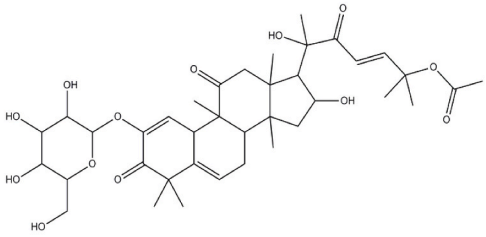
Table 3 (*continued*)

Scientific name (Common name)	Plant part	Pharmacological activity	
Trichosanthes dioica (Pointed gourd)	Fruits	Antihyperglycemic	(Rai et al., 2008)
		Antihyperlipidemic	(Sharmila et al., 2007)
		Antitumor	Bhattacharya and Halder (2013)
		Anti-Inflammatory	Bhattacharya and Haldar (2013)
		Immunomodulatory	Bhadoriyal and Mandoriya (2012)
		Wound Healing	(Shivhare et al., 2010)
Trichosanthes tricuspidata (Bitter snake gourd)	Fruits	Anthelmintic Activity	(Duvey et al., 2012)
		Antioxidant Activity, Antibacterial	(Xavier and Dhanasekaran, 2018)
		Antihyperglycemic	(Kulandaivel et al., 2013)
Sechium edule (Chayote/ Mirliton squash)	Fruits	Antioxidant, α-Glucosidase Inhibitory, Anti-Diabetic	Sulaiman and Ooi (2013)
		Cardioprotective	(Neeraja et al., 2015)
		Anti-Atherosclerotic	(Ragasa et al., 2014)
		Hepatoprotective	(Firdous et al., 2012a)
		Antibacterial	(Ordonez et al., 2009)
		Antiepileptic, CNS Depressant	(Firdous et al., 2012b)
		Antiproliferative	(Aguiñiga-Sánchez et al., 2015)

(34) was isolated from fruits of *Momordica charantia* (Bitter gourd) reported by Supraja and his co-workers (Supraja et al., 2015). A glycol alkaloid, vicine was reported from the seed extract of *Momordica charantia* (Haixia et al., 2004). One alkaloidal compound 1-tert-butyl-5, 6, 7-trimethoxyisoquinolene was isolated from the methanolic extract of *Coccinia grandis* (Ivy gourd) (Choudhury et al., 2013). The cytotoxic effect of the alkaloid rich fraction of the fruits of *Citrullus colocynthis* (Bitter apple) was studied in MCF-7 and HEPG-2 cell lines. A bitter principle colocynthin (35) (a mixture of alkaloid and crystalline alcohol) was reported in *Citrullus colocynthis* (Mukherjee and Patil, 2012).



Momordicin I (34)



Colocynthin (35)

5. Pharmacological and therapeutic potential

Plants from the Cucurbitaceae family provide an excellent source of bioactive functional components with various therapeutic importances. The metabolites obtained are very useful for their extensive biological activity, which includes antidiabetic, anti-inflammatory, cytotoxic, hepatoprotective, antimicrobial effects etc. Various types of the pharmacological and therapeutic potential of Cucurbitaceae family plants have been discussed in this section. Fig. 5 shows the different pharmacological and therapeutic potential of Cucurbitaceae food plants and their pharmacological aspects are listed below (Table 3).

5.1. Activity on the central nervous system (CNS)

The effect of several fruit extracts of the Cucurbitaceae family on the central nervous system (CNS) was studied through *In-vivo* model. The anxiolytic activity of alcoholic extract of *Benincasa hispida* (Wax gourd) fruits was evaluated on various behavioural models such as open field model of anxiety, Hole-board apparatus and Mirror chamber on Swiss albino mice. 200 (medium) and 400 (high) mg/Kg doses of the extract resulted in an increase in central locomotion and an increase in latency to the 1st head dips, number of head dips and time spent in head dips. Experimental animals exhibited anxiety through various observations including freezing, grooming (fear), rearing, head dips (curiosity) and the number of fecal boli (Nimbal et al., 2011). Central nervous system (CNS) depressant activity was evaluated on petroleum ether, chloroform and the methanol extract of leaves of *Lagenaria siceraria* (Bottle gourd) 100 and 200 mg/kg doses of extract inserted through intraperitoneal route and methanolic extract exhibited reduction of spontaneous motor activity, motor coordination (fall of time) at higher doses compare to the other extracts (Pawar et al., 2009). The CNS depressant activity was exhibited in methanolic and aqueous seed extracts of *Momordica dioica* (Spine gourd) through neuropharmacological experimental models such as muscle co-ordination and locomotor activity in swiss albino mice. The extracts showed CNS depressant activity and reduced locomotor activity in a dose dependent manner of 100 mg/kg and 200 mg/kg respectively. The CNS depressant activity was achieved by a decrease in the serotonergic and dopaminergic transmission and an increase in the cholinergic

transmission (Rakh et al., 2011). In another study, fruits of *Sechium edule* (Chayote) and *Cucumis sativus* (Cucumber) have been reported to possess antiepileptic and CNS depressant activity. Ethanolic extract of fruits of *Sechium edule* possesses antiepileptic activity by reduction of the duration of various phases of convulsions in maximum electroshock-induced seizure model at a dose of 100 mg/kg and 200 mg/kg. In the Pentylenetetrazol-induced seizure model the extract exhibited potent anticonvulsant activity and delayed the onset of convulsion. The ethanolic extract also showed CNS depressant activity by decreasing the spontaneous locomotor activity and locomotor coordination (Firdous et al., 2012b). CNS depressant activity of methanolic leaves extract of *Cucumis sativus* (Cucumber) was evaluated by pentobarbitone-induced hypnosis, neuropharmacological experiment by open field and hole cross tests in Swiss albino mice. The extract exhibited the reduction of the onset and duration of pentobarbitone-induced hypnosis, also reduction of locomotor and exploratory activities in a dose dependent manner. CNS depressant activities either dwindle the time for onset of sleep or lengthen the duration of sleep or both. Gamma-amino-butyric acid (GABA) is the major inhibitory neurotransmitter in the CNS and many drugs such as different anxiolytic, muscle relaxant, sedative-hypnotic elucidate their action through GABA. The methanolic extract of *Cucumis sativus* reduced the locomotor activity by GABAergic inhibition in the CNS via membrane hyperpolarization or by activating of the GABA receptor directly (Nasrin et al., 2013). An alcoholic extract of the entire fruit of *Luffa cylindrica* (Sponge gourd) exhibited anti-epileptic activity in adult Wistar albino rats. A behavioural study of experimental animals was performed using diazepam induced sleep, pentylenetetrazole induced seizures, maximal electroshock-induced seizure models. At a dose of 400 mg/kg the alcoholic extract prolonged the duration of sleep, minimized time taken to sleep and also exhibited anti-convulsant activity by reducing the onset and duration of convulsion. Mainly apigenin, vitexin, luteolin, quercetin, linarin identified from the extract through LC-ESI MS/MS study were found to be responsible for the anti-epileptic activity (Mishra et al., 2018).

5.2. Analgesic and anti-inflammatory activity

The analgesic and anti-inflammatory properties of some plants of the Cucurbitaceae family *viz.* leaves of *Citrullus colocynthis* (Bitter apple) (Hussain et al., 2014), fruits of *Cucumis melo* (Musk melon) (Vouldoukis et al., 2004), fruits and seed extract of *Momordica charantia* (Bitter gourd) (Ullah et al., 2012; Gill et al., 2012) have been reported by several researchers. A polyherbal formulation named "Jatyadi Ghrita, "consisted *Jasmine officinale*, *Azadirachta indica*, *Berberis aristata*, *Curcuma longa*, *Picrorrhiza kurroa*, *Rubia cordifolia*, *Trichosanthes dioica*, *Aristolochia indica*, *Hemidesmus indicus*, *Randio spinosa*, *Glycyrrhiza glabra*, and Cow's ghee possesses anti inflammatory activity (Kumar et al., 2012). The *in-vitro* and *in-vivo* anti-inflammatory activity of the *Cucumis melo* (Musk melon) fruit extract was evaluated for its high superoxide dismutase activity. C57BL/6 mice were supplemented orally with the extract and also the wheat gluten (*Triticum vulgare*) and a combination of both of them. During the study, the combination showed protection against the pro-inflammatory properties of IFN-γ and inducing the production of IL-10 by macrophages (Vouldoukis et al., 2004). Methanolic extract of *Cucumis sativus* (Cucumber) leaves exhibited analgesic activity at a dose of 200 and 300 mg/kg by acetic acid-induced writhing test and tail immersion test. At the dose of 300 mg/kg the extract exhibited much better analgesic effects and approximately 64% inhibition of writhing response. Inhibitory effect on the writhing response of the extract might be due to the presence of analgesic principles by inhibition of the production of algogenic substances or the transmission of painful message at the central level (Nasrin et al., 2013). Analgesic and anti-inflammatory activity of ethanolic extract *Momordica charantia* (Bitter gourd) fruits was evaluated in *in-vivo* models. Acetic acid induced writhing model in mice, tail immersion method, carrageenan induced hind paw edema in rats were used in the study. The extract resulted in significant inhibition of pain and moderate anti-inflammatory activity at the dose of 500 mg/kg. It can be assumed that the presence of phytochemicals including flavonoid, alkaloids and tannins may be responsible for analgesic activity by targeting the prostaglandins or inhibiting the pain perception (Ullah et al., 2012). The analgesic activity was evaluated in petroleum ether, chloroform and the methanol extract of *Lagenaria siceraria* (Bottle gourd) leaves by several experimental models such as, acetic acid induced writhing, hot plate, tail flick test. Compared to the other extracts Petroleum ether extract exhibited maximum analgesic activity at a dose of 100 mg/kg and 200 mg/kg (Pawar et al., 2009). Methanolic seed extract of *Momordica charantia* exhibited anti-inflammatory activity in rats and inflammation was induced by carrageenan induced rat paw edema model. The extract exhibited anti-inflammatory activity by 62.79% reduction in the paw volume at 500 mg/kg dose (Gill et al., 2012). It has been also found that seed and leaf extract of *Citrullus lanatus* (Watermelon), *Cucurbita maxima* (Pumpkin) fruit and *Cucurbita pepo* (Field pumpkin) seed extract possesses anti-inflammatory potential (Rajasree et al., 2016). In another study, the aqueous and methanolic extract of *Lagenaria siceraria* (Bottle gourd) fruits exhibited analgesic and anti-inflammatory activity (Shah et al., 2012; Ghule et al., 2006a). Fresh fruit juice of *Lagenaria siceraria* possesses analgesic activity on acetic acid-induced writhing and formalin pain tests in mice and anti-inflammatory activity on ethyl phenylpropionate-induced ear edema, carrageenan and arachidonic acid induced hind paw edema, albumin induced paw edema in rats in a dose dependent manner (Ghule et al., 2006a). The anti-inflammatory activity of petroleum ether and methanolic extract of the fruit of *Benincasa hispida* (Wax gourd) was observed in the carrageenan induced paw edema, histamine induced paw edema and cotton pellet induced granuloma in a rat model. Both the extracts exhibited significant inhibition of inflammation in a dose dependent manner and at the dose of 300 mg/kg showed maximum inhibition (Rachchh et al., 2011).

5.3. Antidiabetic and antihyperglycemic activity

The anti-hyperglycemic effect of Cucurbitacecae family is well reported in the management of diabetes. In a study, hypoglycemic activity was evaluated on storage proteins of *Telfairia occidentalis* (Fluted gourd), *Citrullus lanatus* (Watermelon), *Lagenaria siceraria* (Bottle gourd), *Cucumeropsis mannii* (Egusi melon) and *Cucurbita moschata* (Butternut squash). The storage proteins were extracted using the Bradford method and the hypoglycemic activity was performed on Wistar rats. Isolated globulins from above mentioned plants except *Cucumeropsis mannii* showed hypoglycemic activity by reduction of blood glucose level and possessing insulin-stimulating properties (Teugwa et al., 2013). The polysaccharides obtained from *Cucurbita pepo* (Field pumpkin) exhibited antidiabetic activity by improving the insulin level, reducing blood glucose and enhancing the tolerance of glucose in diabetic induced *In-vivo* model. Ethanolic extract of *Cucurbita pepo* peels showed antidiabetic activity on alloxan induced diabetic rats by downregulating the blood glucose and serum lipid level (Dabaghian et al., 2012). Some peptides, sterol glucosides, saponins isolated from *Momordica charantia* (Bitter gourd) aqueous fruit extract exhibit beneficial effects in diabetes management in both Charles Foster rats and diabetic patients compared to the dried fruit powder (Srivastava et al., 1993). In another report, ethanolic extract of leaves of *Coccinia indica* (Ivy gourd) exerts hypoglycemic effects on rats by altering the activity of the gluconeogenic enzyme glucose-6-phosphatase. The extract inhibited the glucose-6-phosphatase and urea cycle enzyme arginase in the liver of 48 h starved and normal-fed animals. (Hossain et al., 1992). Moreover, the fruit, seed, leaves and whole plant extract of *Momordica charantia* (Bitter gourd) exhibited hypoglycemic activity on *in-vitro* model by inhibiting the glucose utilization. The glucose uptake activity was evaluated and estimated by glucose oxidase method (Saeed et al., 2007). *Benincasa hispida* (Wax gourd), *Cucurbita maxima* (Pumpkin), *Lagenaria siceraria* (Bottle gourd), *Luffa acutangula* (Ridge gourd), *Momordica charantia*

(Bitter gourd) and *Trichosanthes cucumerina* (Snake gourd) fruits were successively extracted with n-hexane, chloroform, ethyl acetate, and methanol and α-Glucosidase Inhibitory assay was performed. Due to the presence of phenolic compound methanolic extract of *Lagenaria siceraria* (Bottle gourd) and the ethyl acetate extract of *Sechium edule* (Chayote) were further studied; Isoquercetin present in the methanolic extract of *Lagenaria siceraria* and Gallic acid in ethyl acetate extract of *Sechium edule* possesses potent α-Glucosidase Inhibitory activity (Sulaiman et al., 2013). Some medicinal plants of Cucurbitaceae family including, aqueous seed extract of *Citrullus colocynthis* (Bitter apple) (Al-Ghaithi et al., 2004), aqueous fruit extract of *Momordica cymbalaria* (Kameswararao et al., 2003), leaves and fruit extract of *Coccinia indica* (Ivy gourd) and fruit extract of *Momordica charantia* (Bitter gourd) (Mukherjee et al., 2006) exhibit anti-hyperglycemic activity on *In-vivo* model. The antidiabetic activity of aqueous seed extract of *Citrullus colocynthis* on male Wistar rats was evaluated by inducing using streptozotocin (i.p.), and evaluation of various biochemical parameters such as plasma level of alanine aminotranferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), lactic dehydrogenase (LDH), Blood urea nitrogen (BUN), blood creatinine and levels of K+, Na+, Ca^{2+} and P. The seed extract significantly reduced the levels of AST and LDH in plasma. No significant changes was observed in the levels of blood creatinine, BUN, K+, Na+, Ca^{2+} and P (Al-Ghaithi et al., 2004). Aqueous fruit extract of *Momordica cymbalaria* possesses anti diabetic activity in alloxan induced diabetic Wistar male rats. At a dose of 500 mg/kg showed significant anti-hyperglycemic activity through increase in plasma insulin levels (Kameswararao et al., 2003). Chanda and his co-researchers studied an *in-vitro* α-glucosidase enzyme kinetic assay on the fruits of *Coccinia grandis* (Ivy gourd). The study highlighted the potential antidiabetic activity of ethyl acetate fraction of *Coccinia grandis* fruit extract by inhibition of α-glucosidase enzyme in a dose dependent manner due to the presence of several phytocompounds such as Cathinone, *p*-Coumaroyl glucose, Sinapic acid, Camptothecin, Caffeic acid, Carnosic acid, Cinnamyl alcohol etc. in the active fraction. α-glucosidase enzyme plays an important role in the metabolism and mainly acts as exohydrolases by breaking down the oligosaccharides into simple glucose molecules and is absorbed through the epithelial brush border into circulation. By inhibiting the enzyme resulted the delaying of carbohydrate absorption from the intestinal epithelia and reduced postprandial hyperglycemia (Chanda et al., 2020a).

5.4. Antioxidant activity

Owing to a large number of phenolic and flavonoid compounds present, they are reported to possess significant antioxidant activity. The antioxidant potential of fresh and dried fruits of *Lagenaria siceraria* (Bottle gourd) was evaluated through *in-vitro* DPPH radical scavenging activity reducing capacity assays. Ethyl acetate and n-butanol extract of the fresh fruits of *Lagenaria siceraria* showed high antioxidant activity against DPPH and reducing capacity assay compared to corresponding extracts from dried fruits. There are minor differences in antioxidant properties between the fresh and dried fruit extracts of *Lagenaria siceraria*. It can be assumed that diabetic patients may be inhibited oxidative stress through consumption of this fruit or its extract (Erasto and Mbwambo, 2009). A report has been found that fruit and aril of *Momordica dioica* (Spine gourd), *Momordica subangulata* (Wild bitter gourd) and *Momordica cochinchinensis* (Gac) is a rich source of antioxidants. In a recent study, the ferric reducing antioxidant power and DPPH radical scavenging activities of methanolic extracts of *Momordica dioica, Momordica subangulata and Momordica cochinchinensis* showed the antioxidant activity of the extract using ascorbic acid as the standard compound due to the presence of polyphenolic compounds (Bharathi et al., 2014). The polyphenols and flavonoids components exert antioxidant activity by free radical scavenging as well as ferric reducing antioxidant power assay. Sabo and his co-workers reported antioxidant activity of the seeds of eighteen varieties of edible Cucurbitaceae family plants such as seventeen varieties of *Lagenaria siceraria* (Bottle gourd) (LS1-LS17) and one variety of *Citrullus colocynthis* (Bitter apple) (CC). The antioxidant activity of those plant extracts especially LS5, LS13, LS14 and CC having superior reducing power of Fe^{3+}. The proposed activity is attributed due the presence of phenolic compounds and largely correlated with their several enzyme inhibition (β glucosidase and lipase) activities (Sabo et al., 2014).

5.5. Anticancer/anti-tumor activity

The anticancer activity of some dietary Cucurbitaceae family plants has been extensively reported (Sharma et al., 2015). A large number of cucurbitacin and cucurbitane-type triterpene glycosides isolated from Cucurbitaceae family plants were found to be effective as cytotoxic and antitumoral agents. Some other triterpenes like α-momorcharin, trichosanthin, cyclic bisdesmosides, gypenosides, ribosome-inactivating protein, trichosanthin etc. Obtained from this species possesses cytotoxic and anti-temporal activity. The antitumor activity of trichosanthin isolated from the tubers of *Trichosanthes kirilowii* (Chinese cucumber) was reported in the literature. Trichosanthin was found to be an effective protein and significantly inhibited the growth of solid type tumors and reticulosarcoma in mice. Some proteins isolated from *Luffa cylindrica* (Sponge gourd) seeds exert inhibitory effects on different tumor cell lines by reducing the proliferation of cancer cells. Proteins Luffaculin, luffins a and b demonstrate a wide range of antitumor activity to a variety of different tumor cell lines by inactivating ribosomes (Ng et al., 1992; Méndez-Cuesta et al., 2018). The cytotoxic efficacies of cucurbitaceous plant viz. Seeds and leaves of *Cucumis sativus* (Cucumber) *Benincasa hispida* (Wax gourd), *Cucurbita maxima* (Pumpkin), *Luffa acutangula* (Ridge gourd) and fruits of *Coccinia indica* (Ivy gourd) were studied on HeLa Cell Line. The study highlighted the anti-proliferative effects of the selected plants at lower concentrations rather than higher concentrations, aqueous and ethanolic extract of *Coccinia indica* fruits have a larger inhibition potential against HeLa cell proliferation than others. Dichloromethane extract of leaves of *Benincasa hispida* and *Cucurbita maxima* was highly effective at very low concentration inhibiting the proliferation of HeLa cells in but less effective at higher concentration. The Cucurbitaceae family plants possesses anti tumor efficacy by inhibiting the proliferation of cervical cancer cells by inducing apoptosis. This is an important homeostatic mechanism that maintained and balanced the cell division, cell death and appropriate cell number in the body. By inducing apoptosis in tumor cells and inhibiting uncontrolled proliferation cancer can be treated easily (Varalakshmi and Rao, 2012). Isolated compounds from the ethanolic seed extract of *Momordica cochinchinensis* (Gac) showed an anti-proliferative effect on human lung cancer lines such as A549, H1264, H1299 and Calu-6. By LC-MS analysis two major saponins were identified such as gypsogenin 3-O-β-D-galactopyranosyl (1 → 2)-[α-L-rhamnopyranosyl (1 → 3)] β-D-glucuronopyranoside and quillaic acid 3-O-β-D-galactopyranosyl (1 → 2)-[α-L-rhamnopyranosyl (1 → 3)]-β-D-glucuronopyranoside. These two compounds reportedly reduced primary lung endothelial cell proliferation in human lung cancer cell lines (Yu et al., 2017). The aqueous fruit extract of *Momordica charantia* (Bitter gourd) was reported to prevent skin carcinoma in swiss albino female mice. The extract successfully delayed the onset of dimethylbenzanthracin and croton oil induced skin papilloma. The extract was also able to enhance the activity of several liver enzymes such as glutathione-S-transferase, glutathione peroxidase, catalase and superoxide dismutase and reduced the carcinogen-induced lipid peroxidation in liver and DNA damage in lymphocytes (Ganguly and Das, 2000). The fruits of *Cucumis sativus* (Cucumber), fronds and young leaves of *Coccinia indica* (Ivy gourd) and fruits of *Momordica charantia* (Bitter gourd) sequentially extracted with hexane, chloroform, and methanol and antimutagenic activities were evaluated against direct-acting 2-2-furyl-3-5-nitro-2-furyl acrylamide (AF-2) and sodium

azide (NaN_3) and indirect-acting Aflatoxin B_1 (AFB_1) and Benz(a)pyrene (B(a)P) mutagens using Salmonella mutagenicity test with *Salmonella typhimurium* TA100 as tester strain; anticarcinogenic activity was evaluated on 9,10-dimethyl-1,2-benzanthracene (DMBA) induced mammary gland carcinogenesis. Leaves of *Coccinia indica* and fruits of *Momordica charantia* contain antimutagens which are capable of inhibiting the mutagenicity of indirect-acting mutagen AFB_1 and B(a)P. *Momordica charantia* fruits partially inhibited mammary gland carcinogenesis in female rats by inducing glutathione-S-transferase activity in rat liver and reduce the level of ultimate carcinogenic metabolites of DMBA that can bind to DNA. (Kusamran et al., 1998). The active phytoconstituents of *Trichosanthes cucumerina* (Snake gourd) root extract and fruit juice, such as bryonolic acid and cucurbitacin B respectively assayed for cytotoxicity against human breast cancer cell lines (SKBR3, MCF7, T47D, and MDA-MB435), lung cancer cell lines (A549 and SK-LU1), and one colon cancer cell line (Caco-2). Bryonolic acid exhibited higher efficacy than the root extract inhibiting three breast cancer cell lines (MCF7, T47D, and MDA-MB435) and one lung cancer cell line (A549); also cucurbitacin B showed more efficacy than fruit juice inhibiting all breast cancer cell lines, one lung cancer cell line and colon cancer cell line. The root extract significantly inhibited the SK-LU1 cell line compare to others and inhibited the two lung and three breast cancer cell lines (SKBR3, MCF7, MDA-MB435) than the fruit juice. The fruit juice and Cucurbitacin B inhibited Caco-2 cell line compare to the root extract and bryonolic acid respectively (Kongtun et al., 2009). The *Citrullus colocynthis* (Bitter apple) leaves also possesses anticancer activity against estrogen-dependent and independent breast cancers. Cucurbitacin B and E isolated from the aqueous extract (leaf) of *Citrullus colocynthis*. Together Cucurbitacin B and E in a combination of 1:1 exhibited inhibitory potential on MCF-7 and MDA-MB-231 human breast cancer cell lines in a dose dependent manner. The cucurbitacin B and E and combination of them induced cell-cycle arrest in the G2/M phase of the cell cycle, reduced the protein level of both p34CDC2 and cyclin B and its also inhibited survivin expression and disrupted actin polymerization resulted induction of apoptosis (Tannin-Spitz et al., 2007). Methanolic extract of aerial parts of *Cucurbita maxima* (Pumpkin) exhibited anticancer and antitumor properties against Ehrlich ascites carcinoma in swiss albino mice. Tumor growth response, Tumor volume and Packed cell volume, Tumor cell count, viable and nonviable tumor cell count, percentage increase in life span, estimation of Red and White blood cell, Haemoglobin, counting of erythrocytes and leukocytes, biochemical estimations (serum glutamic pyruvate transaminase, serum glutamic oxaloacetate transaminase and alkaline phosphatase), antioxidant assay of liver tissue and in-vitro cytotoxicity was evaluated on the extract at a dose of 200 and 400 mg/kg. The extract potentially reduced tumor volume by reducing the ascitic nutritional fluid volume and also inhibited packed cell volume which may activate macrophage and inhibiting the vascular permeability. The life span also prolonged in tumor bearing mice after treatment with the extract due to its cytotoxicity and antioxidant properties (Saha et al., 2011).

5.6. Antimicrobial, antiviral and antifungal activity

Some proteins isolated from Cucurbitaceae family plants were found to be effective as antiviral, antifungal and antimicrobial agents. The antimicrobial activity of both fresh and dried *Momordica charantia* (Bitter gourd) leaves extract was evaluated against *Staphylococcus aureus* (ATCC 12692, 358), *Bacillus cereus* (ATCC 33018) and *Escherichia coli* (10,536, 27). The ethanolic extract of the leaves inhibited the growth of all selected gram positive and negative strains. Ethyl acetate fraction of fresh leaves extract proved to be the most potent fraction against *Escherichia coli* (EC 27) and *Bacillus cereus* (ATCC 33018). Due to the presence of several phytocomponents such as steroids, flavonoids, alkaloids and tannins both the extract exhibited antimicrobial potential. In addition, the *In-vitro* anti-Human immune deficiency (HIV) virus activity of Ribosome inactivating protein (RIPs) such as agrostin, gelonin, luffin, α-momorcharin, β-momorcharin, saporin and trichosanthin were evaluated. Through CD4/gp120 interaction assay, HIV-1 reverse transcriptase (RT) assay, HIV-1 protease assay and HIV-1 integrals assay the activity was examined. Among all the RIPs, luffin and saporin exhibited anti-HIV activity by inhibiting the HIV-1 integrase enzyme in a dose dependent manner (Au et al., 2000). Anti-mycobacterial potential of methanolic extract of aerial parts and ripe deseeded fruits of *Citrullus colocynthis* (L.) Schrad. (Bitter apple) was studied whereas, ripe deseeded fruits extract reported to exhibit potential anti-mycobacterial activity against some drug sensitive and drug-resistant *Mycobacterium tuberculosis* strain H37Rv (ATCC 27294) due to the presence of Ursolic acid, cucurbitacin I 2-0-β-Dglucopyranoside and cucurbitacin E 2-0-β-D-glucopyranoside. Ursolic acid, cucurbitacin I 2-0-β-Dglucopyranoside exhibited potent antitubercular efficacy against seven non-multidrug resistant, eight multidrug resistant and one extensively drug resistant *Mycobacterium tuberculosis* and two Mycobacterium other than tuberculosis clinical isolates (Mehta et al., 2013). Ethanolic extract of the fruit pulp of *Momordica cochinchinensis* (Gac) displayed a significant zone of inhibition against *Micrococcus luteus* compare to the aqueous extract (Chuyen et al., 2015). In a study, successive extraction of the fruits of *Trichosanthes cucumerina* (Snake gourd) was done with petroleum ether, chloroform, ethyl acetate and methanol against various gram positive and gram negative bacterial strains including *Bacillus cereus* (MTCC-1305), *Enterobacter faecalis* (MTCC-5112), *Salmonella paratyphi* (MTCC-735), *Staphylococcus aureus* (MTCC-96), *Escherichia coli* (MTCC-729), *Proteus vulgaris* (MTCC-426), *Klebsiella pneumoniae* (MTCC-109), *Pseudomonas aeruginosa* (MTCC-647) and *Serratia marcescens* (MTCC-86) by agar well diffusion method. The methanolic fraction exposed the largest zone of inhibition against on all organisms which was comparable to the standard antibiotic Tobramycin, gentamicin sulphate, ofloxacin and ciprofloxacin may be due to the presence of phenolic compounds, flavonoids, carotenoids and terpenoids in the extract (Reddy and Jose, 2013). *Trichosanthes dioica* (Pointed gourd) root extract corroborated broad spectrum antibiotic potential against five Gram-positive bacteria *Staphylococcus aureus*, *Streptococcus faecalis*, *Bacillus cereus*, *Sarcina lutea*, *Bacillus subtilis* and two Gram-negative bacteria, *Klebsiella pneumoniae* and *Escherichia coli* using disk diffusion method; minimum inhibitory concentrations by broth dilution method was also estimated. Methanolic, aqueous as well as dichloromethane fraction showed significant inhibition against gram positive and gram negative bacteria in disk diffusion and broth dilution method. The highest and dose-dependent activity was shown by dichloromethane fraction against all gram positive strains, particularly to *Bacillus cereus*, at the highest concentration (Bhattacharya and Halder, 2010). *Sechium edule* (Chayote) leaves were screened for their antimicrobial as well as antifungal activity against *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterobacter cloacae*, *Serratia marcescens*, *Morganella morganii*, Acinetobacter baumannii, *Pseudomonas aeruginosa*, Stenotrophomonas maltophilia, Candida spp. and Aspergillus spp. using Agar-well diffusion method, agar macrodilution method and a broth microdilution method. The extract showed inhibition against bacterial and fungi growth in a dose dependent manner due to the presence of abundant quantity of flavonoids (Ordonez et al., 2009). Leaves and stems of *Cucumis sativus* (Cucumber) extracted with ethanol and chloroform and exert significant antifungal activities against six fungal strains such as, *Aspergillus Niger*, *Blastomyces dermatitides*, *Candida albicans*, *Pityrosporum ovale*, *Trichophyton* spp. And *Microsporum* spp. Both the extracts exhibit potent antifungal activity comparable to standard drug Griseofulvin where, *Aspergillus Niger* is most sensitive and *Microsporum* spp. is least sensitive fungal strain (Das et al., 2012). Several antifungal proteins *viz*. cucurmoschin, hispin, luffacylin, vicillin isolated from Cucurbitaceae plants have been reported as potential biofungicides against *Fusarium oxysporum* fungal strain (Yadav et al., 2013). The synergistic antifungal activity was observed in metronidazole while combining with ethanolic extract of *Momordica charantia* (Bitter gourd) leaves. The combination dose exhibits a three fold lesser minimum

inhibitory concentrations value (Santos et al., 2012). The antifungal activity of the leaves and stems extracts of *Solena amplexicaulis* (Creeping cucumber) were estimated by *in-vitro* antifungal activity and Minimum Inhibitory Concentration by using agar disc diffusion broth dilution method respectively against nine fungal species. In compared to the standard drug tetracycline aqueous and methanol extract showed higher antifungal activity (Moorthy et al., 2013). Cucurmoschin peptide isolated from seeds of *Cucurbita moschata* (Butternut Squash) exhibited antifungal inhibitory potential against *Fusariam oxysporum, Mycosphaerella oxysporum* and *Botrytis cinerea*. N-terminal sequence of cucurmoschin was rich in arginine, glutamate and glycine residues and reduced the mycelial growth in all the fungi strains and it also inhibited translation in a cell-free rabbit reticulocyte lysate system (Wang and Ng, 2003).

5.7. Cardioprotective effect

The cardioprotective activity of the fruits of *Trichosanthes cucumerina* (Snake gourd) and *Lagenaria siceraria* (Bottle gourd) was observed in the case of doxorubicin-induced cardiotoxicity in the rat model. The methanolic extract of *Trichosanthes cucumerina* was able to reduce the doxorubicin induced rise of lactate dehydrogenase and creatine phosphokinase-MB isoenzyme. *Trichosanthes cucumerina* showed inhibition of the action of doxorubicin induced increase in the ST, QT and QRS interval thus reducing arrhythmia in male Wistar rats. Similarly, the powdered fruit of *Lagenaria siceraria* was fed to doxorubicin injected male Wistar albino rats, which were able to maintain the QT and ST level compared to the control group. Lactate dehydrogenase, creatine phosphokinase-MB isoenzyme and aspartate aminotransferase was brought back to normal levels upon administration of fruit powder (Shah et al., 2012; Fard et al., 2008). The cardio-tonic activity of *Lagenaria siceraria* fruit juice was also reported (Dhiman et al., 2012). Fruits of *Citrullus lanatus* (Watermelon) and *Cucurbita pepo* (Field pumpkin) have also been found to be therapeutically active against cardiovascular disease and possess cardio-protective activity (Rajasree et al., 2016).

5.8. Hypolipidemic activity

Several reports are available for therapeutic uses of plants in the Cucurbitaceae family against hyperlipidemia. The lipid-lowering activity of these plants alleviates clinical complications, such as insulin resistance, diabetes, hypertension and dyslipidemia related to lipid metabolism. *Momordica charantia* (Bitter gourd) fruit juice has a prominent effect on lipid metabolism by showing lipolysis, reduced lipid content and decreased mRNA expression of adipocyte transcription factors, thus addressing hyperlipidemia. It was resulted with the reduction of lipid accumulation in primary human adipocytes through regulating adipogenic transcription factors as well as adipocytokine gene expression (Nerurkar et al., 2010). Ethyl acetate fraction of *Momordica charantia* hydroalcoholic fruit extract also expresses the hypolipidemic activity. Due to the presence of several phytochemicals such as, Feruloyl quinic acid, Vanillic acid, Gentisic acid, 4-Hydroxybenzoic acid 4-Oglucoside, vanillin, *p*-Anisaldehyde, Phenylacetic acid, Methyl catechol, Esculetin, Scopoletin, Benzoic acid, Coumaric acid, Protocatechuic aldehyde, Methylgallic acid, [6]-Gingerol etc. in dose dependent manner by inhibition of pancreatic lipase enzyme compared to positive control Orlistat. Pancreatic lipase catalyzes the hydrolysis of triglycerides present in food into glycerol and fatty acids resulting in accumulation of body fat. By inhibiting the enzyme absorption of triacylglycerol and extreme production of free fatty acid can be reduced and this strategy offers a therapeutic target to control obesity (Chanda et al., 2018b). Ethanolic extract of *Coccinia indica* (Ivy gourd) aerial parts and *Melothria maderaspatana* exhibits hypolipidemic activity in streptozotocin-induced Sprague-Dawley diabetic rats. The reduction of blood glucose level and enhanced glycogen formation were observed after treating with both the extracts. The high density lipoprotein level was upregulated due to the increasing activity of lecithin cholesterol acyl transferase and low density liportein and very low density lipoprotein levels reportedly decreased exhibiting the desired anhtihyperlipidemic potential (Balaraman et al., 2010). The ethnopharmacological uses of *Citrullus colocynthis* (Bitter apple) as lipid lowering agents have been well documented. A double blind clinical trial with seed powder of *Citrullus colocynthis* showed a significant decrease in serum triglyceride and cholesterol levels in nondiabetic hyperlipidemic individuals (Hussain et al., 2014). The alcoholic and aqueous extract of fruit of *Lagenaria siceraria* (Bottle gourd) showed potential activity in lowering total cholesterol, triglyceride and low-density lipoproteins and as well as increasing high-density lipoproteins level, thus exhibited hypolipidemic activity (Ghule et al., 2006b). Some other fruits from the plants of this family *viz. Cucurbita maxima* (Pumpkin) (Rajasree et al., 2016), *Luffa cylindrica* (Sponge gourd) (Partap et al., 2012) and *Momordica charantia* (Bitter gourd) also have been reported for their anti-hyperlipidemic effect in streptozotocin induced diabetic rats. (Hossain et al., 2012). Hypoglycemic and hypolipidemic activity were evaluated on an aqueous extract of *Momordica charantia* (MC) fruit and several biochemical parameters viz. Separate Transaminenase (AST), Alanine Transaminenase (ALT), Alkaline Phosphatase (ALP), Total cholesterol (TCh) and Triglyceride (TGA) were measured. The fruit extract showed reduction of the blood sugar level and body weight of experimental animals in a dose dependent manner (Hossain et al., 2012).

5.9. Hepatoprotective activity

Several plants of the Cucurbitaceae family possess significant hepatoprotective activity. The ethanolic extract and fractions of *Sechium edule* (Chayote) fruits and its ethyl acetate and n-butanol fractions has been reported for inhibiting CCl_4 induced hepatotoxicity in the Wistar strain of albino rats by protecting the liver cells through its antioxidative effect on hepatocytes at a dosage range of 100–200 mg/kg. By estimation of serum biochemicals such as aspartate, aminotransferase, alanine aminotransferase, alkaline phosphatase, total protein and histopathological studies the hepatoprotective activity of *Sechium edule* was confirmed in a dose dependent manner (Firdous et al., 2012a). Fruit juice and aqueous seed extract of *Momordica charantia* (Bitter gourd) when fed to male Sprague-Dawley rats under light ether anaesthesia for 30 days, elevated the levels of serum γ-glutamyl transferase and alkaline phosphatase enzymes indicating the hepatoprotective effect (Talukdar and Hossain, 2014). It was also observed that methanolic polyherbal mixture consisting of *Trichosanthes dioica* (Pointed gourd), *Pistacia khinjuk*, *Cheilanthes albomarginata*, *Cheilanthes farinose* and *Clematis gouriana* leaves, fruits and stem prevented gastric ulcers and liver necrosis in Wistar rats by an increase or decrease in the levels of several biochemical parameters. Rifampicin was used to induce hepatotoxicity and antiulcer activity was induced by pyloric ligation. The ulcer healing property of polyherbal mixture was achieved by regeneration of liver cells by the action of the phytoconstituents Tannins, Flavonoids, Vitamin A, Vitamin C and Quinic acid (Kulkarni and Raghavan, 2013). In addition, hydroalcoholic extract of *Luffa acutangula* (Ridge gourd) fruits exhibited hepatoprotective activity against CCl_4 and rifampicin-induced liver toxicity. It marked a significant decrease in the levels of liver enzymes and increased the contents of the endogenous antioxidant glutathione (Jadhav et al., 2010). Other plants of this family, seed and seed oil of *Cucurbita pepo* (Field pumpkin) and *Lagenaria siceraria* (Bottle gourd) fruits also possess hepatoprotective activity (Rajasree et al., 2016).

5.10. Immunomodulatory effect

Several reports on the immunomodulatory effect of plants of the Cucurbitaceae family are documented. The fruits of *Lagenaria siceraria* (Bottle gourd) showed immunoprotective, as well as

immunostimulation potential (Rajasree et al., 2016). The immunomodulatory activity of ethanolic extracts of *Lagenaria siceraria* fruit was evaluated through humoral immune response to sheep red blood cells by haemagglutination response, cell mediated immune response and neutrophil adhesion to nylon fibres on Swiss albino mice. The fruit extract significantly prevented the reduction of humoral immune response, cellular immune response and also percent neutrophil adhesion which were induced by pyrogallol at a dose of 23 mg/kg of extract (Deshpande et al., 2008). The saponin mixtures isolated from the fruit of *Lagenaria siceraria* exhibited immunomodulatory activity in mice through haemagglutination antibody titre, delayed type hypersensitivity and carbon clearance parameters. The extract resulted in an increase in haemagglutination antibody titre and carbon clearance and inhibited the delayed type hypersensitivity response in a dose dependent manner at a dose range of 50–150 mg/kg (Gangwal et al., 2008). In a recent report, the ethanolic fruit pericarp extract of *Luffa acutangula* (Ridge gourd) exhibited immunomodulatory activity in a dose dependent manner by increasing the phagocytosis percentage of neutrophil adhesion in swiss albino mice at dose of 100 and 200 mg/kg (Shendge and Belemkar, 2018). Some phenolic compounds, *viz.* Gallic acid, *p*-Hydroxybenzoic acid etc isolated from the fruits of *Luffa acutangula* Var. Amara showed potential immunomodulatory activity by *in-vivo* phagocytosis assay and neutrophil adhesion test. The ethanolic extract showed comparable phagocytosis of the carbon by the cells of the reticulo-endothelial system with standard drugs. Neutrophil adhesion test in mice corroborated the adhesion of neutrophils in a dose dependent manner and in an increased pattern compared to standard groups, suggesting a possible immunostimulant action (Kalaskar and Surana, 2014). *Luffa cylindrica* (Sponge gourd) also possesses immunostimulatory activity. The petroleum ether fraction of the fruits, leaves and stem extracted with ethanol when ingested orally initiated a phagocytic response of the peritoneal macrophages in mice (Partap et al., 2012). The exploration of immunomodulatory activity of *Cucurbita pepo* (Field pumpkin) and *Momordica dioica* (Spine gourd) was reported in the literature earlier (Rajasree et al., 2016; Jafarian et al., 2012). The methanolic, chloroform and ethyl acetate extracts of *Cucurbita pepo* fruits was studied for evaluating their immune response activity. Sheep red blood cells were injected intraperitoneally in Balb/c mice for inducing paw swelling of experimental animals. All three extracts of *Cucurbita pepo* fruits reduced the paw swelling in a dose dependent manner at a dosage range of 100 mg/kg and 500 mg/kg doses in both innate and acquired immunity models. In the innate immunity model compared to the methanolic and ethyl acetate extracts the chloroform extract was less potent and the activity was attained due to several phytoconstituents especially cucurbitacin-A cyclic triterpenes (Jafarian et al., 2012).

Table 4
List of Cucurbitaceae food plants as in FDA Poisonous Plant Database (https://www.fda.gov/food/science-research-food/fda-poisonous-plant-database) (FDA, 2018).

Sl. No.	Plant name	Common name	Plant part(s)
	Telfairia occidentalis Hook. f.	Fluted Pumpkin	Root, Leaves
	Citrullus colocynthis (L.) Schrad.	Colocynth, coloquinte	Fruits, Leaves
	Luffa operculata (L.) Cogn	Sponge Cucumber	Fruits
	Melothria scabra Naudin	Cucamelon	Fruits
	Lagenaria siceraria (Molina) Standl.	Calabash, Bottle gourd	Fruits, Leaves
	Bryonia dioica Jacq.	Bryony, Red bryony, wild neep	Leaves
	Trichosanthes kirilowii Maxim.	Chinese Cucumber	Seeds, Tubers, Roots
	Cucumis myriocarpus Naudin	Prickly paddy melon	Fruit
	Cucurbita maxima Duchesne	Winter squash	Seeds
	Ecballium elaterium (L.) A. Rich.	Squirting cucumber	All parts especially fruits
	Momordica charantia L.	Bitter gourd	Fruits
	Cucumis melo L. *subsp. melo var. conomon (Thunb.) Makino*	Sweet melon, snake cucumber	Fruits
	Cucurbita moschata Duchesne	Butternut squash	Fruits
	Cucurbita pepo L	Field pumpkin	Seeds
	Cucurbita digitata A. Gray	Coyote Gourd	Seeds
	Cucurbita foetidissima Kunth	Missouri gourd	Seeds
	Cucurbita pepo L. *subsp. pepo*	Zucchini	Fruits
	Marah oregonus (Torr, and *S. Watson) T. J. Howell*	Wild cucumber	seed
	Sechium edule (Jacq.) Sw.	Chayote	Fruits
	Cucumis melo L. *subsp. agrestis (Naudin) Pangalo*	Musk melon	Fruits
	Bryonopsis laciniosa (L.) Naudin	Gooseberry, native bryony	Fruits
	Cucumis aculeatus Cogn.	Kisawasawa	Fruits
	Cucumis africanus L. *f.*	Wild cucumber	Fruits
	Cucumis ficifolius A. Rich.	Wild melon	Entire plant
	Cucumis hirsutus Sond.	Wild cucumber	Fruits
	Citrullus lanatus (Thunb.) Matsum. and *Nakai*	Bijada cake, Watermelon	Fruits
	Trichosanthes dioica Roxb.	Parval gourd, Pointed gourd	Seeds
	Melothria punctata Cogn.		Fruits
	Trichosanthes cucumerina L.	Snake gourd	Root tubers
	Luffa acutangula (L.) Roxb	Ridge gourd	Seeds

6. Toxicity

Although the plants of Cucurbitaceae family are well recognized for their nutritional and therapeutic benefits, sometimes their uses should be restricted due to adverse reactions in both human and animals which may come from different uptakes, processing methods, physical differences and other conditions (Jia et al., 2017). To overcome this problem, the toxicity profile of the plants needs to be checked which may be developed during the use of herbal therapy. There are several reports, which have been found in the toxic effects of Cucurbitaceae family plants. Leaf, fruit and root of *Cucumis africanus* are widely used as an emetic, purgative in Southern Africa. But due to the presence of toxic principle cucurbitacin, it showed many adverse symptoms such as vomiting, bloody diarrhoea, kidney damage, spasms, respiratory arrests and also death (Ndhlala et al., 2013). Due to the presence of some toxic phytocompounds in the ripe fruits and roots of *Cucumis africanus*, *Cucumis myriacarpus*, *Cucumis leptodermis* sudden death with severe lung edema was seen and also was a cause of haemorrhagic diarrhoea in cattle (Botha and Penrith, 2008). Roots of *Kedrostis nana* caused poisoning in rabbits and sheep due to the presence of some irritant. Consumption of large amounts of this plant part resulted in nausea, vomiting, diarrhoea and also death by respiratory paralysis (Deutschländer et al., 2009). The toxicity of juice and alcoholic extract of *Momordica charantia* (Bitter gourd) fruit was observed in normal and alloxan induced diabetic rats. In post-mortem examination, congestion of internal organs and change in blood color was found (Batran et al., 2006). The antifertility activity of the proteins isolated from *Momordica charantia* leaves was reported by altering spermatogenesis and by inducing histological changes in both testis and accessory reproductive organs of albino mice. Some reports have been found that high dose ingestion of the fruit caused abdominal pain and diarrhoea in diabetic patients. Due to the cytotoxic behaviour, it causes significant inhibition of DNA and protein synthesis and thus acts as an anti-metabolite (Jia et al., 2017). In a study, it was observed that high doses of fresh juice of *Momordica charantia* cause death in rats when administered via intraperitoneal route at 6.0 ml/kg (Sharma et al., 1960). In Kenya, *Momordica foetida* (Wild cucumber) root was reported to be poisonous in cattle. (Omokhua-Uyi and Staden, 2020). Adverse effects like dehydration, haemorrhage of the alimentary tract, edema and swelling of the livers, swollen and vacuolated hepatocytes, pulmonary congestion and abdominal pains were seen after consumption of a large quantity of ripe fruits of *Cucumis myriocarpus* (McKenzie et al., 1998). *Bryonia Alba*

(White Bryony) demonstrated severe laxative, emetic and food poisoning symptoms due to the presence of a toxic terpenoid. The dried fruit pulp of *Citrullus colocynthis* (Bitter apple) causes vomiting, colicky pain, bloody diarrhoea, dysenteric diarrhoea, congestion and hyperemia of the mucus in humans. Intranasal consumption of *Ecballium elaterium* (Squirting cucumber) caused angioedema with fatal cardiac and renal failure (Aronson, 2016).

In another study, the ethanolic extract of the fruits of *Citrullus colocynthis* (Bitter apple) extract exhibited liver toxicity by inhibiting the ferric stimulated liver peroxide (LPO) in a dose-dependent manner (Barth et al., 2002). A high dose of *Citrullus colocynthis* set out morphological changes in the liver, such as granulation of the cytoplasm including collagen and reticular fibres. It also induced hepatocyte necrosis and liver fibrosis in rat liver (Dehghani and Panjehshahin, 2006). The toxic effects of the fruit juice of *Lagenaria siceraria* (Bottle gourd) are well reported due to consumption of the high amount of Cucurbitacin, which causes dehydration and gastrointestinal injury and renal toxicity (Puri et al., 2011). In a case report, it was documented that intake of bitter varieties of *Lagenaria siceraria* cause poisoning in humans. Bottle gourds when grown under extreme temperatures and poor soil quality produced a higher amount of cucurbitacin B, D, G, and H. Symptoms such as diarrhoea, hematemesis and hypotension occurred after ingestion (Ho et al., 2013). In another study, the leaf extract of *Coccinia grandis* (Ivy gourd) showed severe toxicity in alloxan-induced diabetic Wistar rats in higher doses (Attanayake et al., 2013). Combined extract of *Cucumis zeyheri* and *Cucumis metuliferus* exhibited toxicity in the kidney and liver of rats at 1000 mg/kg dose but no toxicity was reported in chickens while administered through oral and intraperitoneal routes (Wannang et al., 2007). The fruit juice of *Cucumis anguria* exhibited toxicity in rats, where LD50 was 1.6 mg/kg, whereas the boiled juice of this fruit resulted in lesser toxicity (Omokhua-Uyi and Staden, 2020). In a study, β-Sitosterol, Cucurbitacin E and Cucurbitacin L 2-O-β-glucoside were isolated and identified from chloroform extracts of the fruit of *Citrullus lanatus* var. citroides (Wild melon) which prompted toxicity against brine shrimp larvae (Hasan et al., 2015). Ethanolic fruit extracts of *Citrullus lanatus* exhibited no sign of toxicity on Wistar rats against a 28 day repeated toxicity study. The experimental animals were devoid of any changes in behavioural, gross pathology, body weight, biochemical and hematological parameters (Belemkar and Shengde, 2021). Toxicity study for *Diplocyclos palmatus* (Striped cucumber) methanolic leaf extract was performed with qPCR analysis; up to 600 μg/ml dosage was administered which did not affect the lifespan and healthspan of *C. elegans* proving a non-toxic nature of *Diplocyclos palmatus* (Rajaiah et al., 2019). 70% ethanolic fruit pulp extract of *Benincasa hispida* (Wax gourd) was subjected to acute oral toxicity study according to the OECD guideline and the extract revealed no mortality and behavioural signs of toxicity in experimental animals (Shakya et al., 2020). In a case study, it was observed that excess consumption of the fruit of *Luffa echinata* (Bristly luffa) caused gastrointestinal bleeding, deranged liver function (Giri et al., 2014). A list of poisonous plants from the Cucurbitaceae family has been shown in Table 4 based on the USFDA Poisonous Plant Database (https://www.fda.gov/food/science-research-food/fda-poisonous-plant-database).

7. Ethnoveterinary uses

Plants belonging to the Cucurbitaceae family exhibit several ethnoveterinary uses. The uses of herbal remedies for animal healthcare mostly depend on traditional beliefs and also the shortage of modern medicine. Several reports have been found on the uses of the plants of the Cucurbitaceae family in ethnoveterinary practices. The tuber of *Cucumis ficifolius* is used in the treatment of blackleg, colic and emaciation in cow, bovine, etc (Tamiru et al., 2013). Leaves of *Cucurbita pepo* (Field pumpkin) are used in the treatment of trypanosomiasis in animals. The leaves and seeds of *Lagenaria siceraria* (Bottle gourd) are used to treat rabies and trypanosomosis; *Momordica foetida* (Wild cucumber) leaves are useful in the management of fracture, rabies, trypanosomosis, myiasis, lice and some ectoparasite infestation (Sori et al., 2004) and also useful to treat Babesiosis and Anaplasmosis in sheep, goat and cattle (Eshetu et al., 2015). It also has sedative effects on animals. The root decoction of *Citrullus colocynthis* (Bitter apple) is given to the animals to cure constipation and also used during delivery (Galav et al., 2013). In addition, the application of the juice of *Citrullus colocynthis* was reported in the treatment of skin infection of buffalo, cow, goat (Tariq et al., 2014). In another study, Reang and his co-workers reported the use of a mixture of animal feed along with *Luffa acutangula* (Ridge gourd) to improve indigestion and constipation-related problems in animals (Reang et al., 2016). The uses of some cucurbitaceous plants from Eastern Ghat, *Corallocarpus epigaeus* (leaves) and *Kedrostis rostrate* (roots) have been reported in the management of enteritis in animals (Usha et al., 2015). The seed of *Cucurbita maxima* (Pumpkin) was found very useful in controlling worm's related infection in animals (Rajkumari et al., 2014). Other species viz. *Luffa*, *Cucurbita* etc. are widely used as a complementary dietary ingredient of feed for poultry and increasingly as a protein and vitamin supplement to aqua feeds. Members of this family such as *Momordica* spp., *Cucurbita* spp., *Cucumis* spp. etc. also used as remedies for livestock (Ajuru and Nmom, 2017). Thus, the Cucurbitaceae family plants serve a potential role in providing nutrition, therapeutic benefits, and food security to the livestock to a great extent.

8. Nutritious and economical importance of Cucurbitaceae family

Different parts of Cucurbitaceae family plants are used in the human diet for their several nutritional benefits. Some major species of Cucurbitaceae family, e.g., *Cucurbita* spp. (Pumpkins, squashes, gourds, marrows, courgettes), *Cucumis* spp. (Melons, cucumbers), *Benincasa* spp. (Wax gourd, apple gourd), *Citrullus* spp. (Watermelon), *Sechium edule* (Chayote), *Lagenaria* spp. (Calabash, bottle gourd) and *Luffa* spp. (Sponge gourd, sponge bitter gourd, angular gourd, ridge gourd) are widely used for edible purposes (Ajuru and Nmom, 2017).

Some plants of the Cucurbitaceae family are used for making items of utility such as drinking vessels, cooking pots, utensils, bath sponge, industrial filters and sound insulation. Most of the members of this family contain cucurbitacins, which are bitter and possess high medicinal value. *Benincasa hispida* (Wax gourd) are usually found in a tropical atmosphere with moderate rainfall. The white, chalky wax which covers the fruit prevents the occurrence of microorganisms. The fleshy part of the fruit is used to make the soup stock. *Citrullus colocynthis* (L.) Schrad. or Bitter apple helps in obtaining a balanced diet and the seeds are also edible, but the fruits are very much better (Badifu and Ogunsua, 1991). The leaves, shoots, and immature fruits of *Coccinia cordifolia* or Ivy gourd are used for culinary purposes. Fruits, shoots, and leaves of *Lagenaria siceraria* (Bottle gourd) and *Luffa acutangula* (L.) (Ridge gourd) are widely used in cooking purposes and are used in making icing for cakes. The fruit flesh of *Cucurbita ficifolia* (Black seed squash), is used with sugar to make candy or it can be fermented to make beer. Immature fruits of *Sechium edule* (Chayote) are used to prepare salads and both in cooked form, having a good source of vitamin C. *Cucumis sativus* L. (Cucumber) contains a good amount of water, with small fibre content. In addition, it provides a good source of vitamins A, K, and C, as well as a large amount of potassium (Mukherjee et al., 2013). The fruit of *Cucumis melo* (Musk melon) consists of juicy flesh, used mainly in preparing dessert. The flowers, stems, and fruits of *Cucurbita pepo* (Field pumpkin) are consumed as a vegetable. *Cucurbita maxima* (Pumpkin) contain a high level of vitamin A, thiamin, niacin, vitamin B6, iron, magnesium, and phosphorus. They also contain vitamin C, vitamin E, potassium, copper and manganese. The flesh is a good source of dietary fibre and the seeds of pumpkin possess fat, high levels of protein, magnesium, and zinc (Ajuru and Nmom, 2017). The medium-sized calabashes or *Lagenaria siceraria* (Bottle gourd) fruits are used for the production of ladles, boxes, water jugs, planters, flutes, sitars, and other musical instruments.

The dry rinds are used as containers for palm wine, water, and floats by fishermen for fishnets and rafts, gunpowder and seeds (Jimoh et al., 2013). Another potential use of *Cucumis sativus* (Cucumber) is being observed in the cosmetic industry. The fruits are softening and whitening of the skin; it offers cooling, healing, and soothing effects and is also used to prepare soup. The mature fruits of *Luffa aegyptiaca* (Loofahs) are used for sponges and filters, and also for stuffing pillows, saddles, and slippers (Vouldoukis et al., 2004). The seed oil of *Citrullus colocynthis* or Bitter apple may be used for the production of biodiesel (Ajuru and Nmom, 2017).

9. Conclusion and future aspects

Medicinal food plants belonging to the Cucurbitaceae family are widely used as food since the ancient times. They are also used in the ethnomedicinal system for the treatments of several ailments. The present review highlights the therapeutic uses of the medicinal plants of this important family, including their geographical origin, morphology, phytochemistry, ethnopharmacology, ethnovetrinary, nutritional importance, therapeutic benefits, safety and efficacy and related aspects. The phytochemical and pharmacological potential indicated throughout this review will popularize this plant family as a potential source of novel therapeutic agents and functional foods, which are being used for different health benefits. The Cucurbitaceae family needs to be evaluated based on combined approaches of chemoprofiling and bioexploration to develop the effective lead for further therapeutic applications. They can be developed for sustainable applications in nutritional, medicinal, ethnoveterinary fields as convenient bioproducts. This study will help to validate the therapeutic claims of several ethnomedicinal uses of these plants of the Cucurbitaceae family. This may help in developing the concept of food as medicine in order to develop new generation therapeutics as alternative medicine to rejuvenate the human health worldwide.

CRediT authorship contribution statement

Pulok K. Mukherjee: Design, planning, execution, discussion, critical appraisal, Manuscript checking, Writing – review & editing. **Seha Singha:** Collection of literature, Manuscript writing, Writing – original draft. **Amit Kar:** Design, execution, collection of literature, Graphical representations, Manuscript writing and checking, Writing – original draft. **Joydeb Chanda:** Collection of literature; manuscript writing and checking, Writing – original draft. **Subhadip Banerjee:** Collection of literature; manuscript writing, Writing – original draft. **Barun Dasgupta:** Manuscript editing, Writing – review & editing, Graphical representations. **Pallab K. Haldar:** Manuscript writing and editing, Writing – review & editing. **Nanaocha Sharma:** Manuscript writing, Writing – original draft, checking and editing, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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