MOLECULAR ANNOTATION AND DOCKING OF SECONDARY METABOLITES OF MEDICINAL PLANTS FOR THE INHIBITION OF ENZYMES OF DIABETIC TARGET

A THESIS SUBMITTED TO THE

CENTRAL DEPARTMENT OF CHEMISTRY

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DEAN'S OFFICE

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EXTERNAL EXAMINERS

The Title of Ph.D. Thesis: " Molecular Annotation and Docking of Secondary Metabolites of Medicinal Plants for the Inhibition of Enzymes of Diabetic Target "

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Asst. Dean

DECLARATION

Thesis entitled **"MOLECULAR ANNOTATION AND DOCKING OF SECONDARY METABOLITES OF MEDICINAL PLANTS FOR THE INHIBITION OF ENZYMES OF DIABETIC TARGET"** which is being submitted to the Central Department of Chemistry, Institute of Science and Technology (IOST), Tribhuvan University, Nepal for the award of the degree of Doctor of Philosophy (Ph.D.) is a research work carried out by me under the supervision of Prof. Dr. Niranjan Parajuli of Central Department of Chemistry, Tribhuvan University.

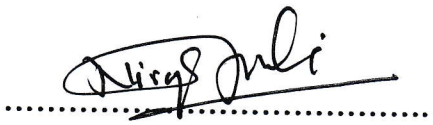
This research is original and has not been submitted earlier in part or in full in this or any other form to any university or institute, here or elsewhere, for the award of any degree.

Basanta Kumar Sapkota

RECOMMENDATION

This is to recommend that **Basanta Kumar Sapkota** has carried out research entitled **"MOLECULAR ANNOTATION AND DOCKING OF SECONDARY METABOLITES OF MEDICINAL PLANTS FOR THE INHIBITION OF ENZYMES OF DIABETIC TARGET"** for the award of Doctor of Philosophy (Ph.D.), in **Chemistry** under my supervision. To my knowledge, this work has not been submitted for any other degree.

He has fulfilled all the requirements laid down by the Institute of Science and Technology (IOST), Tribhuvan University, Kirtipur for the submission of the thesis for the award of a Ph.D. degree.



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August 2023

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Date: August 03, 2023

On the recommendation of **Prof. Dr. Niranjan Parajuli,** this Ph.D. thesis submitted by **Basanta Kumar Sapkota** entitled **"Molecular Annotation and Docking of Secondary Metabolites of Medicinal Plants for the Inhibition of Enzymes of Diabetic Target"** is forwarded by Central Department Research Committee (CDRC) to the Dean, IOST, T.U.

.....

Prof. Dr. Jagadeesh Bhattarai
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It gives me great pleasure and pride to be able to record some of the most memorable moments. I want to express my gratitude to the countless individuals, some of whom have been by my side since the beginning and others who have connected with me later, whose support and love have made this work possible for me.

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Thank you

Basanta Kumar Sapkota

August 2023

ABSTRACT

Type-2 diabetes mellitus (T2DM), a severe endocrine disorder indicated by an unusual elevation in blood glucose levels due to a deficiency in insulin secretion, action, or both, is expected to be the third most common risk factor for premature death. The alarming rise in diabetes prevalence, as well as the surging costs associated with managing diabetes and its complications, highlights the necessity for safer, more efficacious, and cost-effective alternative therapies. Over 1200 plant sources have been documented in ethnomedicine for the treatment of diabetes, providing an additional and valuable source for the discovery of novel antidiabetic compounds. Plants have potential therapeutic properties because of the presence of phytochemicals such as alkaloids, flavonoids, terpenes, phenols, coumarins, and others which reduce cellular stress and prevent cytotoxicity from various agents. Biologically active polyphenols and flavonoids can have antidiabetic properties which could increase insulin secretions or decrease intestinal sugar absorption by inhibiting α-amylase and α-glucosidase. Similarly, lipid metabolism could be controlled by inhibiting pancreatic lipase because lipids are also associated with Diabetes Mellitus. Increased amounts of fatty acids and glucose cause glucolipotoxicity, which decreases insulin production, impairs insulin gene expression, and causes β-cells to die through apoptosis. Moreover, microbial infections cause the body to experience stress, which can lead to hormonal abnormalities that influence insulin production and raise blood sugar levels.

This study, therefore, focuses on the evaluation of the inhibitory activities of enzymes of diabetic targets and secondary metabolites profiling of *Mimosa pudica*, *Bergenia ciliata,* and *Phyllanthus emblica* chosen based on an ethnobotanical knowledge and literature review. Total flavonoid contents (TFC), total phenolic contents (TPC), and antioxidant activities of their extracts were also measured. Additionally, phytoconstituents responsible for inhibiting the enzymes of diabetic targets were identified through *in-silico* analyses.

Higher TPC and TFC have been found in the EA extracts of all three medicinal plants under study. The high TFC value was also shown by the crude methanolic extract of

B. ciliata. Effective antioxidant activities were demonstrated by *P. emblica* and *M. pudica* EA extracts (IC_{50}: 11.98 ± 0.36 µg/mL and 21.39 ± 3.76 µg/mL, respectively), as well as *B. ciliata* aqueous extract (IC_{50}: 16.99 ± 2.56 µg/mL). The IC_{50} values for the EA extract of *B. ciliata* against α-amylase and α-glucosidase were 38.50 ± 1.32 µg/mL and 3.41± 0.04 µg/mL respectively. The hexane fraction of *M. pudica* and *P. emblica* with IC_{50} of 0.49 ± 0.02 and 2.45 ± 0.003 mg/mL showed a higher pancreatic lipase inhibitory activity while methanolic extract of *B. ciliata* inhibited lipase more effectively (IC_{50}: 1.07 ± 0.03 mg/mL). Higher antimicrobial activity was demonstrated by the EA extracts against *Shigella sonnei* ATCC 25931, *Salmonella typhi* ATCC 14028, *Escherichia coli* ATCC 25923, and *Staphylococcus aureus* ATCC 25923. The range of the MIC for the EA extracts of the medicinal plants included in this study was 1.56 to 6.25 mg/mL. Twenty-six significant secondary metabolites were identified through liquid chromatography-high resolution mass spectrometry (LC-HRMS) analysis of various extracts obtained through solvent/solvent partition from three selected plants. *In silico* analysis revealed that procyanidin B3 has the highest binding affinity toward enzymes of the diabetic target with the binding energy of -8.5, -9.1, and -9.5 kcal/mol for α-glucosidase, lipase, and α-amylase respectively. Furthermore, molecular annotated compounds interacted with these enzymes, supporting the efficacy and ethnobotanical uses of the selected plants for their inhibition and showing their therapeutic significance for treating diabetes.

Keywords: Diabetes Mellitus; Medicinal plants; Secondary metabolites, Molecular Docking, Mass spectrometry

LIST OF ACRONYMS AND ABBREVIATIONS

ATCC	: American Type Culture Collection
CFU	: Colony Formation Unit
CNPG	: 2-Chloro-4-nitrophenyl-α-D-maltotrioside
DCM	: Dichloromethane
DMSO	: Dimethyl Sulphoxide
DNA	: Deoxy-ribonucleic Acid
DPP-4	: Dipeptidylpeptidase-4
DPPH	: 2,2-Diphenyl-1-picryl-hydrazyl
EA	: Ethyl Acetate
F-C	: Folin Ciocalteu
GLUT-4	: Glucose Transporter-4
IC_{50}	:The Inhibitory Concentration of the Drug That Gives a Half-Maximal Response
IDF	: International Diabetes Federation
IGF	: Insulin-like Growth Factor
LCMS/MS	: Liquid Chromatography-Mass Spectrometry / Mass Spectrometry
LDL	: Low-density Lipoprotein
MBC	: Minimum Bactericidal Concentration
MeOH	: Methanol
mgGAE/g	: Milligram of Gallic Acid Equivalent Per Gram
mgQE/g	: Milligram Quercetin Equivalent Per Gram
MHA	: Mueller-Hinton Agar

MHB : Muller- Hinton Broth

MIC : Minimum Inhibitory Concentration

mM : Millimolar

NOS : Reactive Nitrogen Species

NPs : Natural Products

OD : Optical Density

PNPB : *p*-Nitrophenylbutyrate

PNPG : *p*-Nitrophenyl-α-D-glucopyranoside

PPA : Porcine Pancreatic α-Amylase

ROS : Reactive Oxygen Species

RNS : Reactive Nitrogen Species

Rpm : Revolution Per Minute

SGLT : Sodium Glucose Co-transporter-2

T2DM : Type-2 Diabetes Mellitus

T2DM : Diabetes Mellitus

TC : Total Cholesterol

TFC : Total Flavonoid Content

TG : Triglycerides

TPC : Total Phenolic Content

VLDL : Very Low-density Lipoprotein

WHO : World Health Organization

ZoI : Zone of Inhibition

LIST OF SYMBOLS

α	: alpha
ß	: Beta
μ	: Micro
Å	: Angstrom
mL	: Millilitre
m/z	: Mass/charge
µL	: Microlitre
nm	: Nanometer
gm	: Gram
℃	: Degree Centigrade
hrs	: Hours

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CHAPTER 1

1. INTRODUCTION

1.1 General introduction

Nature has an infinite variety of molecular entities which serves as an endless source of potential drug candidates, novel chemotypes and pharmacophores, scaffolds for drug amplification into active forms, and other beneficial bioactive compounds. Natural remedies have been the cornerstone of traditional medical practices all over the world since ancient times. Although bioactive natural products (NPs) have been employed for hundreds of years as herbal drug preparations, the application of NPs in the development and detection of modern drugs started only after the 19th century (Veeresham, C., 2012). They have been well-documented to play an essential role in modern drug development (Newman *et al.,* 2007). Over 80% of the overall population relies on NPs because of good efficacy and health safety despite the use of artificial products growing due to their low cost of production, time effectiveness, easy quality control, strict regulation, and quick effects (Luziatelli *et al.,* 2010). NPs are a desirable substitute for synthetic libraries because of their distinct chemical space, particularly in therapeutic areas where there are few lead compounds (Butler, 2004). The potential beneficial properties in medicinal plants are attributed to the occurrence of phytoconstituents like alkaloids, flavonoids, terpenes, phenols, coumarins, etc. which reduce cellular stress and interact with metabolic enzymes. These phytoconstituents are produced in plants as secondary metabolites in the course of metabolic pathways or to combat various stresses during the defense mechanism (Azmir *et al.,* 2013)**.**

Plants restrain a variety of bioactive phytochemicals with advantageous biological effects. So, decoctions can be used to treat complex diseases like type-2 Diabetes Mellitus (T2DM). As a result, a multi-targeted treatment approach that is both effective and affordable could be developed. To treat T2DM, phytochemicals such as polyphenols and flavonoids can scavenge reactive free radicals and reduce oxidative damage (Lv *et al.,* 2021). Additionally, pharmacologically active phytochemicals do have anti-diabetic properties as they enhance the function of pancreatic cells which is

frequently accomplished by stimulating the secretion of insulin or decreasing intestinal glucose absorption by inhibiting vital enzymes concerned with glucose synthesis (Alam *et al.,* 2022). Identifying medicinal plants with bioactive phytochemicals with the capacity of pancreatic lipase, α-amylase, and α-glucosidase inhibition, and possessing antioxidant properties is crucial for treating T2DM in conventional medicine (Kooti *et al.,* 2016). Additionally, over the years, effective antimicrobials have been formed but even so, a dramatic increase in antimicrobial drug resistance has been witnessed (Aslam *et al.,* 2018). The imbalance between the formation of free radical intermediates and the activity of antioxidant defenses is known as oxidative stress, and it has been connected with several chronic illnesses, including cancer, neurodegenerative, diabetes, and heart diseases (Sharifi-Rad *et al.,* 2020). Many investigations have been conducted to investigate the antioxidant with antimicrobial properties of natural products (Angiolella *et al.,* 2018; Bruck de Souza *et al.,* 2020).

The scarcity of chemical entities throughout many medicinal areas and the novel chemical space occupied by NPs have rekindled interest in NPs research. The enormous number of metabolites derived from natural sources at various stages of clinical development highlights the importance and current viability of using natural products as novel candidates. This revived interest, however, can only be preserved if NP research continues to compete with other drug discovery programs. Continuous improvements in the efficiency of compound supply, isolation, dereplication, structure elucidation processes, and careful drug target selection for NP library are thus essential for maintaining competitiveness (Nisbet *et al.,* 1997).

1.2 Natural products

Simply, natural products are low molecular weight molecules (less than 1500 Da) obtained from a biological source such as animals, plants, or microorganisms. Historically, NPs have been a profuse resource of compounds that have been extensively applied in biology, pharmacy, and medication. NPs investigation continues to investigate a diverse range of major structures that may serve as templates for the pharmaceutical industry. Since ancient times, NPs notably from microorganisms and plant sources have been associated with mankind. These have a crucial role in the exploration of novel chemical entities for drug development. Drug

leads for synthetic method optimization were also derived from natural substances. In some therapeutic areas, like antimicrobials, anticarcinogenic, antihypertensive, and anti-inflammatory drugs, the majority of new chemical entities were acquired from nature (Mahidol *et al.,* 1998). It would be preferable to anticipate the identification of new target molecules from various plant sources and assess their utility (S. Priya *et al.,* 2020). Identifying novel molecular tools for studying the biological macromolecules associated with human disease is a critical step toward a more comprehensive understanding of human disease (Molinari, 2009). Thus, NPs research is an excellent strategy for identifying novel biological probes for a variety of diseases. Many of the therapeutic drugs currently in use were first derived from natural sources. Some of the therapeutic drugs isolated from NPs with their applications are listed below in **Table 1.**

Table 1: Some drugs extracted from natural products and their uses

S.N.	Plant	Family	Medicine/Drug	Application/uses
1	*Atropa belladonna*	Styracaceae	Benzoin	Ophthalmology
2	*Cumanmomum Camphora*	Lauraceae	Camphor	Rheumatic pain
3	*Rauvolfia canescens*	Apocynaceae	Deserpidine	Hypertension
4	*Papaver somniferum*	Papaveraceae	Morphine	Analgesic
5	*Papaver somniferum*	Papaveraceae	Papaverine	Antispasmodic
6	*Rauvolfia serpentine*	Apocynaceae	Risperidone	Hypertension
7	*Chinchona pubescens*	Rubiaceae	Quinine	Malaria, Prophylaxis
8	*Filipendula ulmaria*	Rosaceae	Aspirin	Analgesic, Inflammation
9	*Ephedra sinica*	Ephedraceae	Ephedrine	Respiratory, Ailments
10	*Erythroxylum coca*	Erythroxylaceae	Cocaine	Analgesic,Antitussive
11	*Galiga officinalis*	Leguminaceae	Galegine	Diabetes

1.3 Phytochemicals

Phytochemicals (the Greek word 'Phyto' means plants) are naturally occurring bioactive constituents found in plants that are consumed as vegetables, fruits, grains, spices, beverages, and medicines. Due to their positive impact on human health, plant-derived phytochemicals with nutraceutical qualities are quite significant. They are present in various plant parts such as stems, leaves, roots, and fruits, and protect the plants from disease and adverse conditions (against pathogens, predators, and environmental stress). They are also responsible for imparting color and aroma, fragrance or flavor, dye, and pigments in some plants. Some of the phytochemicals act as antioxidants, electron donors, hydrogen donors, enzyme inhibitors, singlet oxygen quenchers, and metal ion chelators. Phytochemicals are generally classified as primary and secondary based on the metabolic functions they provide, with the former including carbohydrates, fat, proteins, nucleic acid chlorophyll, and so on, whereas the latter includes phytochemicals such as alkaloids, flavonoids, saponins, terpenes, lignin, steroids, curcumins, phenolics, and glucosides (Saxena *et al.,* 2013). Although they are non-essential nutrients, phytochemicals have a crucial function in the management of countless ailments such as diabetes, cancer, hypertension, metabolic and neurological disorder, and heart and respiratory diseases as a part of traditional or modern medication.

Phytochemical components in medicinal plants are primarily responsible for the wide-ranging and diverse pharmacological actions of those plants. According to their function in fundamental metabolic processes, the primary and secondary metabolites are the two broad categories used to classify phytochemical components of plants.

1.4 Primary metabolites

Primary metabolites are the small organic molecules that are found in living tissues with a crucial function in growth, respiration, and development. Throughout the course of fundamental physiological processes, they carry out specific activities and assume well-known roles (Springob *et al.,* 2009).

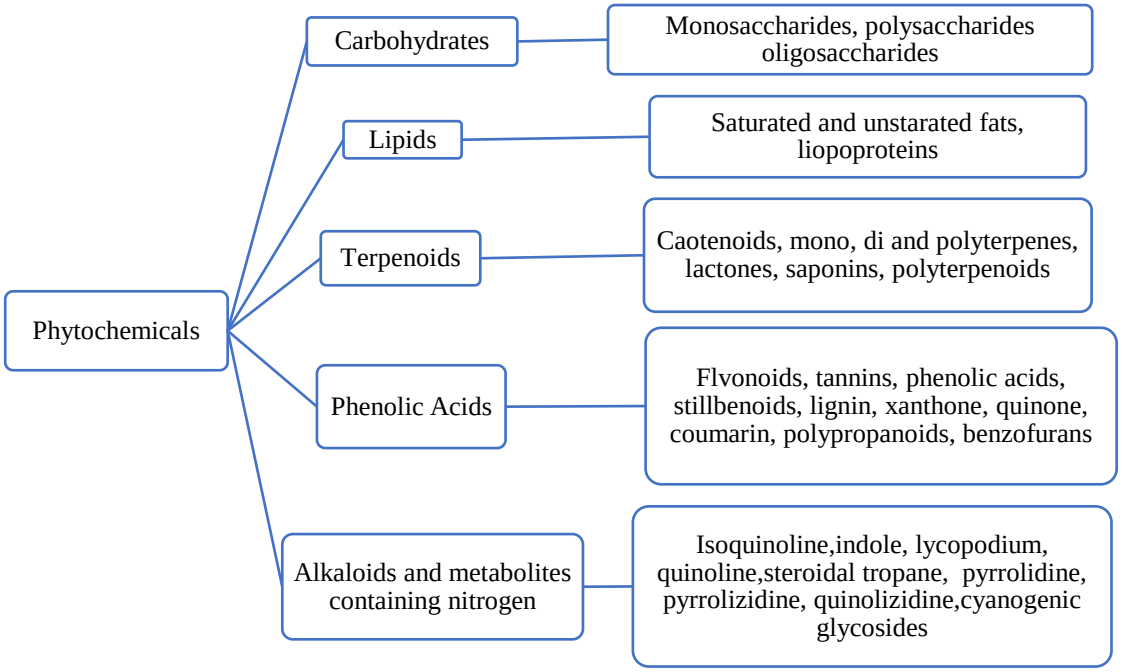


Figure 1: Classification of phytochemicals (Huang *et al*., 2016)

Table 2: Phytochemicals and their mode of activity (Bhushan *et al*., 2010)

Phytoconstituents	**Mode of activity**
Flavonoids	Suppresses glucose level, and plasma cholesterol, and enhances hepatic glucokinases, probably by stimulating insulin release from the pancreas
Alkaloids	α-glucosidase and α-amylase inhibition to reduce glucose absorption through the intestinal lumen.
Imidazole compounds	Enhance the secretion of insulin in a way that is reliant on blood sugar.
Polysaccharides	Increase serum insulin level and improve glucose tolerance.
Dietary fibers	Inhibit α-amylase and reduce glucose absorption and decrease post-prandial sugar level.
Saponins, tannins, and telluric acid	Stimulates the insulin release.

Primary metabolites operate as chemicals that signal to initiate a defense response through signal transduction and pathogen detection processes also. They respond to

pathogen infection by regulating carbohydrates, protein, and lipids (Rojas *et al.*, 2014). Examples are organic acids, sugars, amino acids, fatty acids, nucleosides, and nucleotides

1.5 Secondary metabolites

Plant cells generate innumerable chemical compounds known as secondary metabolites through metabolic routes that divert from the core metabolic processes. The term "Secondary Metabolite" was initially used by Albrecht Kossel, who was awarded the Nobel Prize (1910) in medicine or physiology ("The Imperial Agricultural Research Conference," 1927). Secondary metabolites exert many impacts on the plants themselves and also other living beings. Moreover, they are the byproducts of other processes like the shikimic acid system (Hussein *et al.*, 2019)). These increase their competitiveness within their respective ecosystems. The therapeutic benefits of many contemporary drugs and medicinal plants are based on secondary plant metabolites (Teoh, 2016). The plant kingdom has more than 50,000 secondary metabolites, each one of them possessing certain bioactivity.

In traditional medicine and folk applications, secondary plant metabolites were crucial in treating a variety of illnesses, while in the context of modern medicine, they contributed as the principal molecules in the development and synthesis of medications for multiple ailments, including migraine and cancer (Hussein *et al.*, 2019). They have been reported to be antibiotic, antifungal., and antiviral substances, making them capable of defending plants from infections. Secondary metabolites from plants also act as antioxidants, pheromones, attractants, stimulators, enzyme inhibitors, toxic compounds, etc. (Wink, 2018). The major bioactive components of secondary metabolites including flavonoids, alkaloids, terpenes, and other polyphenols present in the various parts of plants (Nwokeji *et al.*, 2016).

1.6 Types of secondary metabolites

Secondary metabolites are different in chemical, structural, and functional properties. They are classified into the following three major groups.

- Terpenes or Isoprenoids eg., essential oils rubber, carotenoid pigments. steroids,

- Nitrogen-containing compounds eg., alkaloids, glycosides, glucosinolates, and amino acids (non-protein)
- Phenolic compounds eg., tannins, lignin, coumarins, flavonoids (anthocyanins). aflatoxins,

Some important secondary metabolites synthesized by plants are given below.

1.6.1 Phenolics

Phenolics are the largest class of secondary metabolites comprising one or many phenol functional groups. They include phenols, cinnamic and benzoic acid, tannins, lignin, coumarins, and flavonoids (Khoddami *et al.,* 2013). Plant phenolics are considered to act as protective chemicals against harmful environmental factors such as low light and high temperatures, pathogen infection, herbivore attack, and nutrient deprivation (Lattanzio, 2013).

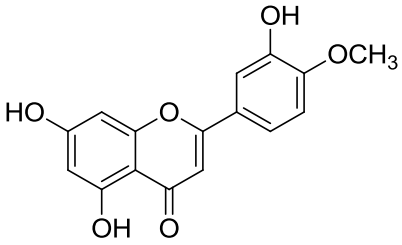
They are found in every plant (like fruits, cereals, vegetables, legumes, chocolate, olives, etc.) and beverages (coffee, tea, etc.) (Dai *et al.,* 2010). Several studies have established that a fruits and vegetable-rich diet can slow down the aging process, reduce oxidative damage, and can control inflammation linked to chronic diseases like cancer, arteriosclerosis, heart disease, diabetes, neurological disorders, cataracts, and issues with cognitive function (Minatel *et al.,* 2017).

1.6.2 Flavonoids

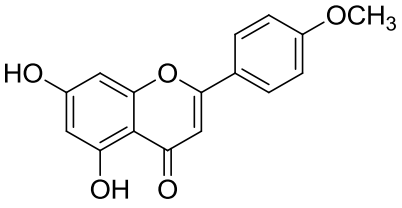
The majority of naturally occurring phenols, known as flavonoids consist of a chromanol ring containing an aromatic ring in C-2, C-3, or C-4 (Serafini *et al.,* 2010). They are typically present in vegetables, fruits, and some drinks. They also serve as the flower's pigments for luring pollination insects and are crucial to the plant's defense mechanisms. Flavonoids provide various beneficial health effects since they contain antioxidative, antidiabetic, anti-inflammatory, anticarcinogenic, antibacterial., and enzyme-inhibitory activities (Panche *et al.,* 2016). Being effective antioxidants and metal chelators, flavonoids also act as enzyme and lipid peroxidation inhibitors (Pourmorad *et al.,* 2006). Based on their chemical nature, extent of unsaturation at the C_3 ring, the oxidation state of oxygen, position, and number of -OH groups, they are categorized into various subgroups which include flavones, flavonols, flavanones, flavanol, flavan, isoflavones, neoflavonoids, and anthocyanidins. They can also be found as methylated derivatives, aglycones, and glycosides.

1.6.2.1 Flavones

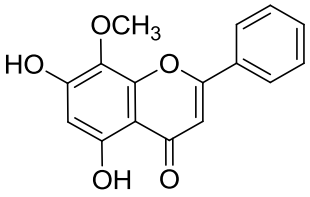
Flavones are organic compounds of the benzopyran class that are extensively found as secondary metabolites and is an important type of oxygen heterocycles (Verma *et al.*, 2010). They have the keto group in the C-4 position and an unsaturation between C-2 - C-3. Such compounds are found in flowers, fruits, leaves, stems, and rhizomes of different plants such as parsley, chamomile, mint, red pepper, etc (Manach *et al.*, 2004). In addition to defending plants from microbial illnesses, flavones have been shown to possess several beneficial biological qualities, such as antioxidant, antimicrobial., and anti-inflammatory capabilities (M. Singh *et al.*, 2014).



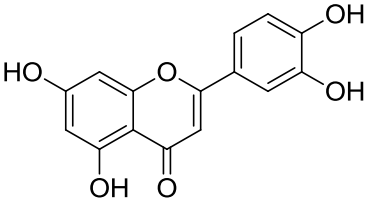
Diosmetin



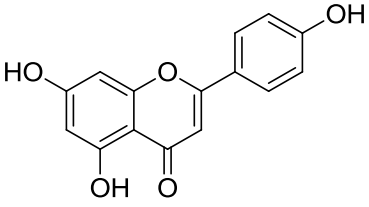
Acacetin



Wogonin



Luteolin



Apigenin

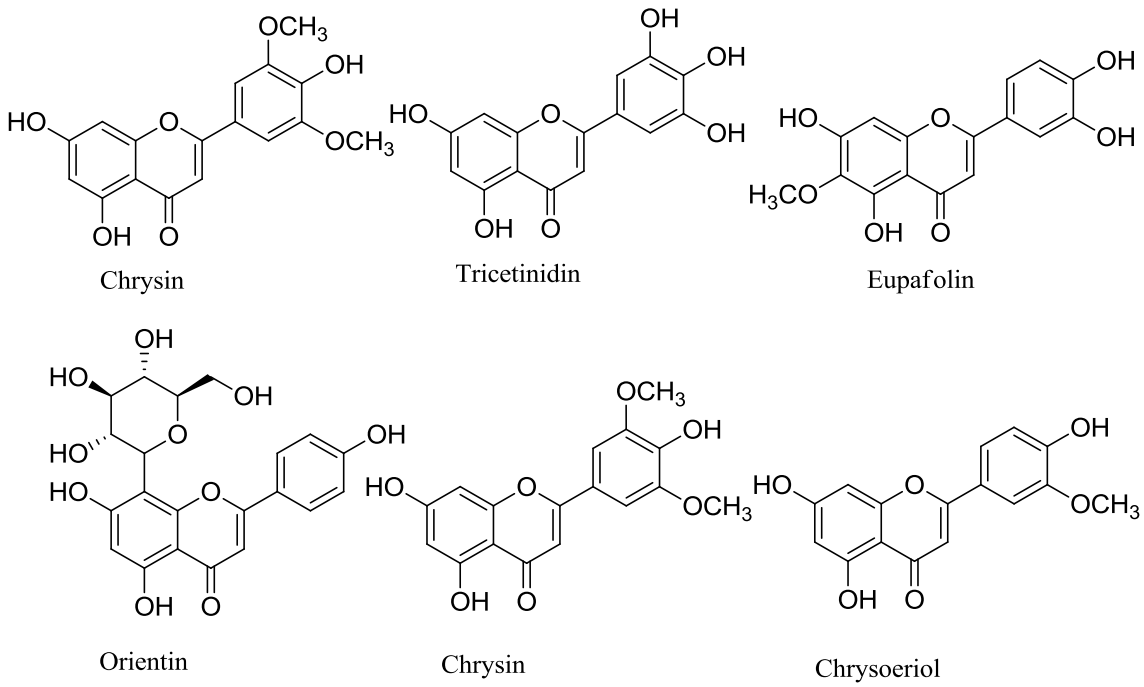


Figure 2: Structure of flavones

1.6.2.2 Flavonols

Flavonoids with the 3-hydroxy flavone backbone are known as flavonols. The primary food sources of flavonols, one of the flavonoid subclasses most prevalent in our diet, are fruit and vegetables including grapes, apples, lettuce, onions, spinach, asparagus, and various types of berries. They have the -OH group at position 3 and a ketonic group at the 4th position in the C ring. Major examples are kaempferol, quercetin, and myricetin. Flavonols have many health advantages including antioxidant, antimicrobial., antidiabetic, etc. (Ullah *et al.,* 2020). Dietary flavonols have been demonstrated to lower the threat of heart disease as well as cancer. (Choi *et al.,* 2008).

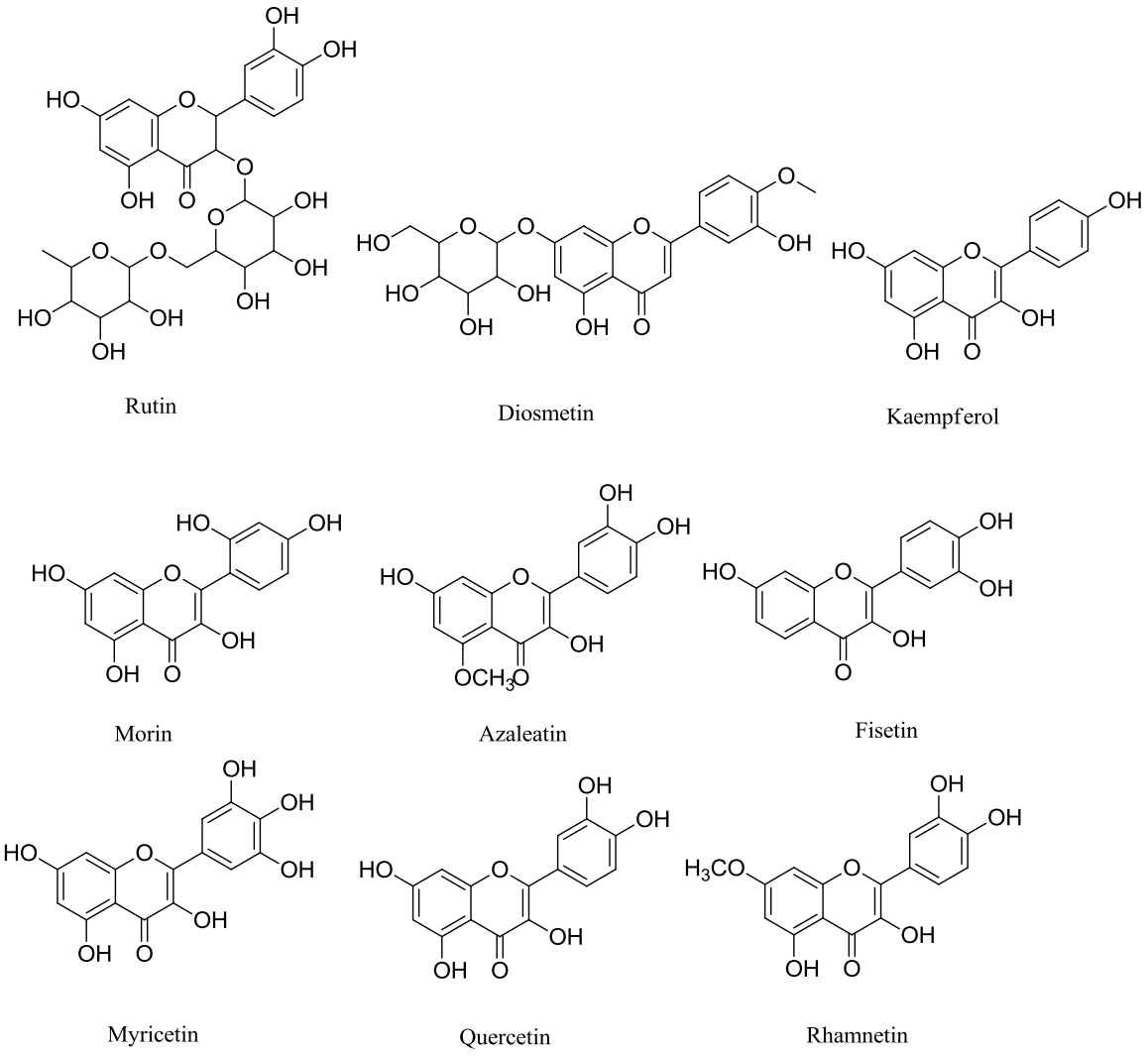


Figure 3: Structure of flavonols

1.6.2.3 Flavanones

One of the main groups of flavonoids is flavanones, which have 350 aglycones and 100 glycosylated forms. A flavan nucleus of flavanones is made up of two aromatic rings attached by a dihydropyrone ring with saturation at C2- C3 positions with a ketonic group at the 4th position Flavanones, in comparison to flavones, have saturated C2- C3 positions with a ketonic group at 4th position. They are frequently found in citrus fruits like oranges and lemons and have several pharmaceutical advantages together with anti-diabetic, anti-inflammatory, and anti-bacterial characteristics (Barreca *et al.,* 2011). These substances defend the body against oxidative stress and help to control cancer, atherosclerosis, and cardiovascular illnesses. Additionally, they exhibit anti-inflammatory, antiviral., and antibacterial effects among other things (Barreca *et al.,* 2011).

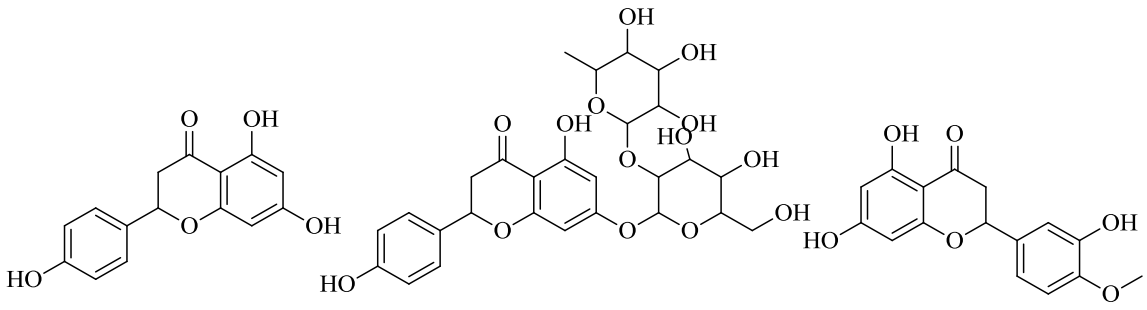


Figure 4: Structure of flavanones

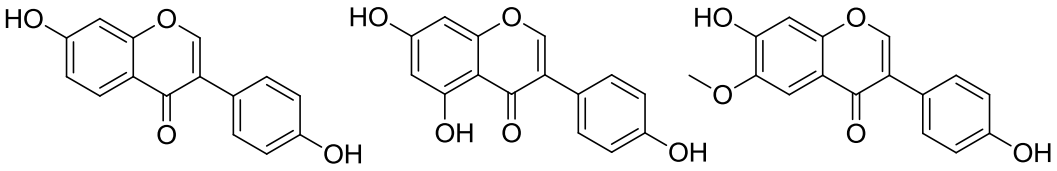
1.6.2.4 Flavanol

Flavanols, often known as flavan-3-ols exist in both oligomeric and monomeric procyanidin forms in various plant sources. Green tea, cocoa, red grapes, and apples are the best sources of flavonols (Hackman *et al.,* 2008). They are found in foods as monomers or oligomers with the fundamental monomer unit of (-)-epicatechin or (+)-catechin. They possess a big array of biological abilities, such as those that act against inflammation, cancer, obesity, allergy, and diabetes (Martin *et al.,* 2021).

1.6.2.5 Isoflavonoids

As secondary metabolites found in plants, isoflavonoids have a 3-phenylbenzopyran skeleton that is biosynthetically produced from the 2-phenylbenzopyran skeleton of flavonoids (Miadoková, 2009). These are the flavonoids having ring B attached at position 3 of the C ring and are very common in soybeans and leguminous plants. Isoflavonoids have a role in the host plant's defense system and offer them a competitive advantage to withstand and grow in unfavorable environmental circumstances while in the context of an animal study, isoflavonoids interact with a variety of signaling pathways and exhibit estrogenic, anti-oncologic, and antioxidant effects *in vivo* (Sajid *et al.,* 2021). The bioprotective properties of isoflavonoids have been described as having antioxidant, anticarcinogenic, antimutagenic, and antiproliferative activity. Breast, prostatic cancers, and uterine-linked hormones may be prevented by isoflavonoids (Birt *et al.,* 2001; Scarpato *et al.,* 2008). These compounds have the potential to act against various diseases. They also act as the precursor for the production of phytoalexins during the interactions between plants

and microbes. Rotenoids, isoflavones, isoflavones, isoflavones, and pterocarpans are examples of isoflavonoids (Foudah *et al.*, 2017).

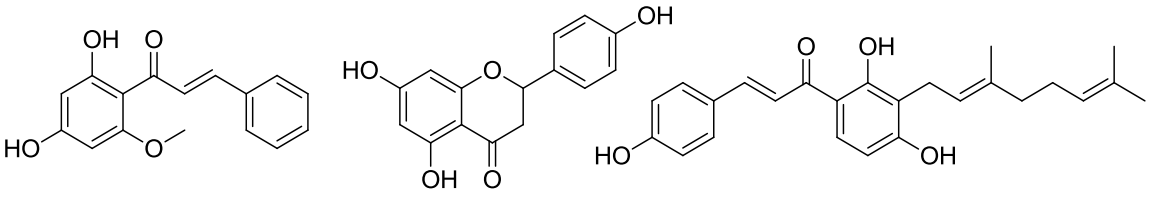


Daidzein Genistenin Glycitein

Figure 5: Structures of isoflavonoids

1.6.2.6. Chalcones

Since ancient times, the therapeutic uses of chalcones for the treatment of many ailments have been known (Ouyang *et al.*, 2021). In a vast range of vegetables, fruits, teas, and many plants, chalcone is a basic chemical scaffold made up of several compounds that are found in nature. (Karthikeyan *et al.*, 2015; Zhou *et al.*, 2015). Chalcones, which are essential., *α,β*-unsaturated ketones, show several pharmacological activities including antioxidant, antibacterial., anticancer, antiviral., antiplasmodial., antitubercular, antileishmanial., anti-inflammatory, immunosuppressant, and more (Rudrapal *et al.*, 2021). Moreover, chalcones are widely present in therapeutic and edible plants and are bio-precursors of flavonoids. They are secondary metabolites that are members of the flavonoid (C6-C3-C6 system) family. Pears, tomatoes, bearberries, strawberries, and some cereals are the main sources of chalcones. (Panche *et al.*, 2016). The following are some typical examples: phloretin, cardamon, arbutin, naringenin phloridzin, and chalconaringenin.



Cardamonin Naringenin Xanthoangelol

Figure 6: Structures of chalcones

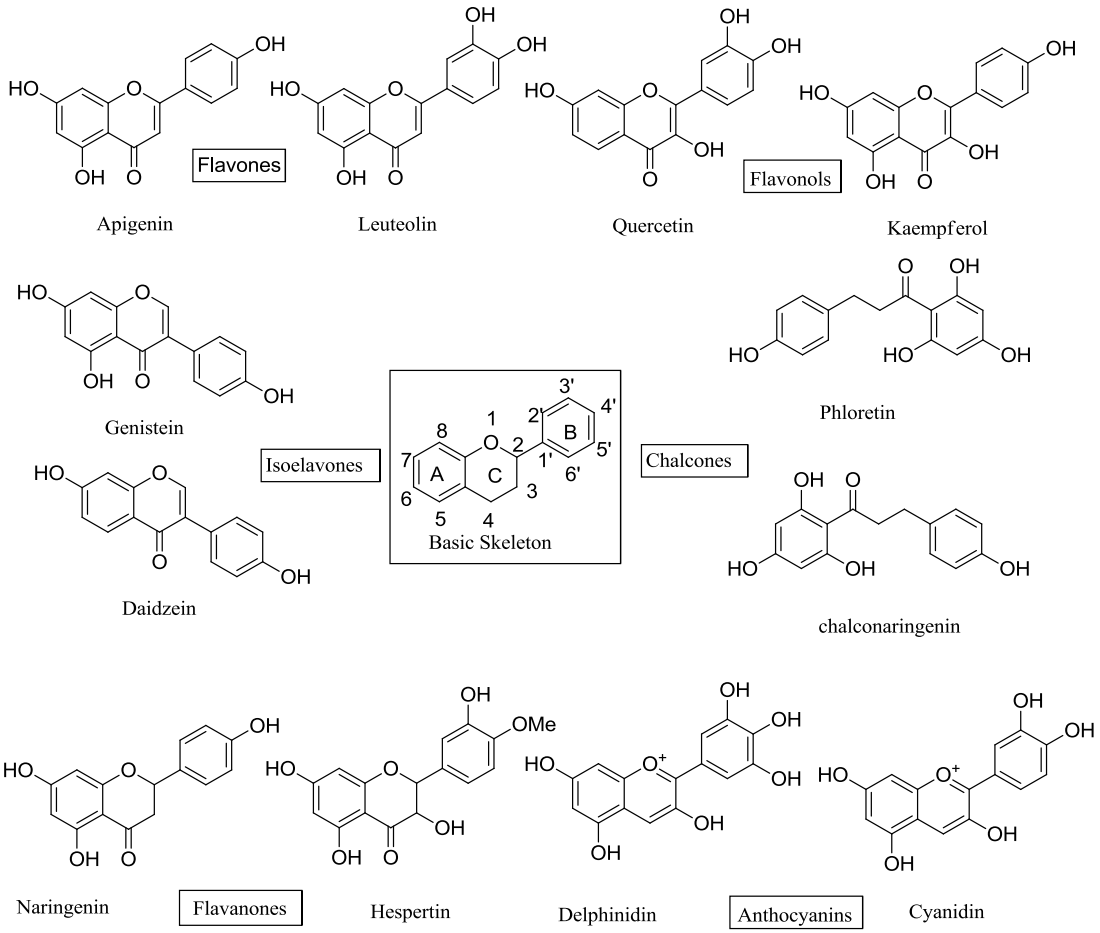


Figure 7: Types of flavonoids

1.6.3 Alkaloids

Alkaloids are the most fundamental secondary metabolites that plants, animals, and certain microorganisms contain (Ziegler *et al.*, 2008). Generally, alkaloids are basic, physiologically active compounds of mostly plant origin with at least one nitrogen atom in their cyclic system. However, some alkaloids eg. adrenaline and ephedrine contain the nitrogen atom at the side chain. Based on structure, alkaloids are classified as phenylethylamines, pyrrolidines, pyridines, tropanes, tropolones, pyrrolizidines, isoquinolines, indoles, quinolines, terpenoids, and steroids. Alkaloids are essential for maintaining human health as well as the body's defense system and are mostly utilized as anesthetics, cardioprotective, and anti-inflammatory components (Kurek, 2019). Therapeutically, the most common alkaloids include morphine, nicotine, ephedrine,

strychnine, and quinine (Heinrich *et al.,* 2021). Most alkaloids possess numerous pharmacological activities like antihyperglycemic, antioxidant, antimicrobial., anti-inflammatory, antimalarial., antiulcer, etc. (Greenwell *et al.,* 2015; Rasouli *et al.,* 2020).

1.6.4 Terpenoids

Terpenes or terpenoids (oxygenated terpenes) are a diverse group of phytochemicals that mostly exist in medicinal plants as essential (volatile) oils. They are comprised of isoprene units having the molecular formula $(C_5H_8)_n$ where n represents the isoprene unit's number. Depending on how many linked isoprene groups are present in their structures, they are classified into monoterpenes, sesquiterpenes, diterpenes, sesquiterpenes, and triterpenes. Some terpenes (eg. many sterols) are primary metabolites whereas others are secondary metabolites such as plant hormones like gibberellins. Because of their aroma, fragrance, and biological and physiological activity, most terpenes are of significant importance in the field of food, pharmaceuticals, cosmetics, vitamins, hormones, and so on (Rajput *et al.,* 2018). Terpenoids exhibit antitumor, antibacterial, anti-inflammatory, antidiabetic, antimalarial., and antiviral effects, prevent or treat cardiovascular diseases, and stimulate transdermal absorption (Yang *et al.,* 2020). They have a vital function in plants' development and growth, physiological processes, defense and immune system, and environmental response (Singh *et al.,* 2015).

1.6.4.1 Monoterpene

Monoterpenes have the chemical formula C_5H_{10} and are made up of two isoprene units. The major natural sources of monoterpenes are essential oils and other oils. Structurally, they may be acyclic, monocyclic, and bicyclic types. The monoterpenes; thymol, carvacrol, and eugenol have key applications in agriculture, fragrance, cosmetics, flavor, pharmaceutical., and other industries. They have significant anti-cancer, anti-inflammatory, antimicrobial., analgesic, and antioxidant properties (Rajput *et al.,* 2018). Some other examples of monoterpenes are *β*-Myrcene, Limonene, *α*-Piene, etc.

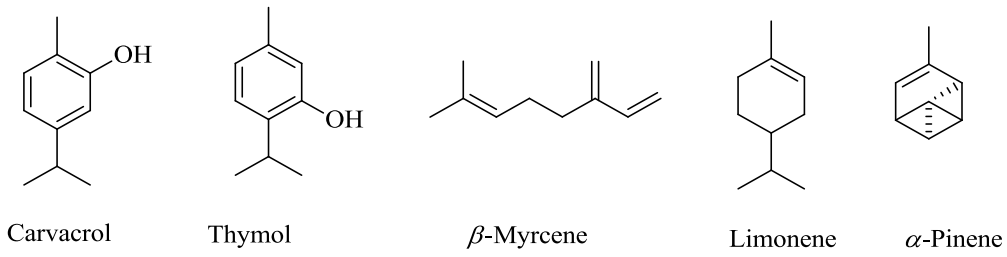


Figure 8: Structures of monoterpenes

1.6.4.2 Sesquiterpenes (C_{15})

Sesquiterpenes constitute three isoprene units having the molecular formula $C_{15}H_{24}$. Some examples of sesquiterpenes are parthenolide, farnesol, zingiberene, and cadinene. Biochemical conversions such as oxidation and rearrangements produce sesquiterpenoids. Lactone rings containing sesquiterpenoids are called sesquiterpene lactone most prominently found in different plants such as carrots, parsley, asters, lettuce, spinach, and daisies. A sesquiterpenoid cadinene is found in potatoes, caryophyllene is found in clove, and lactucin and lactucopicrin are in lettuce and spinach (Ludwiczuk *et al.*, 2017).

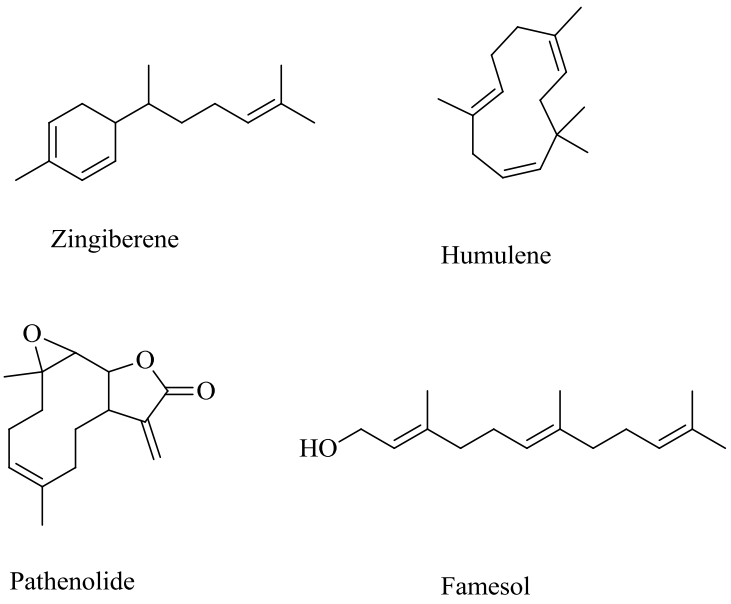
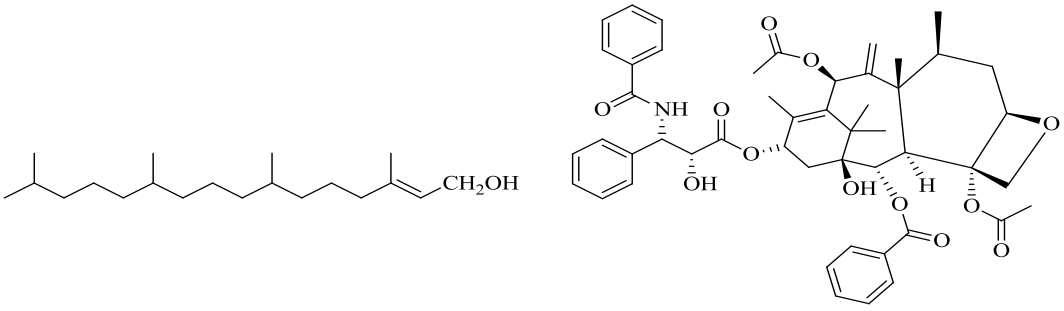


Figure 9: Structures of sesquiterpenes

1.6.4.3 Diterpenes

These are the terpenes with four isoprene units having the molecular formula ($C_{20}H_{32}$). These are chiefly used in the industry of food and cosmetics because they

have preservative and flavoring properties and are reported to have many neurobiological (Islam *et al.*, 2016), anti-inflammatory, antimicrobial, antispasmodic, and cardiovascular activities (Tirapelli *et al.,* 2008). Various diterpenes are reported such as taxol, triptolide, oridonin, and labdane to show potential anticancer activity (Islam, 2017). Some other examples of diterpenes are retinol, phytol, forskolin, and salvinorin. pineolidic acid, sugiol etc.



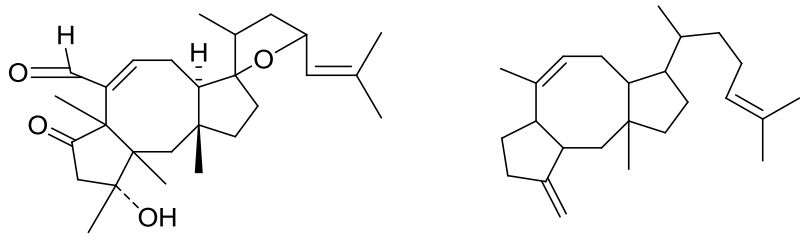
Phytol

Paclitaxel (Taxol)

Figure 10: Structures of diterpenes

1.6.4.4 Sesterterpene

Sesterterpenes are unique terpenes consisting of five isoprene units with the C_{25} skeleton of carbon. Sesterterpenoids are found in insects, higher plant sources, lichens, fungi, marine organisms, and fossil sediments. They originate from geranylfarnesyl pyrophosphate. Some examples of sesterterpenes are ophiobolin, ceroplastol, fasciospongin, cavernosolide, and cheilanthes.



Ophiobolin A

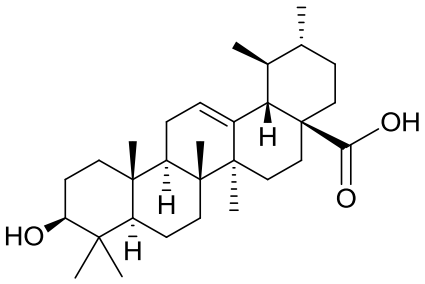
Ceroplastol

Figure 11: Structures of sesterterpenes

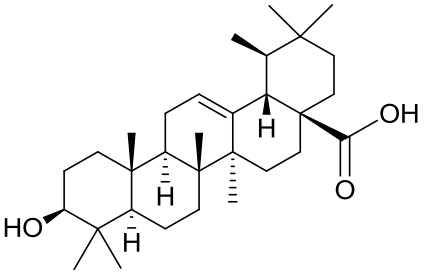
They exhibit significant pharmacological properties like anticancer, antimicrobial, antitubercular, antifungal, anti-inflammatory, and cytotoxic (Wang *et al.,* 2013).

1.6.4.5 Triterpenes

These are terpenes having the molecular formula $C_{30}H_{38}$, which are made up of six isoprene units. Squalane acts as the precursor in the formation of various triterpenoids. The sources of triterpenes are fruits, cereals, vegetables, animals, microorganisms, marine organisms, and fossil rocks. The two important triterpenoids ursolic acid and oleanolic acid and possess a wide range of pharmaceutical benefits including hepatoprotection, antiinflammation, antitumor-promotion, and anti-hyperlipidemia (J. Liu, 1995). Some examples are ursane, lupin, oleanane, hopane, malabaricane, chamaecydin, etc.



Ursolic acid

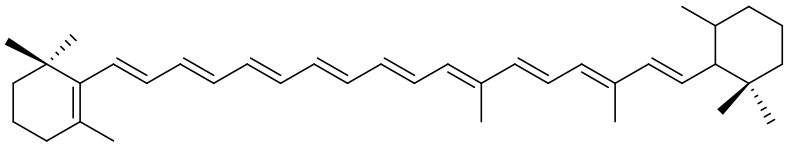


Oleanolic acid

Figure 12: Structures of triterpenes

1.6.4.6 Tetraterpenes

Tetraterpenes are terpenes composed of eight units of isoprene with the general molecular formula $C_{40}H_{64}$. The most important group of these compounds are carotenoids, dyes, and pigments. Tetraterpenes include vitamin A, ß-carotene, lycopene, astaxanthin, cryptoxanthin, etc.



β-carotene

Figure 13: Structure of ß-carotene (Tetraterpenes)

1.7 Secondary metabolites as a medicine or drug candidate

Before the 20th century, diseases in humans and domestic animals were treated with crude extracts of plants, animals, microorganisms, and minerals. But today, instead of using such crude or semi-impure extracts, chemically purified and pharmacokinetically proven (combination) drugs are used for the treatment of various diseases. Several modern or conventional systems of medicine depend majorly on plants because of the occurrence of several phytoconstituents of secondary plant metabolites having their corresponding pharmacological properties (Srivastav *et al.,* 2020). In some drug discovery processes, NPs are also used as starting compounds to prepare their more potent analogs (Newman *et al.,* 2007). The World Health Organization (WHO) reported that over 80% of people worldwide rely on plants as their major source of traditional medicine for basic healthcare needs. Additionally, it's believed that about 40% of pharmaceutical businesses rely only on herbal remedies (Luziatelli *et al.,* 2010). A wide number of modern synthetic drugs are either directly derived or based on natural products. A well-known analgesic drug Aspirin (acetylsalicylic acid) is derived from the natural product salicin, Morphine is an alkaloid derived from the plant *Papaver somniferum*, Zyconitide extracted from *Conus magus*, Atorvastatin (used to lower cholesterol) from the fungus *Penicillium cirtinum,* etc. Most medicines from secondary metabolites are safer and more effective whereas synthetic drugs fail, potentially boosting or synergizing the actions of other constituents in the medicine (Seca *et al.,* 2019). Literature suggests that many secondary metabolites including flavonoids, alkaloids, glycosides, terpenes, polyphenols, etc. extracted from different medicinal plants could be used as medicine for different ailments such as antiviral (Hussain *et al.,* 2017), antidiabetic (Rasouli *et al.,* 2020), antimalarial (Pan *et al.,* 2018), anticancer (Solowey *et al.,* 2014), antitumor (Greenwell *et al.,* 2015), cardiovascular disease (Miao *et al.,* 2020), and antihypertensive (Guerrero *et al.,* 2002).

1.8 Diabetes

T2DM is a serious endocrine disease indicated by chronic hyperglycemia due to a deficiency in the secretion of insulin, action, or both (O'Brien *et al.,* 1996). Scientists have paid the highest attention to the remedy of T2DM because the International Diabetes Federation (IDF) and WHO reported that hyperglycemia is the third-highest

risk factor for early mortality, after hypertension and lung cancer. It is also among the biggest risks of the 21st century (Zimmet *et al.*, 2001).

According to a report (2021) by IDF, 515 million adult population (20-70yrs) worldwide are affected by T2DM and it is predicted that this figure could rise to 643 million by 2030, and 783 million by 2045. It is the major cause of 6.6 million deaths in 2021 worldwide. In Nepal., the population of this disease is 1,333,500 with a prevalence in adults between 20-79 years. Chronic hyperglycemia raises the risk of foot ulcers and amputation as well as impairment of the kidneys, eyes, nerves, blood vessels, and heart (Sanders, 1994). The most typical indications of diabetes are frequent urination, thrust, hunger, muscular fatigue, unusual weight loss, unhealing of cuts and bruises, sexual dysfunctions, tingling, and numbness. There are three types of diabetes Mellitus (DM).

- Type 1. Diabetes resulting from inadequate insulin secretion by the pancreas
- Type 2. Diabetes results from insulin resistance or the cell's inability to insulin. response.
- Type 3. Diabetes is acquired in pregnant women without a history of diabetes ie, gestational diabetes (GDM).

The most general type of the disease is type 2 which covers 90–95 percent of all diabetes cases in western nations. It is a metabolic condition marked by a prolonged rise in blood sugar levels more than normal (hyperglycemia) brought on by a gradual insulin secretory failure against a background of insulin resistance (Ríos *et al.*, 2015). People with T2DM have both insulin resistance and insulin inefficiency. It develops with decreased peripheral tissue insulin sensitivity before changes in pancreatic cell mass and function (insulin resistance). Due to genetic mutations in insulin receptors, T2DM patients have insulin resistance and impaired insulin internalization which results in prolonged insulin existence in circulation causing hyperinsulinemia (Chen *et al.*, 2019). These people can survive without receiving insulin treatment at least initially, and in some circumstances, exercise, unusual weight loss, and/or the use of oral glucose-lowering medications can result in acceptable glycemic control (American Diabetes Association, 2010). Patients who have severe insulin-secreting cells destroyed, on the other hand, need insulin to survive.

Gestational diabetes (Type-3) is related to the metabolic syndrome acquired during pregnancy due to hormonal changes which are characterized by obesity, inflammation, impaired insulin sensitivity, and insulin resistance that lead to hyperglycemia and hyperinsulinemia. Impaired insulin sensitivity and insulin resistance which is also associated with the inflammation affecting the insulin signaling cascade which leads to gestational diabetes, when it is combined with ß-cell dysfunction (Catalano, 2014).

Maternal obesity and an energy-dense diet can cause an increase in insulin and IGF-1 serum levels, producing metabolic disorders, such as insulin resistance, GDM, and high birth weight (> 4,000 g) due to a higher level of body fat Women with GDM have a high risk of developing type 2 diabetes in the future. The pathogenesis of GDM is not completely understood, nevertheless, two factors could contribute to its development: β-cell dysfunction and failure in insulin secretion in response to insulin resistance induced by gestation. Both processes, together with the physiological activities of the insulin-like growth factors (IGFs), play a crucial role in glucose transport to the fetus and hence, fetal growth and development (Martin & Ramos, 2021).

1.9 Causes of diabetes

If the postprandial glucose level in blood serum increases, pancreatic cells are promoted to generate and release more insulin which binds with the receptors in target organs such as adipose tissue, muscle, or the liver before entering the bloodstream. When insulin binds to a target cell, a series of intracellular signal transduction pathways are activated. These pathways inhibit lipolysis in adipose tissue, inhibit glucose synthesis in the liver, and promote glucose absorption into target cells (muscle and fat). But if insulin resistance develops due to many factors, including genetics, aging, and obesity, which are typically present before the start of diabetes, this will lead to hyperglycemia later. Excess visceral adiposity, dyslipidemia, and hypertension are some other causes that frequently go hand in hand with insulin resistance. Other studies report decreased fibrinolysis and increased platelet aggregation, endothelial dysfunction, premature atherosclerosis, and vascular inflammation (Fonseca, 2003).

Due to the loss or dysfunction of insulin-forming pancreatic ß-cells, type-1 diabetes manifests as a complete lack of insulin. Type-2 diabetes, however, shows two defects: insulin inactivity and insulin insufficiency. In most patients with T2DM, the glucose level rises, and it sends a signal to ß-cells to secrete insulin into the bloodstream. Insulin binds with insulin receptors which give intracellular signaling to pass the glucose into the cell which is used by several target tissues like the adipose tissue, muscle, the liver, and throughout the body (Cheng, 2005). If a pancreatic insulin output decreases and various tissues (including muscle, liver, and adipose tissue) are resistant to the insulin action, and leads to weakness in the uptake of glucose. Although the precise cause of insulin resistance is uncertain, it is thought that a lower amount than required in post-insulin receptor intracellular signaling pathways may be a factor (Virkamäki *et al.,* 1999). Furthermore, eating habits, a sedentary lifestyle, and consequent obesity are also factors that result in T2DM.

1.10 Enzymes of diabetic target

Digestive enzymes are globular proteins that catalyze the hydrolysis of carbohydrates and convert them into smaller units to absorb through the gut wall and then mix into the bloodstream to transport into the different body parts. Various organs such as the salivary gland, pancreas, liver, small intestine, and stomach produce digestive enzymes. The most important metabolic enzymes for the digestion of dietary carbohydrates and lipids are *α*-glucosidase, *α*-amylase, and pancreatic lipase. During the digestion of starch, *α*-amylase breaks down the starch into oligosaccharides and *α*-glucosidase converts the α-amylolytic products into D-glucose which is absorbed by the sodium-glucose cotransporter (SGLT) found in enterocytes' luminal surface (Zhang *et al.,* 2013). Although enzymes are essential for digestion, the abnormally excessive activity of the enzyme can lead to hyperglycemia. Using the inhibitors against the diabetic target enzymes *α*-amylase, *α*-glucosidase, and pancreatic lipase can inhibit or delay glucose absorption, allowing for the maintenance of a healthy blood sugar level (Imam, 2015). One of the most effective therapeutic approaches for the management or control of diabetes is to lessen postprandial hyperglycemia. The use of inhibitors that reduce glucose absorption by inhibiting the key enzymes of diabetic targets related to carbohydrate and lipid metabolism ultimately retard the increment in the post-prandial plasma glucose (Tundis *et al.,* 2010).

1.10.1 α-Amylase

The *α*-amylase is found in pancreatic secretion and saliva (Kazeem *et al.*, 2013) which hydrolyses 1-4-*α*-bonds of polysaccharides yielding glucose and maltose which easily enter the bloodstream (Alagesan *et al.*, 2012). The molecular mass of porcine pancreatic α-amylase is about 51 to 54 kDa (Cozzone *et al.*, 1970). This enzyme is a polypeptide chain comprising, 475 residues with four disulfide bridges, two-SH groups, and firmly bound calcium ions that are essential for stability (Granger *et al.*, 1975). For greatest action, it needs a medium with a pH of 7.0 or a slightly alkaline pH (7.0 to 7.4) at 37 ℃ (Henry *et al.*, 1960). *α*-Amylase inhibition slows down digestion by delaying or impeding the breakdown of starch in the gut and is, therefore, a useful treatment approach for the control of diabetes

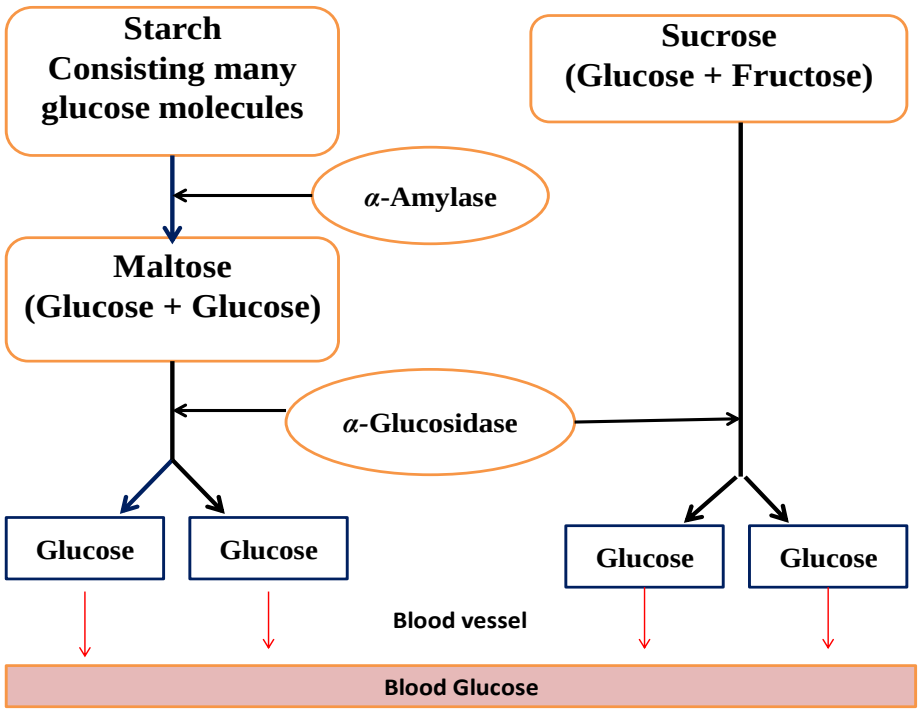


Figure 14: Hydrolysis of starch catalyzed by *α*-amylase and *α*-glucosidase (Yang *et al.*, 2019)

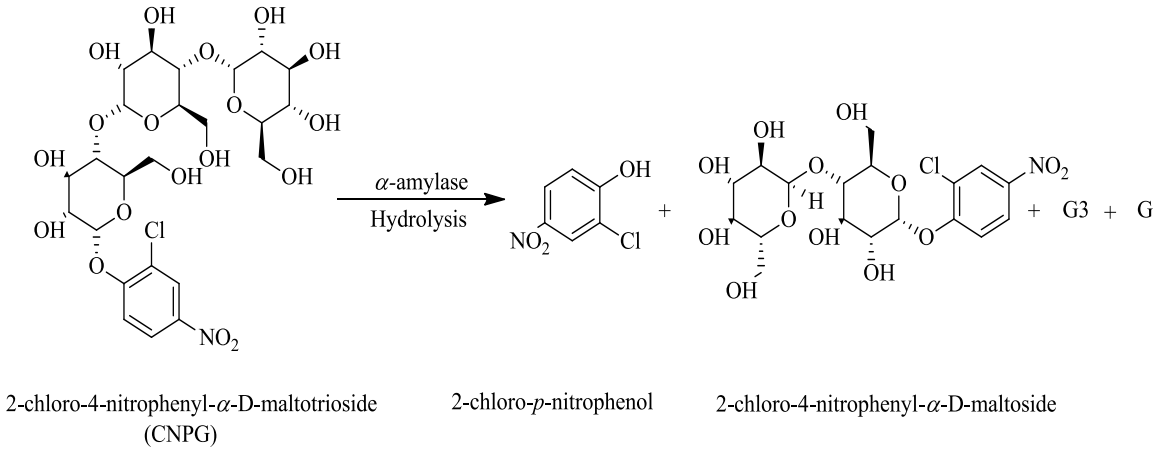


Figure 15: Hydrolysis of 2-chloro-4-nitrophenol-α-D-maltotrioside by α-amylase

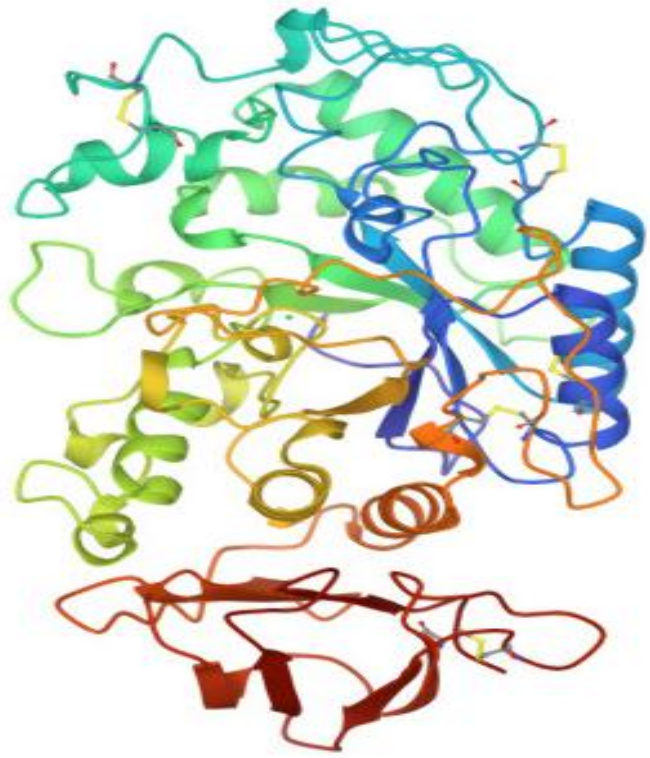


Figure 16: 3D-Structure of α-amylase (PDB ID: 1HNY) [Source: RSCB PDB]

1.10.2 α-Glucosidase

The enzyme α-glucosidase, which is membrane-bound, is present in the epithelial wall of the small intestine and hydrolyses the oligosaccharides or disaccharides by cleaving terminal 1-6 α-glycosidic bonds into monosaccharide (glucose) for intestinal

absorption. The inhibitors of α-glucosidase slow down the process of hydrolysis of oligosaccharides and push the digestion process toward the lower parts. This slows down the complete rate of absorption of glucose into the onset of late diabetic complications (Standl *et al.*, 2012).

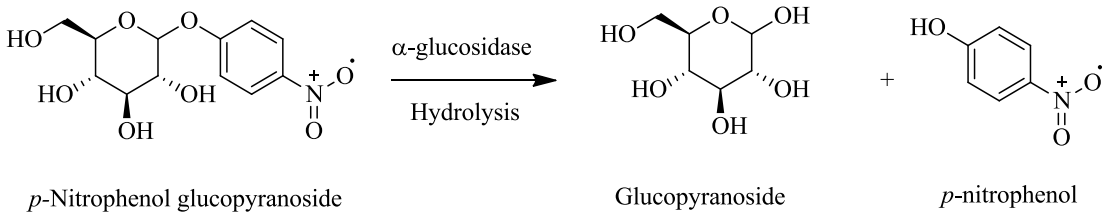


Figure 17: Hydrolysis of *p*-Nitrophenol glucopyranoside by α-glucosidase

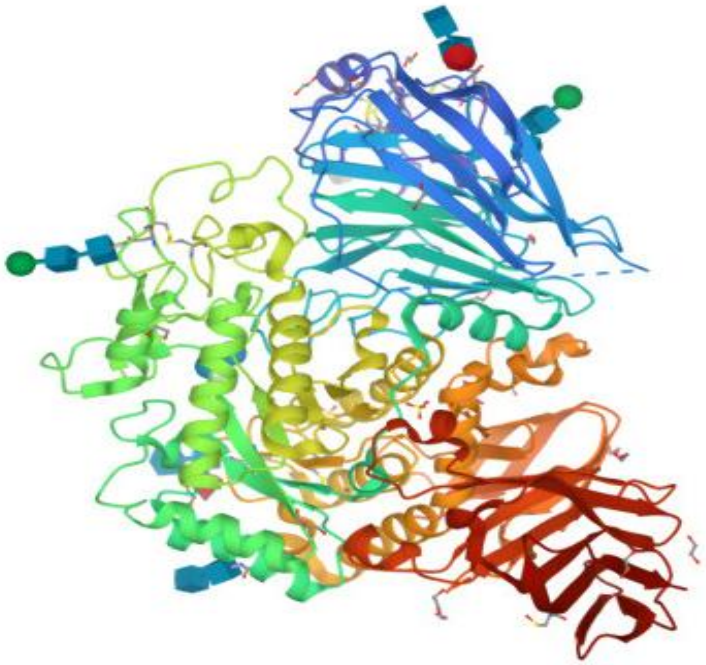


Figure 18: 3D Structure of α-glucosidase (PDB ID: 5NN8) [Source: RSCB PDB]

1.10.3 Pancreatic lipase

Intestinal lipase is an enzyme that hydrolyzes dietary fat in the intestinal epithelium to produce glycerol and free fatty acids. Due to modern lifestyles and an increase in the ingestion of fat-rich foods and carbohydrates, the occurrence of diabetes and obesity has rapidly increased (Hu, 2011)) and there will be around one billion obese people worldwide by 2030 (Lobstein *et al.*, 2022). Several metabolic risk factors cause abdominal obesity that also contributes to T2DM, cardiovascular, and fatty liver

disease (Brahe *et al.*, 2016). Pancreatic lipase inhibition is an important strategy for curing metabolic syndrome because it accounts for 50 to 70% of the total dietary fat metabolism (Liu *et al.,* 2020). Hepatic lipases, hormone-sensitive lipases, lipoprotein lipases, and pancreatic lipases are some of the various types of lipases. Dietary triglycerides are broken down by pancreatic lipase, which is present in the small intestine (Pirahanchi *et al.,* 2022). The excessive accumulation of lipids in the pancreas is one of the primary causes of T2DM, which leads to the malfunctioning of pancreatic *ß*-cells that produce insulin (Galicia-Garcia *et al.,* 2020). Up to 90% of diabetic individuals worldwide have type 2 diabetes because of being overweight or obese. Reducing dietary fat or inhibiting carbohydrate digestion and absorption is an essential strategy for lowering obesity and weight gain (Sellami *et al.,* 2017). As a result of their mode of action, safety, and metabolic activity, NPs are preferred over synthetic drugs for the control of obesity. Therefore, the untapped potential of medicinal plants could bring about the development of alternative lipase inhibitors with fewer adverse effects

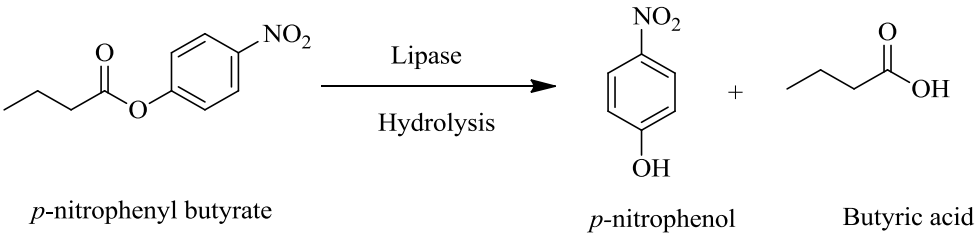


Figure 19: Hydrolysis of *p*-Nitrophenol butyrate by pancreatic lipase

1.11 Enzyme inhibition

Compounds that inactivate the enzymes and slow down the rate of enzymatically catalyzed reactions are called enzyme inhibitors. They are typically low molecular weight compounds that interact with the target enzyme to produce an inhibitor-enzyme complex, hindering the enzymatic activity and slowing down the rate of reaction (Roberts *et al.,* 2013). Most of the inhibitors interact with particular enzyme(s) of the target by blocking their activity towards their corresponding natural substrates without interfering with other beneficial enzymes (Ata, 2012). Both reversible and irreversible inhibition is possible. Reversible inhibition occurs when an inhibitor binds to an enzyme non-covalently and the enzyme's activity is restored even if the inhibitor is removed, it is called reversible inhibition. If the inhibitor strongly

binds with an enzyme covalently causing the change in conformation in the active site and destroying the enzyme's catalytic activity, it is called irreversible inhibition (Roskoski, 2007).

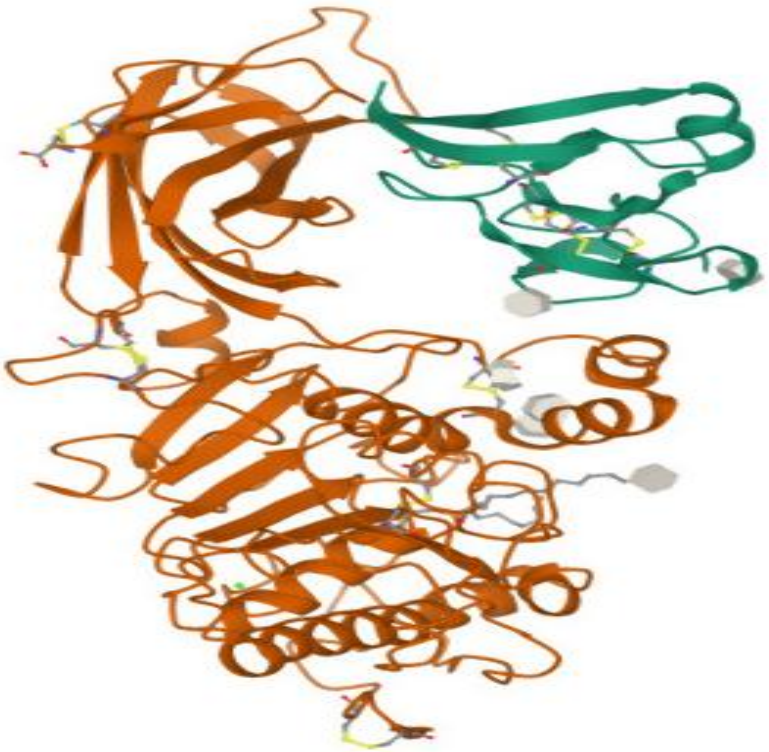


Figure 20: 3D Structure of human pancreatic lipase (PDB ID: 1LPB) [Source: RSCB PDB]

Based on the mode of binding, the inhibitor with the substrate, inhibitions are of three types.

- Competitive inhibition: An inhibitor that mimics the substrate binds reversibly to the same place on the enzyme as the substrate binds. When the enzyme binds to a substrate, an inhibitor competes with it. The concentration of an inhibitor and the substrate determines the degree of inhibition.
- Non-competitive inhibition: The inhibitor binds to a region of the enzyme other than its active site (allosteric site) making its conformational change with no competition with the substrate for binding. The substrate and the inhibitor don't have any structural similarities. Increasing the substrate concentration does not alter the rate of inhibition.

- Uncompetitive inhibition: The free enzyme and the inhibitor do not bind together. An inhibitor only binds to the enzyme-substrate complex (ES) after the substrate has already bonded to the enzyme. It causes structural distortion of the enzyme's active site causing catalytically inactive. Increasing substrate concentration favors inhibition.

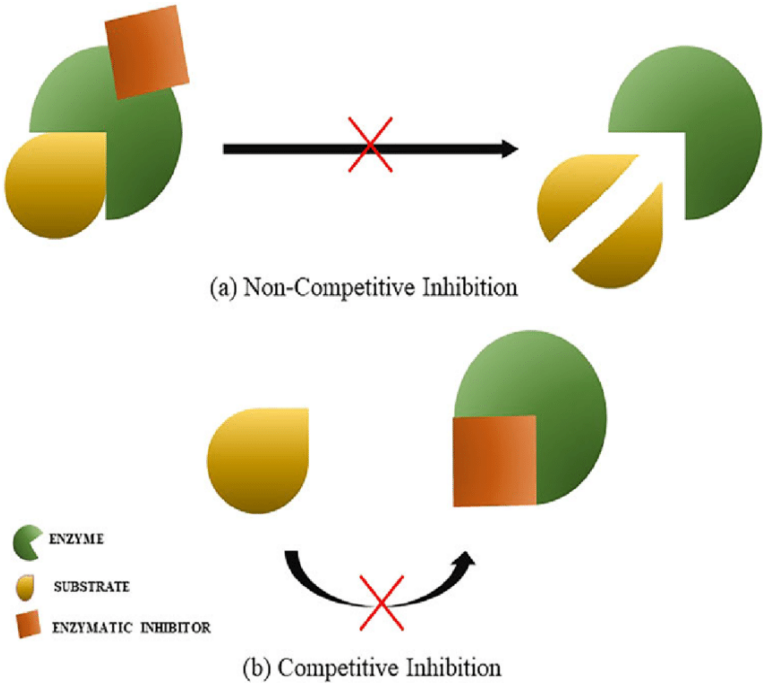


Figure 21: Mechanism of inhibition (Cristina Oliveira de Lima *et al.*, 2019)

1.12 Role of medicinal plants in the treatment of diabetes

Today, the majority of diabetic treatments make use of medicinal plants since they contain antioxidants and anti-diabetic phytochemicals such as flavonoids, phenolic, tannins, alkaloids, etc. which can raise the production of insulin by decreasing the glucose absorption in the gut, which results in the generation of an anti-diabetic effect (Kooti *et al.,* 2016). Inhibitors obtained from plants inhibit or retard the digestion of carbohydrates and fat via competative inhibition mechanism by blocking the some of active sites of the taget enzymes (Nair *et al.,* 2013). According to reports, 70–80 percent of the global utilizes traditional herbal treatment for their basic medical requirements (Kunwar *et al.,* 2009). Approximately, 800 plants are utilized as traditional treatments as per ethnobotanical data. Several traditionally used medicinal herbs are still employed to treat T2DM due to their numerous beneficial functions,

including improving glucose uptake and utilization, maintaining-cell integrity and function, insulin-secreting activity, and correcting altered carbohydrate metabolism (Tiwari *et al.,* 2002). Herbs contain a number of bioactive hypoglycemic compounds and antioxidants that stimualte insulin secretion, inhibit glucose absorption in the intestine and promote glucose uptake in muscles and adipose tissues. Phtoconstituents in herbs activate pancreatic *ß*-cells to release insulin, reduecs the reactive oxygen and nitrogen species, inhibit the apoptosis of *ß*-cells and increase insulin sensitivity with glucose transporters (GLUT-4). GLUT-4 receptors are insulin sensitive and bind with the insulin and initiate signalling cascade by activating phosphatidylinositol kinase for the intake of glucose into the cell (Nair *et al.,* 2013). According to reports, about 25% of medications rely on plant sources. Among the drugs developed in the previous 25 years, 5% are prepared from natural products, and 27% from their derivatives (Shrestha *et al.,* 2018).

NPs are plant-based chemical compounds of pharmacological activity that are produced by primary and secondary metabolism pathways (Newman *et al.,* 2007). Over 900 medicinal plants are used in diabetes by different therapeutic systems such as Ayurveda, Sidha Amchi, Homeopathy, Unani, Chinese medicine, Folklore, etc. worldwide (Sheng-Ji, 2001). In Nepal, various ethnic communities employ more than 700 medicinal plants in diverse treatment systems to treat or prevent various ailments, some of which are indigenous to Nepal (Aryal *et al.,* 2021). The documentation of traditional knowledge and the study of it using more scientific techniques may result in the development of new drugs.

1.13 Antioxidants properties of natural products

One of the most health-boosting beneficial effects of natural products is due to their significant antioxidant potential**.** An antioxidant is a substance that prevents oxidation by removing oxygen from other molecules, scavenging or suppressing reactive oxygen species (ROS), reactive nitrogen species (RNS), and binding metallic ions necessary for the catalysis of ROS and RNS generation (Halliwell, 1996)**.** RNS and ROS such as superoxide ($O_2^{\bullet}$), peroxyl ($ROO^{\bullet}$), hydroperoxyl ($HO_2^{\bullet}$), hydroxyl ($OH^{\bullet}$), nitric oxide ($NO^{\bullet}$), alkoxyl ($RO^{\bullet}$), and lipid peroxyl ($LOO^{\bullet}$); and non-radicals such as hydrogen ozone (O_3), nitrous acid (HNO_2), singlet oxygen ($O^{\bullet}$), dinitrogen trioxide (N_2O_3), peroxynitrate ($ONOO^-$), lipid peroxide (LOOH), and non-radicals also act as

oxidizing agents and initiate reactions of radicals in living organisms easily (Sen *et al.*, 2010). The lack of balance between the free radicals generation and the utilization of endogenous and exogenous antioxidants characterizes the complicated process known as oxidative stress in a biological system (Shalaby *et al.*, 2019). The majority of research has demonstrated a link between oxidative stress and diabetes, as well as heart, liver, kidney, and eye diseases. Many investigations have shown that oxidative stress plays a significant role in the development of diabetes, including the impairment of insulin action and the elevation of the risk of complications. Elevated levels of ROS and RNS have been associated with lipid peroxidation, non-enzymatic protein glycation, and glucose oxidation, all of which are factors in diabetes mellitus and its consequences (Asmat *et al.*, 2016). Oxidation reaction can generate free radicals which may trigger the chain reaction for the destruction of the cells. In addition to the body's normal metabolic processes (endogenous sources), exogenous causes such as stress, harmful radiation, as well as pollution, can contribute to the generation of reactive free radicals. By eliminating the reactive radical intermediates, antioxidants stop these chain reactions, and by being oxidized themselves, they also stop further oxidation processes. Exogenous antioxidants that are present in frequently consumed cereals, vegetables, drinks, and fruits may enhance the natural antioxidant defense system of the body (Lourenco *et al.*, 2019). Plants contain a diverse group of compounds including secondary metabolites like polyphenols with a minimum of one aromatic ring substituted with methoxyl, hydroxyl, and/or glycosyl groups (de Mello Andrade *et al.*, 2014).

1.14 Antibacterial activities of natural products

Individuals with DM are more susceptible to infections due to the increased virulence of some pathogens, a decreased production of interleukin in response to infection, a reduction in chemotaxis and phagocytic activity, immobilization of polymorphonuclear leukocytes, glycosuria, and gastrointestinal and urinary dysmotility (Casqueiro *et al.*, 2012). Phytochemicals have exerted potential antibacterial activities against pathogens via inhibiting bacterial cell-wall biosynthesis, cell membrane destruction, retardation of nucleic acid replication and repair, and inhibition of a metabolic pathway (Khameneh *et al.*, 2019). Additionally,

bacterial infection causes the body to alter metabolic pathways and the development of oxidative stress which results in diabetic complications.

Since many synthetic antibiotics have developed significant resistance in microorganisms, the use of biologically active compounds from isolated plant species is quite popular these days (Septama *et al.,* 2020). Plant extracts have been utilized for generations in addition to conventional therapy for conditions like diabetes, microbial infections, stress-related disorders, antioxidant sources, and more (Egharevba *et al.,* 2019). Many plants employed in traditional medicine, such as *Anethum graveolens*, *Foeniculum vulgare*, *Viola odorata*, *Dioscorea dracaena*, *Vernonia colorata*, and others, have been tested for their ability to treat infectious diseases (Omari *et al.,* 2022). Several multidrug-resistant microorganisms such as *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus cereus*, *Enterobacter aerogenes*, *Shigella dysenteriae*, and *Salmonella typhimurium* are susceptible to the secondary metabolites such as anonaine, nornuciferine, and liriodenine found in the extracts of *Annonaceae spp*. (Harahap *et al.,* 2022). The bioactive molecules including flavonoids, terpenoids, and phenolic acids have been identified to have potent anti-bacterial effects in a variety of medicinal plants (Kumar *et al.,* 2022). These molecules have various mechanisms of action that can affect structural., cellular, and molecular levels, making them strong contenders for the creation of natural antibiotics (Monserrat-Martinez *et al.,* 2019; Pancu *et al.,* 2021).

Biologically active secondary metabolites in plants including tannins, terpenoids, alkaloids, flavonoids, and phenolic compounds, may have a distinct physiological impact on human health. The synergistic interaction of the active compounds in medicinal plant extracts is also a factor in how well they work to prevent bacterial growth. Synergism leads to the creation of multi-target mechanisms, the presence of substances that can impair bacterial defense systems, physicochemical or pharmacokinetic actions that improve solubility, bioavailability, and resorption rate, neutralization of adverse effects, and decreased toxicity (Wagner *et al.,* 2009).

The broad antimicrobial activity range of bioactive metabolites is influenced by the morphology, position of various substituent groups, the existence of various linkages, -OH groups, alkylation, and the topography of their location (Arima *et al.,* 2002; Assob *et al.,* 2011). The horse radish (*Armoracia rusticana*) root-derived

isothiocyanates were tested for their antibacterial ability against oral pathogens, and the findings revealed that these substances had the best antimicrobial effects (Park *et al.,* 2013). Vegetables such as *Brassica rapa*, and *Brassica campestris* contain phenethyl isothiocyanate which has antimicrobial properties against Gram-positive bacteria (Aires *et al.,* 2009; Jang *et al.,* 2010).

1.15 Molecular annotation

Molecular annotation refers to the identification of chemical compounds produced by living systems. Among different hyphenated analytical tools, liquid chromatography-mass chromatography (LC-MS) is popularly being used to annotate various chemical compounds present in the sample. To analyze further and validate the bioactivity of the plant, the identification of the secondary metabolites is another important aspect. This method enables to increase the value of bioactivities of plants through analyses of a variety of metabolites (Dias *et al.,* 2016). Using chromatographic techniques like preparative high-performance liquid chromatography (HPLC), active metabolites are isolated and purified from crude extracts through bioassay-guided fractionation (Wang *et al.,* 2018). The detailed characterization and, eventually, structural elucidation of these agents have been made possible by new technologies, including mass spectrometry (MS), nuclear magnetic resonance spectroscopy (NMR), infrared spectroscopy (IR), and ultraviolet-visible spectroscopy (UV-Vis) (Thomford *et al.,* 2018).

LC-MS, a complementary analytical technique, is employed to evaluate a variety of plant metabolites (Kruve *et al.,* 2011; Santilio *et al.,* 2019). The advantages of LC-MS in the metabolite analysis include a fine resolution of the complex sample that enables an accurate assessment of the molecular diversity content (Huan *et al.,* 2015). High-resolution masses are used as queries in metabolomics databases like Metlin for compound annotation (Domingo-Almenara *et al.,* 2018), and analyzed using Mestre Nova 12.0 (Zanatta *et al.,* 2021). Additionally, it allows comparison with standards, boosts authentication in structural annotations, distinguishes isomeric and isobaric metabolites that cannot be resolved by fragmentation pattern, and provides accurate molecular mass in addition to providing more specific details about the metabolic makeup of the test samples through various chromatographic characteristics like peak shape and retention time of the analytes (Brodsky *et al.,* 2010; Wernisch *et al.,* 2016).

Numerous LC-HRMS/MS applications have been described in the literature, and many discoveries made as a result of these applications have expanded the scope of analytical research and the study of metabolites (Lima *et al.*, 2022). This method has been extensively used to annotate flavonoids in the roots and shoots of *Selaginella convolute* (Reginaldo *et al.*, 2021), seeds of *Erythrina velutina* (Chacon *et al.*, 2022), and many other metabolites in different plants extracts.

Recent advances in analytical techniques, combined with advanced technology for data processing, enable the simultaneous determination of hundreds of chemically distinct metabolites, as well as a more in-depth investigation of metabolic network regulation to analyze its impact on complex traits (Carvalho *et al.*, 2019; Lima *et al.*, 2022; Murphy *et al.*, 2022).

1.16 Molecular docking

Natural products have a long history and tradition of usage especially in underdeveloped nations, in the treatment of numerous ailments. It is generally accepted that chemicals of natural origin can be used as lead compounds to create conventional drugs. Utilizing *in vitro* or *in vivo* assays to isolate and characterize pure secondary metabolites from plant extracts for drug development has some drawbacks that are generally difficult, time-consuming, and expensive. In traditional drug development processes, molecular docking is a helpful technique for anticipating interactions between tiny molecules and therapeutic targets. During the drug discovery process, medical scientists use this method to predict and screen a large number of phytochemicals from different databases that are likely to exhibit pharmacological activity, saving time, effort, and money. Additionally, it may be used to identify new applications for medicinal plants as well as to explain and justify some traditional uses (Asiamah *et al.*, 2023).

The recent methodologies for drug development involve strenuous target identification, validation, the discovery of hypothesized lead compounds, and their optimization, followed by demanding pre-clinical and clinical trials (Tang *et al.*, 2006).

Computer-aided drug discovery (CADD) techniques have recently gained popularity because of their time-efficient aspects encountered by traditional scientific disciplines.

CADD encompasses the identification of bioactive drug targets computationally, the virtual screening of huge chemical libraries, and *in silico* evaluation of their potential toxicity candidates (Vamathevan *et al.,* 2019). CADD employs two distinct approaches: structure-based or ligand-based drug discovery (Aparoy *et al.*,2012). Molecular docking is frequently employed in structure-related drug discovery and has transformed clinical research in a variety of fields (Meng *et al.*, 2011).

When the term "docking" was first used in the late 1970s, it referred to improving a model of a complex structure by maximizing the distance between partners while maintaining constant relative orientations (De Ruyck *et al.,* 2016). Docking is the crucial method for studying the interaction between two molecules such as medications and enzymes (Ferreira *et al.,* 2015; Kirkpatrick, 2004). It is a technique of molecular modeling that forecasts the perfect orientation between two molecules during the formation of a stable complex. By using scoring functions, we may determine how strongly two binding molecules will bind (binding affinity) to one another (Lengauer *et al.,* 1996). The docking process predicts and obtains the binding energy and mode of interaction with the ligand and target by simulating conformation according to complementarity and pre-organization (Dong *et al.,* 2018). In general, molecular docking uses the following two molecular models:

- Lock and key model: This describes the rigid docking process in which ligands seek the proper orientation to fit in the complementary structure of the target protein.
- Induced fit model: It uses the flexible docking method, in which the protein and ligand change shape to fit one another.

When discussing molecular docking algorithms, the three broad categories can be used to classify the scalability of ligand and receptor docking techniques (Dias *et al.,* 2008). They are:

- Rigid docking: This method assumes both ligand and protein as rigid entities and only three transitional and rotational degree of freedom was taken into consideration during sampling. Rigid docking is applicable for studying macromolecular complexes, like protein-protein, and protein-nucleic acid composites (Kuntz *et al.,* 1982).

- Flexible docking: It is predicated on the assumption that during binding, a protein is not a passive, rigid entity, rather, it interacts with the ligand as flexible counterparts (Pagadala *et al.*, 2017).
- Semi-flexible docking: In this approach, only the ligand is considered a flexible molecule, while the protein is rigid. Semi-flexible docking is best for analyzing the patterns of interactions between proteins and small molecules (Andrusier *et al.*, 2008).

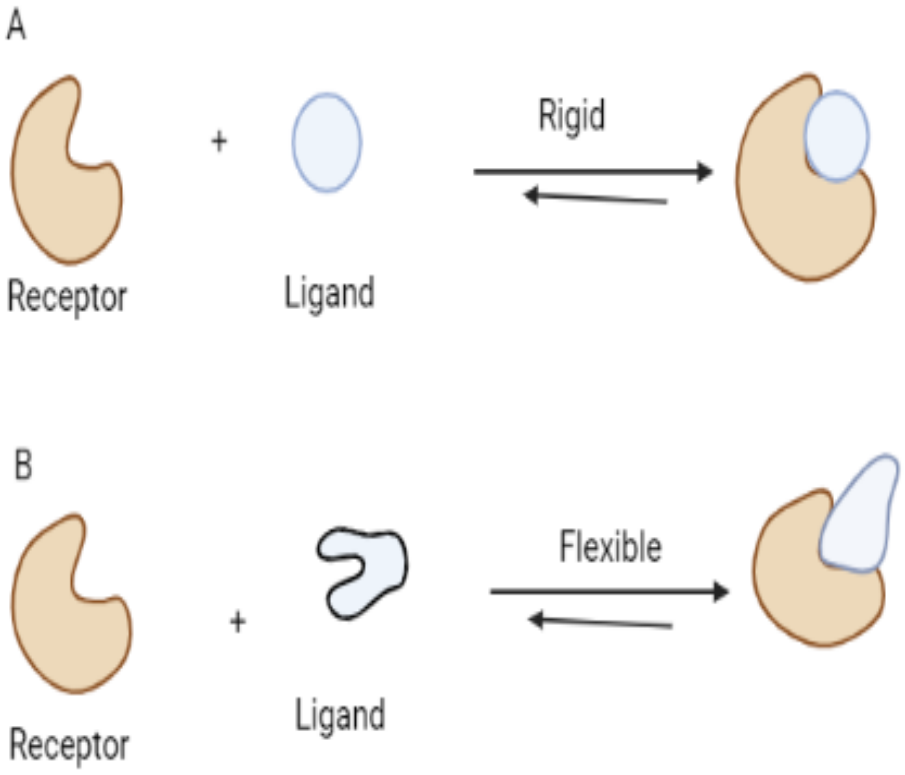


Figure 22: Different modes of molecular docking process: **A**. Rigid docking, **B.** Flexible docking

Three broad categories can be used to categorize the scalability of ligand and receptor Based on the types of ligands, docking can be divided into ligand-protein docking, protein-protein docking, and nucleic acid-protein docking (Kirkpatrick, 2004). So depending on the docking process, algorithms, and scoring function, many software like Flex X, Gold, Glide, AutoDock, Autodock vina, Gdock, and Rdock are available **(Table 3)** which predict the interaction between the target and ligand molecules (Andrusier *et al.*, 2008).

Table 3: Some examples of the molecular docking process (Chaudhary *et al.,* 2016; Fan *et al*., 2019)

Program	**Algorithm**	**Features of docking**
Flex X	Fragmentation algorithms	Flexible-rigid
GOLD	Genetic algorithm	Flexible
AutoDock	Genetic algorithm	Flexible -rigid
Glide	Exhaustive systematic search	Flexible
Dock	Fragmentation algorithm	Flexible
Autodock vina	Genetic algorithm	Flexible -rigid
Ligand fit	Monte Carlo	Flexible

Molecular recognition on both a structural and energy tier is understood and predicted by automated molecular docking software, which also predicts binding affinity. To score the numerous possible binding modes, all docking algorithms require a scoring system and a search mechanism to explore the state variables. Search methods are divided into two categories: systematic and stochastic. Scoring functions are empirical., forcefield-based, knowledge-based, and classical force-field-based. Scoring functions evaluate binding affinity by adding together non-bonded electrostatic and van der Wall interactions (Kollman, 1993). Hydrophobic effect, ionic interaction, hydrogen bond, and binding entropy all contribute to the binding energy in empirical scoring methods like ChemScore (Head *et al.,* 1996). Knowledge-based scoring systems analyze the protein-ligand complexes to determine the interatomic distance between the ligand and protein (Feher *et al.,* 2003).

The popular molecular docking software is AutoDock suite, which comprises AutoDock (Morris *et al.,* 2008) and AutoDock Vina (Trott *et al.,* 2009). AutoDock

Vina is frequently suggested as a first-line bioinformatics tool for molecular docking implementation due to its docking rate and accuracy (Goodsell *et al.,* 2021). Rapid and precise ligand-bound conformation and binding energy predictions for macromolecular targets are possible with autodock tools. To quickly find binding space that is accessible to ligands around the protein, auto dock tools employ the grid base method (Morris *et al.,* 1998). The assessment of the ligand's binding free energy to the target molecule is performed using a semi-empirical method (Morris *et al.,* 2009). In addition to databases on target genes and proteins, structural chemical databases with several chemotypes will help in the development of novel chemical entities for computational molecular modeling-based pharmacological evaluation (Nisbet *et al.,* 1997).

1.17 Procyanidin: Bioactive compound

Procyanidin is a proanthocyanidin made up of two B-ring hydroxyl groups and epi-catechin. Based on the extent of polymerization and the stereochemistry between the monomer, it is classified as either an A-type or B-type (Gu *et al.,* 2002). Legumes, fruits, grapes, kiwis, pears, bananas, lettuce, cinnamon, and tea leaves are abundant sources of procyanidins (Ferni *et al.,* 2022). Numerous biological effects of procyanidins include antioxidant, anticancer, antibacterial, anti-inflammatory, antidiabetic, anti-allergy, and preventive effects against chronic illnesses and metabolic disorders. Procyanidins significantly reduce the oxidative effect by recovering DNA damage, limiting lipid peroxidation, and activating signaling pathways (Wang *et al.,* 2020). Procyanidin B1-B3 from Japanese azuki beans (*Vigna angularis*) was found to have substantially higher antioxidant capabilities than common conventional antioxidants such ascorbic acid, γ-oryzanol, gallic acid, L-tryptophan, (+)-catechin, and D-IX-tocopherol (Ariga *et al.,* 1988). Studies have shown that procyanidins exhibit protective mechanisms against oxidative stress produced by ROS in the environment and possess antioxidant capacities that are 50 times more than vitamin E and 20 times more than vitamin C, respectively (Zandi *et al.,* 2022). Procyanidin is also reported to influence the suppression of NF-B, p38, ERK, and JNK phosphorylation as well as the inhibition of the NF-B and MAPK pathways. These effects prevented the m-RNA pro-inflammatory cytokines production and COX-2 inflammatory prostaglandin products (Chu *et al.,* 2016;

Siomek, 2012). Procyanidins are reported to significantly raise the levels of pancreatic glutathione (GSH) and reduce alloxan-induced lipid peroxidation. Additionally, they dramatically reduced the amount of total nitrate/nitrite in the pancreas and showed antihyperglycemic effects by preventing alloxan-induced hyperglycemia (Sun *et al.,* 2016). Pancreatic lipase, pancreatic α-amylase, and phospholipase A2 are also inhibited by cocoa extracts and procyanidins in a dose-dependent manner (Gu *et al.,* 2011).

The bark of *Pinus massoniana* contained procyanidin B3, which significantly reduced the migration of HeLa cells (Wu *et al.,* 2011). In OEC-M1 cell studies, it also lowers the expression of MMP2, and MMP-9 (Lin *et al.,* 2012). This shows that procyanidin B3 may have therapeutic potential for the management of metastatic cancer. Molecular docking analysis revealed that Procyanidin B3 from *V. vinifera* showed effective binding affinity towards β-catechin, a therapeutic target protein that is involved in carcinogenesis, with a binding affinity of -6.170 kcal/mol. Procyanidin B3 shows hydrogen bonding interaction with amino acid residues, i.e., GLN 28, GLN 24, GLU 33, ASN 387, and $\pi - \pi$ stack bonding with TRP 383 indicating that it could be effective for the cancer treatment (Adebesin *et al.,* 2022).

Likewise, Procyanidin B3 was reported to show anti-inflammatory activity in mice (Oizumi *et al.,* 2010). The therapeutic potential of B3 in the management of human osteoarthritis (OA) and heterotopic ossification has been also reported (Aini *et al.,* 2012; Ray, 2012).

Different extracts of the plants *M. pudica, B. ciliata,* and *P. emblica* showed effective antioxidant, antimicrobial., and enzyme inhibitory activity toward the digestive enzymes. The EA extract of *M. pudica* is found to contain procyanidin B3 as a potent phytochemical (Sapkota *et al.,* 2022). Additionally, procyanidin B3 demonstrated pancreatic lipase inhibitory activity with IC_{50} μM (Moreno-Córdova *et al.,* 2020). Therefore, different results documented emphasis on the biological importance of procyanidin B3 as an effective metabolite against different diseases including diabetes.

1.18 The Hypothesis

Nepal is boomed with biodiversity in a wide altitudinal variation ranging from 80 m to 8,848.86 m and it provides habitats for thousands of medicinal plants, animals, and microbes. It has its own traditional and ethnic system of using medicinal plants following diseases. The extracts of many medicinal plants retard enzyme overactivity such as α-glucosidase, α-amylase, lipase, glycogen synthase kinase, etc.

If bioactive compounds from the selected medicinally important plant extracts could inhibit the catalytic activity of α-glycosidase, α-amylase, and pancreatic lipase then they may play a significant function in the control of diabetes and its major complications.

1.19 Statement of the problem

People with T2DM are becoming ever more prevalent as time goes on. In Nepal., one out of five people suffers from diabetes due to their sedentary lifestyle, overeating, unhygienic and adulterated food, and environmental and genetic factors. The medicines from medicinal plant extracts have slight or no side effects but synthetic medicines like acarbose, miglitol, and sitagliptin and many of them have some side effects. Thus, despite some significant results, some additional research is needed to develop a valuable alternative antidiabetic therapy by inhibiting the enzymes of diabetic targets which include α-amylase and α-glucosidase and lipase. In Nepal., some previous work has also been carried out but no potential inhibitors of those enzymes were identified using *in-vitro* with *in-silico* methods. Many of the previous studies were carried out to the crude extract levels focusing on profiling of phytochemicals. So, the *in-silico* and *in-vitro* evaluation of the bioactivity of various extracts of different medicinal plants for the identification of secondary metabolites followed by the annotation and profiling of their phytochemicals required.

1.20 Objectives

1.20.1 General objectives

To identify potential antidiabetic drug candidates for the inhibition of enzymes of diabetic targets through molecular annotation and docking of secondary metabolites of Nepalese medicinal plants.

1.20.2 Specific objectives

- To prepare methanol (MeOH) extracts of medicinal plants selected based on the ethnobotanical study.
- To determine the total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity of plant extracts and their correlation study.
- To investigate the half inhibitory activity (IC_{50}) of plant extracts and their solvent fractions against α-glucosidase, α-amylase, and pancreatic lipase.
- To conduct LC-HRMS profiling and molecular annotation for the identification of potent secondary metabolites from active fractions of plant extracts.
- To carry out molecular docking of selected secondary metabolites in the enzymes of diabetic targets.
- To study the antimicrobial effects of plant extracts for the determination of zone of inhibition (ZoI), minimum bactericidal concentration (MBC), and minimum inhibitory concentration (MIC).

1.21 Justification of the Study

Natural products are also used in the development of medicines to cure a wide range of diseases. Over 70% of people worldwide still rely on medicinal herbs for their primary healthcare, and around 800 regenerative herbs are used to avoid diabetes. Active phytoconstituents including flavonoids, phenols, alkaloids, and others found in medicinal plants are utilized to treat diabetics.

Diabetes is a chronic hyperglycemic condition that results from the disability of the body's tissue to carry out the normal metabolism of carbohydrates, lipids, and protein. Long-term hyperglycemia leads to substantial damage to nerves, blood vessels, heart, eyes, kidneys, and many tissues which results in neuropathy, retinopathy, nephropathy, and heart disease, raising the risk of death. In recent years, the use of synthetic or semi-synthetic antidiabetic drugs has increased extensively resulting in very adverse side effects and drug resistance. Hence researchers have focused on developing anti-diabetic medications from the components of natural plants that inhibit digestive enzymes.

The phytoconstituents of medicinal plants inhibit digestive enzymes and scavenge free radicals. Increased cellular oxidative stress as a result of the generation of reactive oxygen and nitrogen species (ROS and NOS) is thought to be the chief cause of diabetic problems. Therefore, plants with strong antioxidant capacity may effectively inhibit enzyme activity. Overexpression of α-glucosidase, α-amylase, and pancreatic lipase, which are causes of high blood glucose levels, are presumed to be inhibited by high levels of phenolic, flavonoids, alkaloids, and other phytoconstituents. The primary emphasis of current research is the screening of natural compounds derived from medicinal plants, the identification, and assessment of antioxidant activity, digestive enzyme inhibition, and molecular annotation of active phytoconstituents.

In our previous research work, more than twenty-four medicinal plants were investigated for their ability to inhibit α-amylase and α-glucosidase along with the determination of TPC, TFC, and antioxidant activity. Among the selected plants, the methanolic extracts of *P. emblica*, *B.cilliata*, and *M. pudica* inhibited 98%, 97.99%, and 99.98% for α- glucosidase and 32.87%, 96.81%, and 90.71% respectively for α-amylase (Parajuli, 2020). So, based on the results of our prior research work, information gathered from books and literature related to Nepal., and the knowledge obtained from the ethnic people during the ethnobotanical survey, these three plants *Mimosa pudica, Phyllanthus emblica,* and *Bergenia cilliata* were chosen for this study. Additionally, in Nepal., no potential enzyme inhibitor from these plant extracts have identified based on molecular docking and annotation using LC-HRMS spectra.

So, this study is aimed to provide the scientific validity of the application of medicinal plants for the treatment and management of hyperglycemia and to investigate the possible drug candidate compound which is confirmed by the identification and molecular annotation of their active phytoconstituents as well as by molecular docking with the enzymes of the diabetic target.

CHAPTER 2

2. LITERATURE REVIEW

2.1 Background

Plants are employed as an important natural resource for both traditional and contemporary medicine. Since prehistoric times, humans became aware of the medicinal importance of plants and started to utilize them to treat common diseases like fever, diarrhea, cough, and common cold and as wound healers. A diverse array of plants with therapeutic properties are referred to as medicinal plants. These plants offer a plentiful supply of chemicals that can be applied to develop new medications for several therapeutic applications (Abdul, 2012).

Natural products could be a good source of novel drugs since they have different phytoconstituents containing different compounds with structural complexity. As mentioned in the reports of WHO, plant extracts and their bioactive components of them are utilized in medications in conventional therapies by more than 80% of people worldwide (Kirbağ *et al.,* 2009). There are 1,950 types of medicinal plants in Nepal., including 1,614 native species. More than 900 plants are utilized to treat diabetes globally in various traditional medicinal systems (Ghimire *et al.,* 2008).

Several synthetic medications have been created to treat diabetes. Meglitinide, biguanides, sulfonylureas, inhibitors of dipeptidyl-peptidase-4 (DPP-4), and sodium-glucose cotransporter-2 (SGLT-2), thiazolidinedione (TZD), and α-amylase and α-glucosidase inhibitors are the main groups of oral antidiabetic drugs (Chaudhury *et al.,* 2017). Many synthetic inhibitors have also been developed against carbohydrate and fat hydrolyzing enzymes. However, the treatment of diabetes patients using these synthetic drugs is less effective due to several unfavorable side effects and the insufficient efficiency of these medications. Additionally, they were found to inhibit the enzymes severely resulting in less starch digestion causing "carbohydrate dumping" into the colon leading to gastrointestinal disorders (Lim *et al.,* 2022). For instance, both biguanides and thiazolidinediones can both result in weight gain and kidney impairment (He *et al.,* 2019). Thus, long-term use and adverse effects of the

present hypoglycemic drugs are creating a tremendous need for effective, and affordable medications for diabetes with fewer side effects.

The majority of research on natural products has found that they have more anti-diabetic benefits than synthetic drugs with a cheaper cost and with minimal side effects. In comparison to synthetic compounds, they usually have greater molecular weights, more sp^3-hybridized carbon and oxygen atoms, some halogen and nitrogen atoms, more hydrogen bond donors and acceptors, and less estimated octanol-water partition coefficients (cLogP values) showing more hydrophilicity. These distinctions may be favorable, for instance, the greater rigidity of NPs may be helpful in the development of drugs to address protein-protein interactions (Atanasov *et al.,* 2021).

Numerous anti-diabetic herbs can stimulate insulin release from the islets of Langerhans enhancing beta-cell activity and having significant antioxidant power. Various enzymes act a specific function in the diabetic pathway for the enhancement of the disease. As every enzyme has a distinct function, it affects the autophosphorylation of insulin receptors, the pathophysiology of the disease, and the glucose intake by glucose transporter type-4 (GLUT-4) (Alam *et al.,* 2019).

Traditional medical practices in Nepal provide the foundation of the basic idea for the development and spread of modern medicine (Gaire *et al.,* 2011). Nepal's traditional medical systems make extensive use of medicinal herbs., which include Shamanistic practices (Pundit, Lama, Jyotish, Gurau, Pujari, Dhami-Jhankri, Jharphuk), Folk medicine (community medicine, ethnomedicine, domestic medicine, and other forms of the local system), and scholarly medical systems (Ayurveda, Unani, homeopathy, and the Tibetian system). The folk medicine system uses more medicinal herbs than the rest of them combined (Gewali *et al.,* 2008).

Various antidiabetic drugs are derived from natural sources. The most prescribed antidiabetic drug metformin is synthesized from the guanidines obtained from *Galega officinalis* and is also used for obese prediabetic patients. Metformin raises glucose tolerance, and insulin sensitivity, and improves peripheral glucose absorption while reducing levels of low-density lipoprotein (LDL) cholesterol, triglycerides, and the insulin-induced suppression of fatty acid oxidation (Pernicova *et al.,* 2014). Acarbose, an *α*-glucosidase inhibitor is extracted from the broth culture of various actinomycetes (Osadebe *et al.,* 2014).

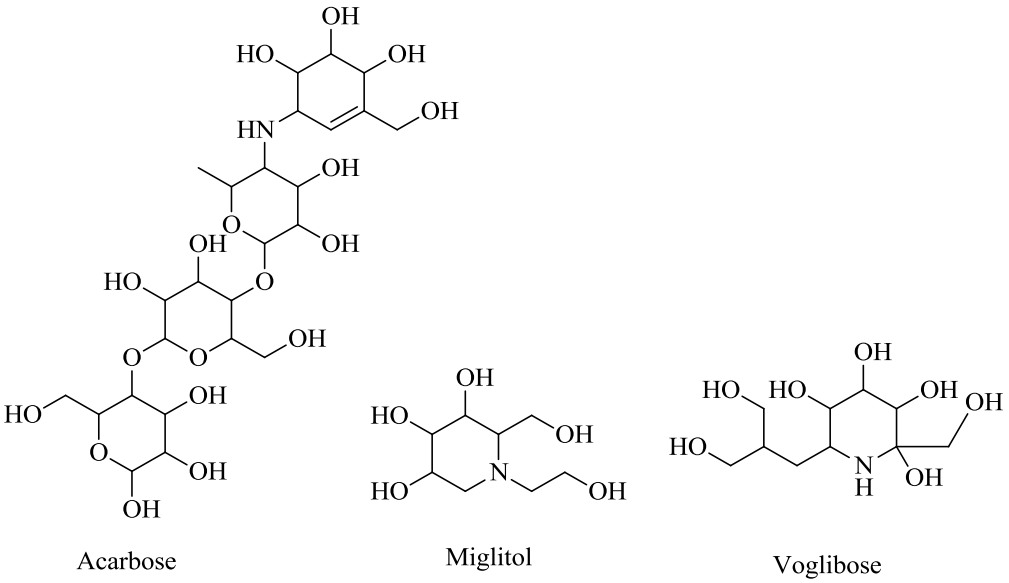


Figure 23: Molecular structure of *α*-glucosidase inhibitors

The N-glycosidic bond of the acarvoisyl moiety is responsible for the inhibitory effect based on the competitive mechanism (AG, 1994). Shrestha *et al.* surveyed 52 antidiabetic plants of the Kaski district belonging to 35 families and found that *Momordica charantia, Asparagus racemosus, Syzygium cumini, Berberis aristata, Gymnema sylvestre, Aegle marmelous*, were used as the major component of herbal and ayurvedic formulations for the antidiabetic medication (Shrestha *et al.,* 2018). Alkaloids, coumarins, polyphenols, terpenes, flavonoids, glycosides, and other plant secondary metabolites play a crucial role in determining the ability of various plants to treat or prevent various illnesses. There are eleven polyphenolic compounds found in the Nepalese medicinal plant *Aconogonon molle,* flower including gallic acid, protocatechuic acid, rutin, quercetin, quercetin 3-O-(6"-O-galloyl)-*ß*-D-galactopyranoside, quercetin 3-O-(6"-O-caffeoyl)-*ß*-D-galactopyranoside, 3-O-*ß* -D-galactopyranoside, quercetin 3-O-*ß*-D-glucopyranoside, quercetin 3-O-*α*-L-arabinopyranoside, quercetin 3-O-*α*-L-arabinofuranoside, and chlorogenic acid (Joshi *et al.,* 2014).

The extracts of *Dioscorea bulbifera, Acacia catechu,* and *Swertia chirata* exhibited potent *α*-amylase inhibition (IC_{50} values: 296.1 µg/mL, 49.9 µg/mL, and 413.5 µg/mL). The antioxidant activity and *in-vitro* and *in-vivo* experiments making use of mouse models showed that these medicinal herbs could be a valuable resource for the development of antidiabetic medications (Khadayat *et al.,* 2020). The extracts of

Tinospora. cordifolia contains many phytochemicals, such as flavonoids, quercetin, kaempferol, luteolin, and epicatechin, and regulates glucose metabolism by increasing the sensitivity and secretion of insulin. Rats with diabetes caused by streptozotocin were administered aqueous extracts of this plant found to produce a considerable rise in serum insulin levels and hepatic glycogen levels while lowering serum levels of triglycerides, total cholesterol, DPP-4, and reactive thiobarbituric acid compounds (Sharma *et al.,* 2019). The hypoglycemic potential compounds such as rose oxide, epicatechin, epigallocatechin gallate, glycyrrhetinic acid, strictinin, isostrictinin dehydrotrametenolic acid, pedunculagin, and christinin were isolated from the plants *Allium sativum, Citrullus colocynthis, Gymnema sylvestre, Trigonella foenum greacum, Ficus bengalensis* and *Momordica charantia* (Patel *et al.,* 2012).

Numerous investigations into the actions of isolated compounds and plant extracts as enzyme inhibitors have been conducted. Glucose uptake through GLUT-4 was enhanced by a methanolic extract of areal portions of *Artemisia pallens* in a dose-dependent manner, whereas intestinal glucose absorption inhibited the intestinal reabsorption of glucose (Subramoniam *et al.,* 1996). The compound myricetin extracted from *Abelmoschus moschatus* inhibited both the enzymes α-glucosidase stigmasterol, and α-amylase (IC_{50} of 0.38 mM), by 29% and 64%, respectively (Tadera *et al.,* 2006).

Table 4: List of antidiabetic plants with their references

S. No.	Medicinal plant	Local name	References
1	*Ficus religiosa*	Peepal	(Olakon *et al.,* 2013)
2	*Acacia catechu*	Khayr	(Ikarashi *et al.,* 2011; Rungsiyothin *et al.,* 2008; M. Kumar, 2014)
3	*Swertia chiraita*	Chiraito	(Phoboo *et al.,* 2013, Umashanker *et al.,* 2011)
4	*Azadirecta indica*	Neem	(Raju *et al.,* 2007; Atanwho *et al.,* 2009)
5	*Momordica charantia*	Tite Karela	(Vitaminoi *et al.,* 2001)
6	*Justicia adhatoda*	Ashuro	(Gulfaz, *et al* ., 2002)
7	*Bergenia ciliate*	Pashanbed	(Bhandari *et al.,* 2008)

8	*Xanthoxylum armatum*	Timur	(Nagarjan, *et al.,* 2016)
9	*Nyctanthes arbortristis*	Pomegranate	(Hung *et al.,* 2006)
10	*Mimisa pudica*	Lajjawati	(Sutar *et al.,* 2009)
11	*Cinnamomum tamala*	Sinkauli	(Das, S. 2015)
12	*Nycanthes arbortristis*	Parijat	(Rangika *et al.,* 2015)
13	*Imperta cylindrical*	Siru	(Villase *et al.,* ,2006)
14	*Phyllanthus emblica*	Amala	(Sultana *et al.,* 2014)
15	*Syzigyum cumini*	Jamun	(Farswana *et al*., 2009)
16	*Acorus calamus*	Bojho	(Si *et al.,* 2010)
17	*Shorea robusta*	Sal	(Soni, *et al.,* 2013)
18	*Utrica parviflora*	Sisnoo	(Farzami *et al.,* 2003)
19	*Aloe vera*	Gheukumari	(Rajasekaran, *et al.,* 2004)
20	*Ficus semicordata*	Khaniyu	(Rai *et al*., 2010)
21	*Ficus lacor*	Kabhro	(Khan, K. Y., 2012)
22	*Discorea bulbifera*	Bhyakur	(Niu *et al.,* 2010)

2.2 Enzyme inhibitors from natural products

Nps contain various enzyme inhibitors involved in diabetic pathways. They are essential for the suppression of enzymes of diabetic targets such as lipase, α-amylase, and α-glucosidase which helps to prevent hyperglycemia after a meal by delaying glucose absorption. For the cure of numerous gastrointestinal, urinary, and respiratory conditions, *Ficus carica* fruit is utilized as a traditional medicine. With the IC_{50} for glucosidase (255.57 ± 36.46 μg/mL) and for amylase (315.89 ± 3.83 μg/mL), the ethanolic extracts of this plant (fruit) demonstrated the inhibition of both enzymes (Mopuri *et al.,* 2016).

The inhibitory activity of ethanolic extract of *Helicteres isora* against sucrase and maltase was reported with significant IC_{50} of 3.71 ± 0.21μg/mL and 2.15 ± 0.48 μg/mL, respectively (Rungprom, 2014). Intestinal glucosidase and amylase were inhibited by the compounds myricetin and quercetin isolated from *Hovenia dulcis* (Meng *et al.,* 2016).

Obesity promotes the emergence of several metabolic illnesses, including diabetes, hypertension, and cardiovascular conditions (Buchholz *et al.,* 2016; Singla *et al.,* 2010). According to WHO, more than one billion people are obese worldwide, and since the numbers are constantly increasing, 167 million people are expected to be in worse health as a consequence of being overweight or obese by 2025. Inhibiting Lipase, which modifies lipid metabolism and absorption, is one of the efficient methods for preventing and treating obesity. The anti-lipase properties of *Vitis vinifera*, *Rhus coriaria*, and *Origanum dayi* demonstrated their potential for treating obesity (Ado *et al.,* 2013). A huge number of bioactive molecules were found to have potential pharmacological agents acting against the enzymes of diabetic targets which include acetyleugenol from *Syzygium aromaticum*, apigenin from *Petroselinum crispum*, cinnamic acid from *Cinnamomum loureiroi*, eriodictyol from *Lippia graveolens*, myrcene from *Myristica fragrans*, piperine from *Piper nigrum* and rosmarinic acid from *Mentha piperita* (Tolmie *et al.,* 2021). In comparison to commercially available medications, *Bergenia pacumbis*, a medicinal plant from Nepal's Himalayan highlands, exhibited significant activity in inhibiting digestive enzymes, scavenging free radicals, effective in skin issues, and neurological illnesses. The methanol extract of this plant was observed to contain significant amounts of bioactive compounds like bergenin, arbutin, catechin, syringic acid, gallic acid, hyperoside, protocatechuic acid, methyl gallate, quercetin, afzelechin, astilbin, paashaanolactone, phloretin, kaempferol-7-O-glucoside, morin, and diosmetin. The methanol extract of this plant displayed significant enzymes inhibitions with IC_{50} values comparable to standard drugs; *α*-glucosidase (IC_{50}: 0.29 ± 0.00 μg/mL), *α*-amylase (IC_{50}: 14.03 ± 0.04 μg/mL), lipase (IC_{50}: 67.26 ± 0.17 μg/mL), elastase (IC_{50}: 74.00 ± 3.03 μg/mL), tyrosinase (IC_{50}: 58.25 ± 1.63 μg/mL), butyrylcholinesterase (IC_{50}: 11.69 ± 0.14 μg/mL) and acetylcholinesterase (IC_{50}: 31.52 ± 0.58 μg/mL) (Pandey *et al.,* 2020).

The hydroethanolic extracts of the plant *Withania frutescens* leaf demonstrated potent antihyperglycemic activities in both *in-vitro* and *in-vivo* assays utilizing diabetic rats (alloxan-induced) (Mechchate *et al.*, 2021). The compounds 3,5-caffeoylquinic acid, 3,4-caffeoylquinic acid, and 4,5-caffeoylquinic acid extracted from the flower buds of *Tussilago farfara*, and chebulagic acid, chebulinic acid, and chebulanin extracted from *Terminalia chebula* showed strong to moderate *α*-glucosidase inhibitory power (Kumar *et al.*, 2011).

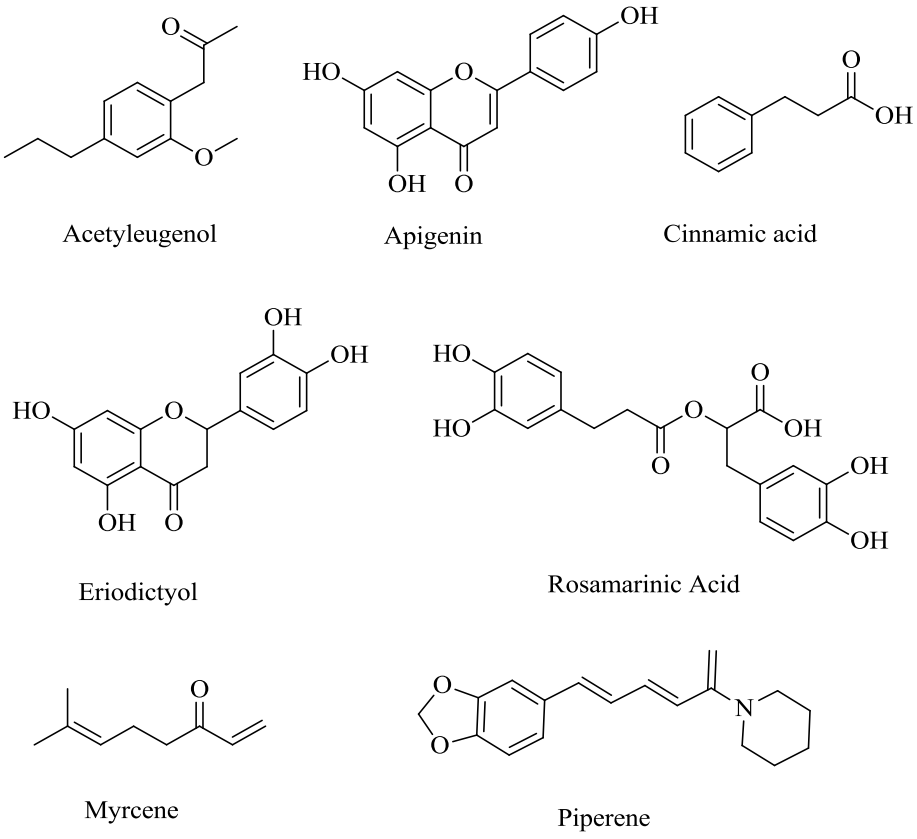


Figure 24: Chemical structures of some herbal enzyme inhibitors

2.3 Medicinal plants used under study

2.3.1 *Bergenia ciliata*

Bergenia cilliata, also known as "Pashanved," is a perennial herb of the Saxifragaceae family that is found in Nepal's temperate Himalayan and mountain ranges at elevations between 800 and 3200 meters. The whole plant can be used to cure a variety of diseases. Due to its unsustainable harvesting and unauthorized export to India and other nations, it is regarded as an endangered plant species.

Figure 25: *Bergenia ciliata* plant

2.3.1.1 Habitat

B. ciliata is an evergreen perennial wild herb that grows under forest shade on a moist rock. The leaves are thick, 20-35 cm long, dark green, alternate, opposite suborbicular, rounded at apex and base, and rhizomatous. Flowers are bisexual, whitish-pink, with obviating petals, acute lobes, and lenticular close to the apex.

2.3.1.2 Geographical distribution

It is abundant in the Himalayan ranges from Afghanistan to Tibet with a height of 800-3200 m throughout India, Nepal, Bhutan, Pakistan, and China.

2.3.1.3 Ethnomedicinal uses

A powder or juice of the whole plant is applied to cure leucorrhea, piles, pulmonary infections, kidney disorders, and urinary troubles. It is frequently used for ulcers, spleen enlargement, dysuria, antiscorbutic, laxative, and tonic purposes. The root extract is applied in the medication for coughs and colds, hemorrhoids, diarrhea, asthma, and urinary disorders (M. Ahmad *et al.*, 2018).

2.3.1.4 Medicinal properties of *B. ciliata*

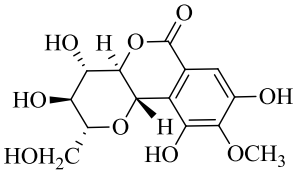
Many rural populations in the Himalayas region have been traditionally using this plant as a medicine in the treatment of various human disorders, including urinary, gastrointestinal., inflammatory, dermal, respiratory, orthopedic, gynecological, and

infectious problems. This species has been claimed to treat 104 types of diseases, according to traditional healers, it has the greatest ability to treat kidney and gastrointestinal illnesses. Almost all plant parts (most typically the rhizome) are utilized in powdered form to treat various illnesses. According to the research findings, it has antioxidant, antifungal, antibacterial, antitussive, anti-ulcer, antiviral, anti-neoplastic, and anti-inflammatory activities (M. Ahmad *et al.,* 2018). Furthermore, the docking analysis of the potent phytochemicals of this plant with digestive enzymes showed strong inhibitory activity against those enzymes (Bohara *et al.,* 2022).

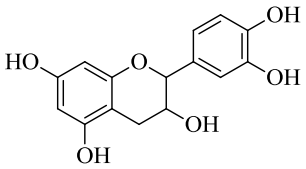
2.3.1.5 Phytochemical constituents of *B. ciliata*

Active phytochemicals extracted from *B. Ciliata* are found to be effective for the management of T2DM. Two bioactive compounds, (-)-3-O-gallocatechin, and (-)-3-O-galloylepicatechin from the extract of *B. Ciliata* were found to contain inhibitory effects against α-glucosidase and porcine pancreatic *α*-amylase in a dose-dependent manner (Bhandari *et al.,* 2008). Among three bioactive phytoconstituents pyrogallol, rutin, and morin were isolated active from the rhizome extract of *B. ciliata,* pyrogallol was found to possess more antioxidant and anticholinesterase potentials (Zafar *et al.,* 2019). Different solvent fractions and subfractions of methanolic extracts of *B. ciliata* leaves from the Sikkim Himalayas were found to contain high levels of flavonoid and phenolic components which showed significant antibacterial and antioxidant activity. Gallic acid, bergenin, and catechin were found to be major ingredients in an HPLC examination (Singh *et al.,* 2017).

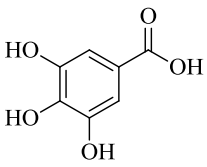
The aerial plant parts also revealed the presence of the phytoconstituents such as (+)-afzelechin, (+)-catechin, hydroquinone (benzenoids), bergenin, quercetin-3-O-L-arabinofuranoside, quercetin-3-O-dxylopyranoside, arbutin, eryodictiol-7-O-D-glucopyranoside, and other phytochemicals. Protocatechuic acid, p-hydroxybenzoic acid, 11-O-p-hydroxybenzoylbergenin, 62-oprotocatechuoylarbutin, 11-O-proto catechuoylbergenin, and 62-O-phydroxybenzoylparasorboside, etc Bergenin and phenolic substances such as gallic acid, leucocyanidin, catechin, methyl gallate, (+)-catechin-3-gallate sterols, (+)-catechin, 11-O-galloylbergenin, sitoindoside, sitoindoside-D-glucoside, β-sitosterol, and β-sitosterol-D- glucoside were also found in the *B. ciliata* rhizome's extract (Kour *et al.,* 2019).



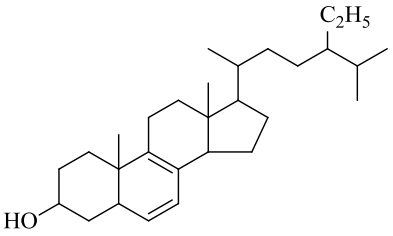
Bergenin



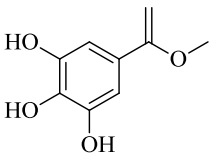
Catechin



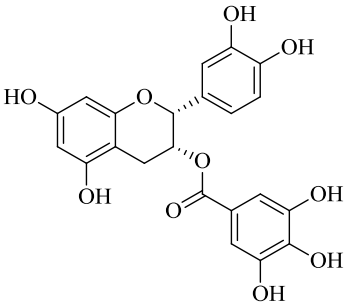
Gallic acid



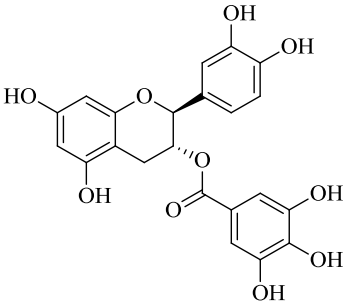
β-Sitosterol



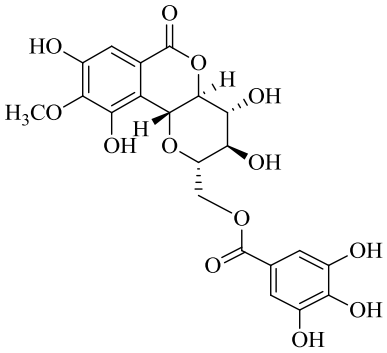
Gallicin



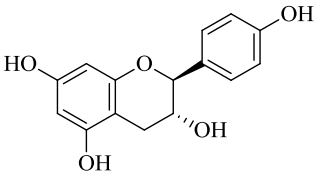
(-)-3-O-galloylepicatechin.



(-)-3-O-galloylcatechin.



11-O-galloylbergenin



(+)Afzelechin

Figure 26: Structures of some compounds isolated from *B. ciliata.*.

2.3.1.6 Research on various plant parts of *B. ciliata*

In traditional therapeutic systems, dried powdered or fresh form of *B. ciliata* was utilized in the medication for cough, fever, vomiting, diarrhea, asthmatic disorders, and pulmonary and lung diseases. The pharmacological investigation of *B. ciliata* was reported to have antiinflammatory, antidiabetic, antipyretic, anti-tussive, anti-bacterial, anti-ulcer, anti-malarial, antioxidant, and antiurolithic activity (Khan *et al.,* 2016). The research performed on the antihyperglycemic properties of the Nepalese medicinal herb *B. ciliata* identified two bioactive constituents, (-)-3-O-galloylcatechin and (-)-3-O-galloylepicatechin, as the active ingredient (Bhandari *et al.,* 2008). Moreover, the methanolic rhizome extract of the plant was reported to demonstrate a considerable hypoglycemic effect and anti-tussive action in a dose-dependent manner. It also had a powerful antioxidant activity with the ability to reduce lipid peroxidation (Pokhrel *et al.,* 2014). The research also revealed that the phenolics and flavonoids content of various extracts of *B. ciliata* is responsible for the inhibitory potential against α-amylase and α-glucosidase which provides the basis to develop a potential antidiabetic drug candidate (Sapkota *et al.,* 2022). The silver nanoparticles (Zia *et al.,* 2018)) and zinc nanoparticles (Dulta *et al.,* 2021) synthesized from the rhizome extract of *B. ciliata* exhibited significant cytotoxic and antibacterial activity against various bacterial pathogens. Numerous bioactive phytoconstituents in the plant are thought to have potent wound-healing properties (Kour *et al.,* 2021) and anti-urolithic activity against calcium oxalate stones (Gupta *et al.,* 2022).

2.3.2 *Mimosa pudica*

Mimosa pudica, an annual or perennial creeping prickly herb belongs to the taxonomic family Mimosaceae also referred to as 'Lajjawati' or 'Touch me not' as its leaves show vary rapid seismonastic movement when triggered by touch or heat or mechanical stimuli. All the parts of this plant are useful in the treatment of various diseases such as asthma, leprosy, vaginal and urinary track diseases, diabetes, leucoderma, inflammation, and fatigue (Ahmad *et al.,* 2012). In Nepal, it grows invasively in dry and moist climates of Terai and mid hills.

Figure 27: *Mimosa pudica* plant

2.3.2.1 Habit

The stem is slender, branched, pricky, and erect in the young plant but creeping with growth. Leaves are bipinnate with 10-26 leaflets per pinna, pedunculate, fold together in the evening, and reopen on sunrise. The leaflets show very rapid semimonastic movement i.e., the leaves droop as a result of stimuli such as heating, touching, or shaking, and the leaves show a very slow periodical movement called nyctinasty movement. Flowers are pink or purple, arising from the leaf axis, calyx is very small with 4 lobes and 4 stamens, the ovary is sessile, and the ovules are numerous. Fruits are 1-2 cm in length with 2-8 pods.

2.3.2.2 Geographical distribution

M. pudica is the native plant of Brazil but is also abundantly distributed in Asian countries eg. Nepal., India, Thailand, Indonesia, Japan, Malaysia, Bangladesh, Philippines, and many Pacific islands. The plant is also called a "Shame plant", "Prayer plant", "Humble plant", "Touch me not", and "Sleeping grass". The three species *M. pudica, M. himalayana, M. hamata, and* are grown together. It is extensively grown in open, wastelands and grasslands, humid places mainly from low to medium altitudes. In Nepal, it is an invasive weed that grows from the lowland Terai to the highland mountains (75-1300 m).

2.3.2.3 Ethnobotanical uses

M. pudica possesses many pharmacological activities such as antibacterial., antivenom, antifertility, anticonvulsant, antidepressant, aphrodisiac, and analgesic. It is applied for the treatment of cough, bronchitis, fever, cholera, dyspepsia, diarrhea, smallpox, jaundice, dysentery, syphilis, tuberculosis, and various gastric and urological disorders (Genest *et al.,* 2008).

2.3.2.4 Medicinal properties of *Mimosa pudica*

All portions of the *M. pudica* plant are of therapeutic value. Traditionally, it was used as a decoction and as an essential ingredient of Samangaa for the treatment of piles, hypothermia, female genital diseases, insomnia, tumor, diarrhea, and persistent dysentery, urogenital infections. It is found to have the major pharmacological activities are aphrodisiac, antiviral, antifungal, antimicrobial, antivenom, antihepatotoxic, anti-diabetic, antidepressant, anticonvulsant, diuretic, antioxidant, and wound healing activity. Phytochemical investigations on *M. pudica* have shown the occurrence of flavonoids, alkaloids, flavonoids, C-glycosides, non-protein amino acids (mimosine), terpenoids, tannins, sterols, and fatty acids (Ahmad *et al.,* 2012).

Alkaloids, glycosides, flavonoids, and tannins with therapeutic benefits have reportedly been found in the plant's various parts. These compounds can be applied in the medication of various illnesses, including dysentery, biliousness, leprosy, uterine and vaginal complaints, asthma, inflammations, burning sensations, exhaustion, leucoderma, and blood diseases. The *in vitro* and *in vivo* pharmacological activity of the crude extracts and isolates of *M. pudica* include anticancer, antiinflammatory, osteoporosis, hypertension, and neurological anomalies. (Kumar *et al.,* 2021).

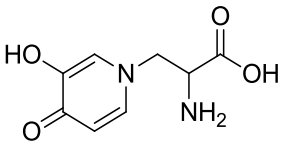
2.3.2.5 Phytochemical constituents of *Mimosa pudica*

Alkaloids, flavonoids, terpenoids, glycosides, quinines, saponins, phenols, tannins, quinines, and coumarins were reported to contain in *M. pudica* leaves It was also discovered that the root of the plant extract subjected to phytochemical testing contained alkaloids, flavonoids, amino acids, phytosterol, glycosides, tannins, and fatty acids. The four compounds 7,8,3',4'-tetrahydroxyl-6-C-[α-L-rhamnopyranosyl-(1→2)]-*ß*-D-glucopyranosylflavone, catachin, 5,7,3',4'-tetrahydroxyl-6-C-[α-L-rham nopyranosyl-(1→2)]-*ß*-D-glucopyranosyl flavone, and 5,7,4'-trihydroxyl-8-C-[α-L-rhamnopyranosyl-(1→2)]-*ß*-D-glucopyranosyl flavones were isolated from *M. pudica*

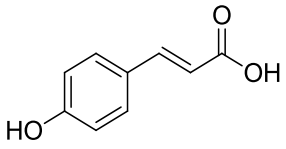
(Ahmad *et al.,* 2012). The extracts of *M. pudica* were also reported to contain secondary metabolites such as norepinephrine, p-coumaric acid, bufadienolide, mimopudine, D-pinitol, mimosine, potassium-5-O-*β*-gluco-pyranosygentisate, etc. (Ahuchaogu *et al.,* 2017). Five compounds, two fatty acids, one steroid, and two flavonoids were extracted from aerial parts of the *M. pudica* plant. Three of them namely quercetin, stigmasterol, and avicularin exhibited potent inhibitory activity against *α*-glucosidase (Tasnuva *et al.,* 2019).

Furthermore, *M. pudica* was found to contain many phytochemicals with numerous pharmacological properties such as mimosine, *β*-sitosterol, stigmasterol, betulinic acid, mimopudine, quercetin, 2-hydroxymethyl-chroman-4-one, *p*-coumaric acid, and avicularin (Bohara *et al.*, 2022).

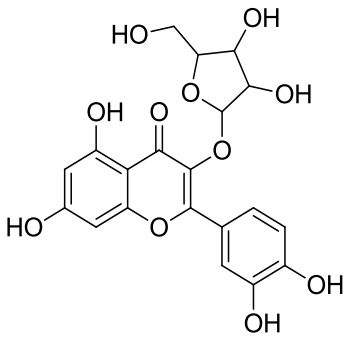
The bioactive compounds such as betulinic acid, stigmasterol, *β*-sitosterol, and a novel sterolglucoside called 2,4-dimethylcholest-7-en-3-D-glucoside were also reported from the roots of *M. pudica* (Muhammad *et al.,* 2016).



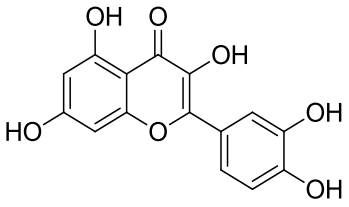
Mimosine



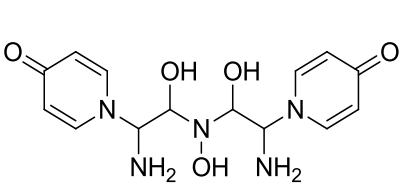
p-Coumaric acid



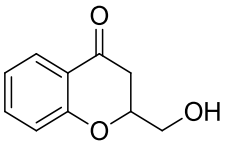
Avicularin



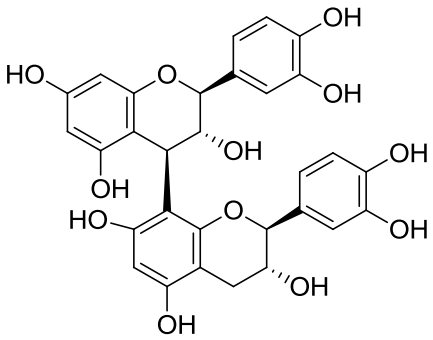
Quercetin



Mimopudine



2-Hydroxymethyl-chromam-4-one



Procyanidin B3

Figure 28: Structures of some secondary metabolites from *M. pudica*

2.3.2.6 Research on various plant parts of *Mimosa pudica*

Due to the availability of valuable bioactive phytoconstituents throughout all plant parts, including the fruits, roots, and leaves, folk medicinal systems employ all of these plant parts to treat a variety of ailments. Streptozotocin-induced diabetic rats were given various dosages of ethanolic *M. pudica* leaf extract, and their effects were analyzed with those of the standard medication metformin (200 mg/kg/b.wt) over 30 days and the results were found to change their elevated glucose, lipid profile, glycosylated hemoglobin, and decreased levels of insulin.in diabetic rats and it was seen to be most effective at the dosage of 300 mg/kg/b.wt. against a high-fat diet (Rajendiran *et al.*, 2019). Likewise, on streptozotocin-treated diabetic rats, the methanol extract of *M. pudica* showed antihyperglycemic and antihyperlipidemic properties. In comparison to the diabetic control rats, the glibenclamide and methanol extract of *M. pudica* treated rats showed a substantial decrease in total cholesterol (TC), triglycerides (TG), glucose levels, very low-density lipoprotein (VLDL), and low-density lipoprotein (LDL) (Parasuraman *et al.*, 2019).

EA extract of *M. pudica* from the Terai area of Nepal was examined for its physicochemical qualities and was found rich in flavonoid and phenolic components and had considerable antibacterial and antioxidant activity. Based on *in-silico* research, L-mimosine was found a powerful antibacterial molecule that could be employed as a candidate for a new antibacterial drug (Mandal *et al.*, 2022).

The *in-silico* study for the phytoconstituents of *M. pudica* against different diabetic enzymes showed a good docking score in two bioactive compounds orientin and isovitexin. The orientin exhibits the best docking value (-7.9 kcal/mol), with three hydrogen bonds interacting with the SER 254, LYS 317, and ARG 320 residues, with bond lengths of 2.76 Å, 3.01 Å, and 2.66 Å respectively. Similarly, the isovitexin exhibits a docking value of (-7.7 kcal/mol), with three H-bonds interacting with the residues THR 360, GLY 253, and TRP 255 (Gopal *et al.,* 2021).

The methanolic *M. pudica* extract has a strong cytotoxic impact by inducing apoptosis in breast cancer cells via an intrinsic route. Bcl-2's expression of the anti-apoptotic gene and protein showed considerable reduction following treatment with the plant extract (John *et al.,* 2020).

2.3.3 *Phyllanthus emblica*

Phyllanthus emblica, commonly known as 'Amala' or ' Indian gooseberry' belongs to the family Phyllanthaceae. It grows naturally or may be planted for its fruit and medicinal benefits in a garden. Although it grows best in temperate climates with temperatures ranging from 20 ℃ to 30 ℃ degrees, it can be found up to 2500 m above sea level. It is reported as a good natural source of vitamin C, minerals, and amino acids. The raw fruits of the plant have a sour, bitter, or astringent taste.

2.3.3.1 Habit

P. emblica is a small to medium-sized (1–8 m) deciduous tree. Leaves are light green, simple, alternate, subsessile, stalked, and closely set along branchlets. Flowers are unisexual., greenish-yellow, and densely clustered in leaf axils. The fruit is fleshy, and slightly spherical., with six vertical stripes or furrows. hard and yellowish-green, and ripens in autumn which is edible with a bitter or sour taste.

2.3.3.2 Geographical distribution

P. emblica grows in tropical and subtropical regions of southern China, Nepal., India, Sri Lanka, Pakistan, and Bangladesh. It is wild or may also be cultivated for its fruits and medicinal benefits, particularly in India, the West Indies, and Japan.

Figure 29: *Phyllanthus. emblica* plant

2.3.3.3 Ethnomedicinal uses

Fruits can be utilized to make chutney, murabba, and other sweets and sauces. For skin conditions, a leaf decoction is employed, and seeds are used to cure biliousness, bronchitis, and asthma, while flowers are said to have laxative and refrigerant qualities. Its fruit pulp is applied to the head to relieve headache and dizziness brought on by high temperatures and fever. The fruit juice is applied to cure colic and other gastrointestinal ailments as well as red, swollen eyes. Its fruit is used to cure hemorrhages, dysentery, diarrhea, jaundice, fever, anemia, dyspepsia, scurvy, diabetes, bronchitis, and cough. It is also regarded as a refrigerant, diuretic, and antibiotic. It is a key ingredient in many medications used in Ayurvedic systems**.**

2.3.3.4 Medicinal properties of *P. emblica*

Amla is one of the potent herbal remedies that have been used for centuries to control and treat illnesses. Arabic, Tibetan, and Egyptian writings as well as the Sidha (Indian), Ayurvedic, and Unani medical systems have all documented its usage as medicine. All parts including the leaves, seed, fruit, root, bark, and flowers, are utilized both fresh and dried. As the best natural source of vitamin C, fresh and dried fruits are usually used as laxatives. It is a component of Triphala and Chayavanprasha according to the Ayurvedic scriptures. Antibacterial, antifungal, and antiviral

activities are found to exist in fruits. Fruits are diuretics and are used to cure nausea, scurvy, ulcers, gastrointestinal inflammation, diarrhea, fever, wounds, premature greying of hairs, and skin sores (Lanka, 2018). Traditional Chinese and Indian systems of medicine still use the plant for its medicinal properties such as anti-diabetic, antioxidant, antidiabetic, hepatoprotective, antihyperlipidemic, and anticancer components (Mirunalini *et al.*, 2010).

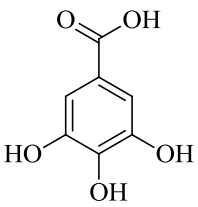
There are notable analgesic, antibacterial., anti-inflammatory, anti-diarrheal., and cytotoxic characteristics of *P. emblica.* It is observed from the experiments that the ethanolic extract of fruit displayed analgesic and anti-inflammatory activity. In mice, it considerably reduced the development of ear edema and produced 38.67% and 19.07% protection or writhing inhibition (Hossen *et al.*, 2014).

2.3.3.5 Phytochemical constituents of *P. emblica*

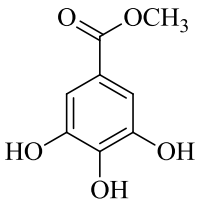
The research findings reveal that the dry fruit of this plant contains carbohydrates (70-75%), proteins (2.5-3.5%) including many amino acids, lipids (1.5-2.0%), and other compounds (2.5-3.5%). It is the biggest source of vitamin C, including carotene, riboflavin, niacin, and thiamine.

Previous phytochemical investigations found the presence of anthocyanins, flavonoids, hydrolyzable tannins (both gallotannins and ellagitannins), flavonols, and phenolic acids, which are the main bioactive constituents contained in amla fruits. Ten phenolic compounds namely gallic acid, methyl gallate, 1-O-galloyl-glucoside, mucic acid 3-O-gallate, corilagin, 1,6-di-O-galloylglucoside, ellagic acid, and ellagic acid-4-Orhamnoside were isolated from amla juice or powder (Rose *et al.*, 2018). Similarly, the ethanolic leaves-extract and shoots-extract of *P. emblica* were reported to contain the two bioactive flavonoids compounds: kaempferol-3-O-L-(6''-ethyl)-rhamnopyranoside, and kaempferol-3-O-L-(6''-methyl)-rhamnopyranoside (Rehman *et al.*, 2007). Various studies on the phytochemical analysis of the fruits extract of *P. emblica* reported the presence of corilagin, geraniin, chebulagic acid, tercatain, dimethyl neochebulagate, methyl chebulagate, chebulic acid 1-O-galloyl-*β*-D-glucose trimethyl ester, 1,6-di-O-galloyl-*β*-D-glucose, digallic acid, ellagic acid, and gallic acid and showed remarkable therapeutic potential against different diseases (Yang *et al.*, 2020). Additionally, eighteen phytoconstituents were detected from the bark extract of *P. emblica.* The *in-silico* study of them revealed isochiapin B, 16α-

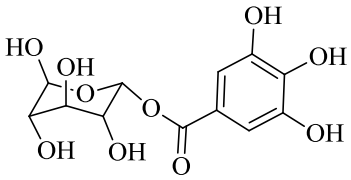
hydroxycleroda-3, Z-dien-15,16-olide, and 14-beta-H-pregna were bioactive to inhibit the diabetes-related enzymes (Quranayati *et al.*, 2022).



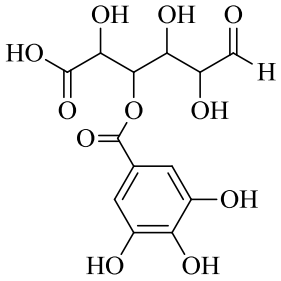
Gallic acid



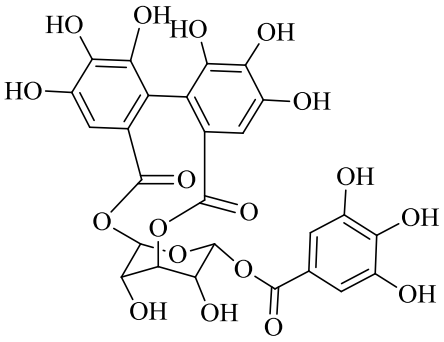
Methyl gallate



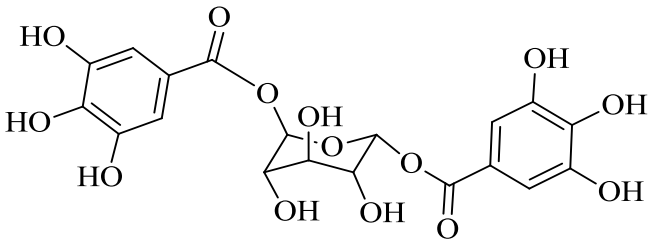
1-O-Galloyl glucoside



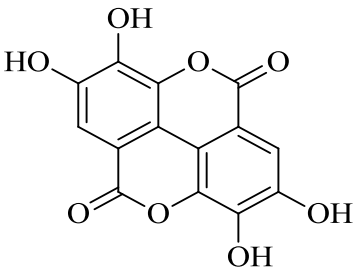
Mucic acid 3-O-gallate



Corilagin



1,6-di-O-galloyl glucoside



Ellagic acid

Figure 30: Structures of phenolic compounds from *P. emblica*.

2.3.3.6 Research on various plant parts of *Phyllanthus emblica*

According to studies, amla has numerous health benefits, such as analgesic, antioxidant, anti-cancer anti-tussive, cardioprotective, cytoprotective, immunomodulatory, and anti-diabetic properties. Amla fruit is commonly used as a hair tonic diuretic, antipyretic, and ulcer preventive either alone or in combination with other herbs (Priya *et al.*, 2019). In a study to evaluate the antimicrobial potentials of plant extracts against some pathogenic bacteria, such as *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis,* and *Escherichia coli*, the plant species were found effective against both gram-positive and gram-negative bacteria, indicating broad-spectrum antibacterial activity of *P. emblica* (Dhale *et al.,* 2011). Shu-Hui Wang identified 14 bioactive compounds that had varying inhibitory effects on the creation of inflammatory factors such as TNF, NO, IL-6, and MCP-1 (Wang, 2019). In another *in vivo* study conducted on streptozotocin-induced diabetic rats, upon the intake of various doses of *P. emblica* fruit extract, diabetic rats showed a significant lowering of blood sugar, an elevation of plasma insulin, and HDL-C, declination in TC, LDL-C, VLDL-C, PL, FFA, TG, and an elevation in, demonstrating the fruit extract's potential to be both anti-diabetic and anti-hyperlipidemic (Krishnaveni *et al.,* 2010). In addition, *in silico* study of the flavonoid quercetin also reveals its antidiabetic activity possessing a significant binding affinity against protein targets, such as peroxisome and glycogen phosphorylase (Srinivasan *et al.,* 2018). *P. emblica* extract has also been shown to have many compounds with anticancer and antitumor properties against most cell lines (Zhao *et al.,* 2015).

CHAPTER 3

3. METHODS AND MATERIALS

3.1 Collection and authentication of plants

The ethnomedicinal plants of Nepal were thoroughly reviewed through the various sources that were available such as books, databases (internet), in-person discussions with tribes and herbal medicine practitioners, etc. The information gathered from them was the main basis for the selection of medicinal plants. It was discovered that certain native plants including *B. ciliata*, *M. pudica*, and *P. emblica* have significant medicinal values but have not been well studied using *in vitro* and *in-silico* enzyme inhibition experiments.

The fresh rhizomes of *B. ciliata* (3 Kg) were taken from Shantipur, Gulmi (82°13′48″ E; 28°11′24″ N), Nepal, while the whole plant of *M. pudica* (2 Kg), and the fruits of *P. emblica* (3 Kg) were obtained from Butwal (83°28′52.3″E; 27°39′48.3″N), Shankarnagar, Rupandehi (Nepal) in October 2020. The National Herbarium and Plant Laboratories, Godawari-3, Lalitpur, Nepal authenticated and taxonomically recognized the gathered plant samples, and allocated voucher specimens BS-02, BS-04, and BS-05 (**Table 5**).

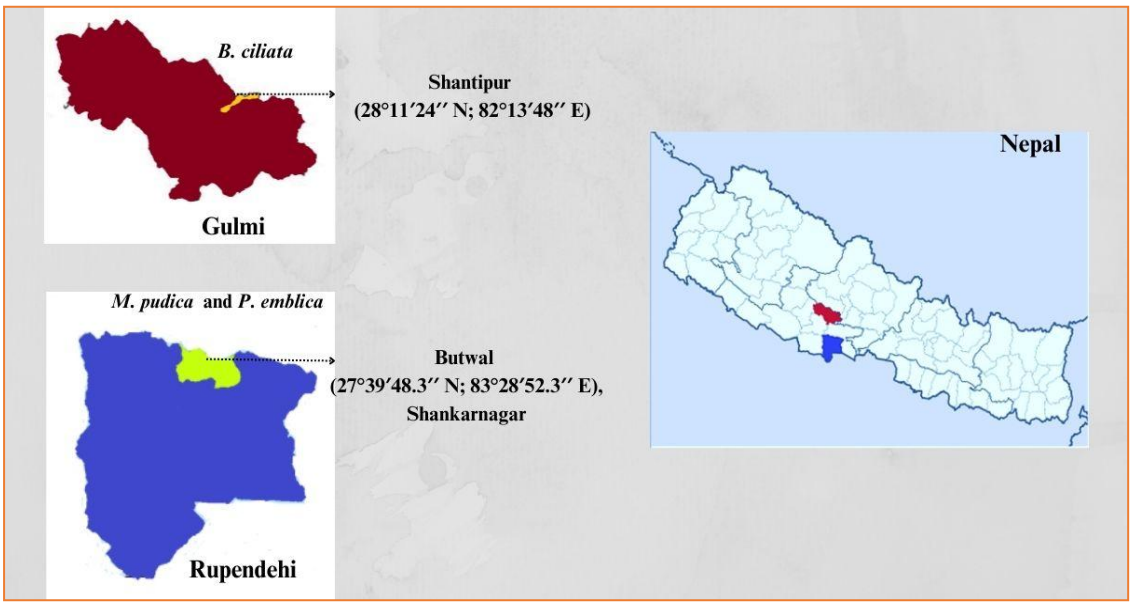


Figure 31: Location of medicinal plants collection

Table 5: Details on the medicinal plants under research, including their locations for collection and parts utilized

Voucher specimens	Scientific Name	Family	Local name	Collection District	Parts used	Month of collection
BS-05	*B. ciliata*	Saxifragaceae	Pakhanved	Gulmi	Rhizome	October
BS-02	*M. pudica*	Fabaceae	Lajjawati	Rupandehi	Whole plant	October
BS-04	*P. emblica*	Phyllanthaceae	Amala	Rupandehi	Fruit	October

3.2 Preparation of herbarium

Herbariums are essential for future reference to study the plant taxonomy, geographic distributions, and stabilizing of nomenclature. The herbarium was prepared at room temperature (27 ℃) by pressing plant parts between newspapers and the newspaper was changed every day for 4 successive days then at the interval of 3 days until 3 weeks to dry out plant parts completely.

3.3 Chemicals and equipments

Porcine pancreatic α-amylase, α-glucosidase, porcine pancreatic lipase, acarbose, 2-chloro-4-nitrophenyl-α-D-maltotrioside (CNPG), *p*-nitrophenyl-α-D-maltotrioside (PNPG), orlistat, neomycin and *p*-nitrophenyl butyrate (PNPB) were purchased from Sigma-Aldrich (USA). 2,2-Diphenyl-1-picrylhydrazyl (DPPH), resazurin, and gallic acid were taken from Molychem and Hi-Media (India). Quercetin, methanol (MeOH), ethanol (EtOH), ethyl acetate (EA), dichloromethane (DCM), dimethyl sulfoxide (DMSO), nutrient agar, aluminum chloride, ascorbic acid, folin–ciocalteau reagent (FCR), hexane, ferric chloride ($AlCl_3$), and ferrous chloride ($AlCl_3$) were purchased from ThermoFisher Scientific (Mumbai, India). All the chemical reagents and glassware used in the study were obtained commercially and were of analytical grade.

The dry methanolic extracts prepared by using a rota-evaporator (IKA RV 10) were used for spectrophotometric measurements for quantitative and qualitative phytochemical analysis, antioxidant, antimicrobial, and the enzyme assays making use

of a 96-Well plate reader (BioTeck, USA), an incubator, a pH meter, an electronic balance, UV laminar, etc.

3.4 Preparation of extracts

3.4.1 Drying and pulverizing

The different plant parts were collected and washed with water to eliminate any dust or other debris, and then it was left to dry at room temperature**.** Soon after the complete drying, it was powdered in a grinder. The process is known as pulverization. The finely powdered plant materials were stored in an airtight jar below 4 ˚C for later use.

3.4.2 Preparation of methanolic extracts and their sub-fractions

The maceration technique was used to make methanolic extracts of medicinal plants in which powered forms of different plants were placed in a different conical flask and methanol was added in the required volume (100 mL/100 gm). After 24 hours, filtration was done with the muslin cloth followed by the filter paper (Whatman no 1). Then, filtrates were recovered in another conical flask and the residue was resuspended, and the procedure was repeated for 3 successive days. Each extract was concentrated under reduced pressure by a vacuum rotary evaporator operated at 40 ˚C and all the extracts were ensured free from the solvent. Each primary methanolic extract was kept separately in an air-tight container below 4 ˚C for later use. Depending on the polarity, various solvents pull out distinct groups of phytoconstituents. Hence, to obtain the various solvent sub-extracts, fatty acids, wax, and other similar components were removed by first extracting with methanol, then fractionating with hexane followed by DCM then EA, and finally with water (Abubakar & Haque, 2020). In this study, the crude extracts were retained in a separate funnel with an equivalent volume of solvent after dissolving in 200 mL water. The two solvent layers were separated by violently shaking the mixture for about four hours and then allowing it to settle overnight. Hexane, DCM, and EA were the solvents on increasing polarity used in fractionation. The percentage yield was measured by the use of the following formula:

$$Percentage\ yield = \frac{dry\ mass\ of\ extracts}{The\ dry\ mass\ of\ plant\ material} \times 100$$

3.5 Phytochemical screening

Standard qualitative methods were applied to identify major phytoconstituents present in the crude extracts of all plant extracts as previously described (Bhandari *et al.,* 2021; Gul *et al.,* 2017; Iqbal *et al.,* 2015). The following tests were carried out to identify major phytoconstituents in plant extracts and the results are illustrated in **Table 7.**

3.5.1 Detection of alkaloids

A little amount (2mL) of the extract was added in a few milliliters of the dilute HCl and then filtered. Following the procedures below, the filtrate was carefully examined to identify the presence of alkaloids.

- **Mayer's test**

A small volume (2 mL) of the extract solution was added to two drops of solution of potassium mercuric iodide (Mayer's reagent) from the side of the test tube, and a brown or orange-colored precipitate was observed.

- **Wagner's test**

Iodine potassium iodide solution (Wagner's reagent) was added to 2 mL of methanolic extract, and the existence of a reddish-brown precipitate was checked**.**

- **Hager's test**

A noticeable yellow color was seen when 2 mL of the methanolic extract solution was treated with an equal amount of saturated picric acid (Hager's reagent) solution.

- **Dragendorff's test**

On addition of 2 mL of extract solution to some drops of potassium bismuth iodide solution (Dragendorff's reagent) causes a reddish-brown or orange-red precipitate to form.

3.5.2 Detection of flavonoids

- **Lead acetate test**

Lead acetate solution (2 mL) was added to the methanolic extract solution, and yellow precipitation resulted, indicating the occurrence of flavonoids.

- **Ferric chloride test**

The extract's alcoholic solution was added with a few drops of $FeCl_3$ solution. The appearance of a greenish solution shows the presence of flavonoids.

- **Alkaline test**

The existence of flavonoids was confirmed by adding about 2 mL of 10% NH_4OH to the methanolic extract solution and watching for the emergence of a yellow color.

- **Shinoda test**

When concentrated hydrochloric acid and a piece of magnesium ribbon are introduced to 2-3 mL of methanolic extract, the resultant production of a pink-red or red hue shows the presence of flavonoids.

3.5.2 Detection of tannins

- **Ferric chloride test**

Two milliliters of the extract and 10% alcoholic $FeCl_3$ solution were mixed in an equal ratio, the presence of greenish-grey or dark blue color in the mixture shows the existence of tannins.

- **Gelatin test**

The appearance of a white precipitate after treating the extract with 10% NaCl and 1% gelatin solution suggests that the tannin was present.

3.5.3 Detection of steroids

- **Salkowski test**

The extract was individually shaken with chloroform and added conc. H_2SO_4 and the development of reddish-brown coloration imply the occurrence of steroids.

3.5.4 Detection of terpenoids

- **Liebermann-Burchard test**

A test tube containing 3 mL extracts and 2 mL of chloroform and a few drops of acetic anhydride was heated in a water bath and quickly chilled in cold water. A brown ring at the intersection of the two liquids appears when conc. H_2SO_4 was mixed from the wall of the test tube. The top layer becoming green showed the

existence of steroids, and the creation of a dark red color indicated the presence of terpenoids.

3.5.5 Test for phenol

- **Ferric chloride test**

Two milliliters of extracts were first mixed with distilled water, and then 10% $FeCl_3$ solution. The appearance of dark green coloration shows the occurrence of phenolic compounds.

3.5.6 Detection of carbohydrates

- **Fehling's test**

The equal volumes (2 mL each) of Fehling 1 and Fehling 2 solutions were combined, and tiny amounts of crude extracts were added to the mixture. Slight heating further forms a brick-red coloring at the test tube's bottom indicating a successful presence of carbohydrates.

- **Benedict's test**

A tiny amount (1 mL) of the extract solution was added with some amounts of the Benedict reagent, followed by heating in the water bath for two minutes. The presence of brick red-colored precipitate denotes the occurrence of reducing sugar.

- **Molisch's test**

The Molisch reagent was treated with two milliliters of filtrate and 1 mL of conc. H_2SO_4 was mixed into the solution from the side of the test tube and then cool. The conformation for a carbohydrate was formed by a violet color ring at the intersection of the two layers.

3.5.7 Detection of glycoside

- **Borntrager's test**

About 2 mL of 5% H_2SO_4 was mixed with the 2 mL extract solutions and filtered after boiling in the water bath. The filtrate recovered was then mixed with an equal amount of chloroform and left for five minutes. Half of the volume of the bottom layer of chloroform was mixed with diluted ammonia. Anthraquinone glycosides are indicated by the appearance of rose pink to red color.

- **Keller-Killiani test**

Ferric chloride (1 mL) and glacial acetic acid (1 mL) were mixed with the extract's aqueous solution (2 mL). When a few drops of H_2SO_4 were added from the test tube's side, a brown-colored ring formed at the intersection of the two liquids, signifying the presence of cardiac glycoside.

- **Bromine water test**

Test solutions were mixed with bromine water and examined for the development of a yellow-colored precipitate which represents the positive test of glycosides.

3.6 Determination of total phenolic content (TPC)

Preparation of reagents

To make 1 M sodium carbonate, 5.29 gm of it was dissolved in 50 mL distilled water, and Folin Ciocaltau reagent (1:10 v/v) was prepared by diluting 10 mL of it in 100 mL distilled water.

Preparation of standard gallic acid solution

The standard gallic acid (500 µg/mL) stock solution was made by mixing 5 mg gallic acid in 10 mL ethanol which is then diluted with distilled water to get the solutions of various concentrations (10, 20, 30, 40, 50, 60, 70, and 80 µg/mL).

Preparation of plant extracts

Similarly, the stock solutions of each extract (50 mg/mL) were diluted ten times in 50% DMSO to form test solutions of plant extracts (500 µg/mL).

Procedure

The TPC of the extracts was evaluated using the Folin Ciocalteu reagent (FCR) via 96 well plate techniques (Ainsworth & Gillespie, 2007; Liu, *et al.*, 2008) with slight modification. Briefly, 20 µL of standard gallic acid solutions (10, 20, 30, 40, 50, 60, 70, and 80 µg/mL) and 20 µL of each extract (500 µg/mL) were placed in triplicate on a 96-well plate. Then, 100 µL of 1 : 10 v/v FCR prepared in distilled water was added to take initial readings in the spectrophotometer. Then, 80 µL of 1 M Na_2CO_3 was added separately in each well containing standard gallic acid and plant extract

solution. Then this mixture was placed in the dark for 15 minutes at normal temperature and the final absorbance was taken after 15 minutes at 765 nm in a microplate reader (Synergy LX, BioTek, Instruments, Inc., USA). The absorbance of Gallic acid was utilized for drawing a standard calibration curve (10-80 µg/mL) and the total polyphenolic compound concentration in the extracts was measured using a standard calibration curve and represented as milligrams of gallic acid equivalent per gram of dry mass (mgGAE/g) of the extract.

3.7 Determination of total flavonoid content (TFC)

Preparation of Reagents

1 gram of $AlCl_3$ was dissolved in 10 mL of distilled water to make 10% aluminum trichloride, and 0.98 grams of potassium acetate was dissolved in the same amount of water to make 1 M potassium acetate.

Preparation Standard quercetin solution

One milligram of quercetin was dissolved in ten milliliters of methanol to prepare a stock solution (0.1 mg/mL). The standard solution was then prepared at various concentrations of 10, 20, 30, 40, 50, 60, 70, and 80 µg/mL by diluting the 0.1 mg/mL stock solution.

Preparation of plant extracts

Each plant extract solution was diluted to a concentration of 500 µg/mL in 50% DMSO solution from a stock solution of 50 mg/mL.

Procedure

The TFC of each extract was assessed by applying the $AlCl_3$ colorimetric technique (Chang *et al.*, 2002) using a 96-well plate reader. In brief, 130 µL of different concentrations of standard quercetin solutions (10, 20, 30, 40, 50, 60, 70, and 80 µg/mL) were obtained by diluting the stock solution (100 µg /mL) with distilled water were loaded on 96-well plates in triplicate. A 96-well plate was then loaded with 20 µL (500 µg/mL) of each plant sample in triplicate. Then, the total volume of each well holding a plant sample was 130 µL after the addition of 110 µL of distilled water. Then, in each well holding the standard quercetin and plant sample, 60 µL of ethanol,

5 µL of $AlCl_3$, and 5µL of potassium acetate were added individually. Next, after being kept in the dark for 30 minutes, this combination was tested for absorbance at 415 nm using a 96-well plate reader. (Epoch2, BioTek Instruments Inc., United States). The amount of total flavonoids is estimated using the quercetin standard calibration curve (10–80 µg/mL) and is represented as milligrams of quercetin equivalent per gram of dry extract mass (mgGAE/g).

3.8 Measurement of antioxidant activity (DPPH Assay)

The DPPH test is predicated on the antioxidant's capacity to provide an H-atom or an electron to the DPPH• radical., a commercially available dark violet-colored stable free radical. The color of the solution changes from original deep-violet to light-yellow when the DPPH radical accepts hydrogen or an electron from an antioxidant agent (radical scavenger), reducing DPPH• radicals to DPPH-H form (Paixao *et al.*, 2007). By using spectrophotometry, one may detect the disappearance of DPPH• in test samples or the amount of absorbance decrease at 517 nm which is proportional to the antioxidant content.

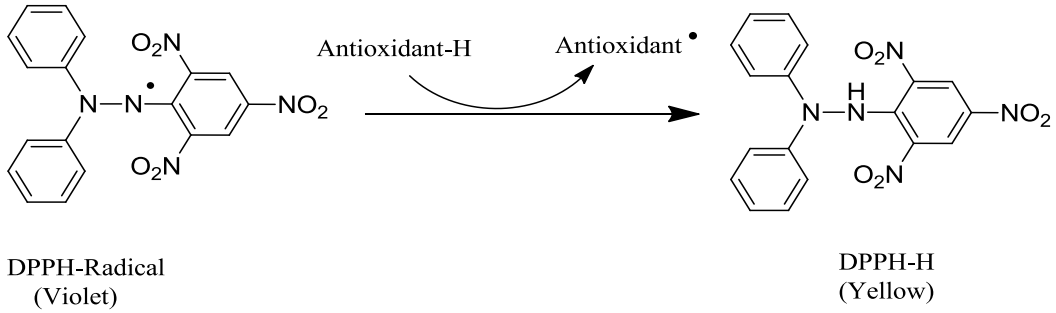


Figure 32: Reduction of DPPH radical by an antioxidant

Preparation of DPPH solution

In a volumetric flask, 3.9 mg of DPPH was dissolved in 100 mL of methanol to create the 0.1 mM solution. After that, the solution was sealed in aluminum foil and kept in the dark for future use.

Preparation of standard quercetin solution

One milligram of quercetin in one milliliter of methanol was dissolved to create a 1 mg/mL quercetin stock solution. Subsequently, various final concentrations (20, 10, 5, 2.5, and 1.25 µg/mL) were produced by diluting a stock solution.

Preparation of plant extracts

To create the final concentrations of 1000, 500, 250, 125, and 62.5 µg/mL by serial dilution, plant extract's 50 mg/mL stock solution was created by dissolving 50 mg of plant extracts in 1 mL 50% DMSO.

Procedure

According to the previously mentioned methodology (Sabudak *et al.*, 2013), using a 96-well plate method, the antioxidant potential of various extracts was assessed against DPPH radicals. A 96-well plate was filled with each of 100 µL quercetin solutions of various concentrations (positive control) or 50% DMSO (for a negative control), and 0.5 mg/mL of plant extracts. Then, each well was added 100 µL of DPPH reagent followed by the incubation in dark for 30 minutes. Using a microplate reader, the absorbance was then recorded at 517 nm. The following equation was used to calculate the ability to scavenge the DPPH-radical.

$$\%\ Inhibition = \left(\frac{A_{control} - A_{sample}}{A_{control}}\right) \times 100 \qquad \ldots\ldots\ldots\ldots\ldots\ldots \quad (i)$$

Where A_{sample} and $A_{control}$ denote the OD of the sample and control respectively.

The results were represented as percentage scavenging activity (% inhibition) or IC_{50} which is the lowest concentration of antioxidant required to scavenge the DPPH-radical by 50%. The IC_{50} values for those extracts having more than 50% inhibition were calculated using GraphPad Prism (Version 8) software.

3.9 *In-vitro* enzyme inhibition assays

3.9.1 α-amylase inhibition activity

The ability of plant extracts to inhibit α-amylase was investigated using different solvent fractions in the manner outlined in our prior work (Khadayat *et al.*, 2020; Senger *et al.*, 2012). Initial absorbance was taken after 15 minutes of incubation at 37 °C with a mixture of 20 µL extracts in 30% DMSO, and 80 µL of the α-amylase enzyme (1.5 U/mL) dissolved in phosphate buffer saline (pH 7.0, NaCl 0.9%). The reaction was started by mixing 100 µL of CNPG (1 mM in the same buffer) after 15 minutes of incubation at the same temperature, and the change in absorbance (at 405

nm) caused by the formation of *p*-nitroaniline was recorded in a microplate reader (Epoch2, BioTek, Instruments, Inc., United States). The absorbance was measured in triplicate in 200 µL of final volume taking 30% DMSO as control. The following equation was applied to determine the % inhibitory activity of α-amylase.

$$\%\ Inhibition = \left(\frac{A_c - A_t}{A_c}\right) \times 100 \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ (ii)$$

Where A_c and A_t represent the absorbance of the control (with 30% DMSO) and the absorbance of the sample (with plant extract) respectively.

3.9.2 α-glucosidase inhibition activity

Different plant extracts were subjected to measure their capability of inhibiting α-glucosidase using the usual methodology with a few minor modifications. (Fouotsa *et al.*, 2012). A 96-well plate containing 20 µL of α-glucosidase (0.2 mM), 120 µL phosphate buffer solution (pH: 6.8), and plant extracts 20 µL (500 µg/mL) was used for the reaction, which was kept for 15 min in incubation at 37 °C. The resulting mixture (200 µL) after the addition of 40 µL of substrate PNPG (0.7 mM in the same buffer) was kept in incubation at 37 ℃ for 15 minutes. Then, the amount of *p*-nitrophenol generated during the hydrolysis of PNPG was measured at 405 nm to determine the degree of α-glucosidase inhibition.

A solution of 30% DMSO, and standard drug Acarbose (20-100 µg/mL) were utilized as negative and positive controls, respectively. The percentage inhibition was determined using the above-mentioned equation (ii) for α-glucosidase after each absorbance was measured in triplicate.

3.9.3 Pancreatic lipase inhibition activity assay

Lipase inhibition activity was assayed by following the procedure by Lankatillke *et al.* with some variations (Lankatillake *et al.*, 2021). Briefly, 20 µL of plant extracts of different concentrations, 100 µL of 0.1 M sodium phosphate buffer solution (pH = 8.0), and 40 µLof enzymes were loaded in 96-well plates. Initial absorbance at 405 nm was taken after 30 minutes of incubation at 37 ℃. Then, 40 µL substrate p-nitrophenyl butyrate (0.7 M in ethanol) was added to each well. After 30 minutes of incubation at 37 ℃, the final absorbance for the hydrolysis of PNB to *p*-nitrophenol was measured. All the measurements were performed in triplicates using 30% DMSO and orlistat as the negative and positive control respectively.

3.10. Antimicrobial activity test

Antibacterial activity is the capability of plant extracts or chemicals to inhibit or stop the development of harmful microorganisms. It explains why some possibly bioactive substances were chosen. According to the methodology given by Daoud *et al.,* and Balouiri *et al.,* the anti-bacterial screening for plant extracts was conducted employing the Agar-Well-Diffusion technique (Balouiri *et al.,* 2016; Daoud *et al.,* 2019). In this approach, the diameter of the zone of inhibition (ZoI) produced by plant extracts against certain pathogenic bacteria was assessed to estimate the antibacterial activity of the extract.

3.10.1 Materials required

Table 6: Materials required for antimicrobial activity test

S. No.	Materials
1	Autoclave
2	Oven
3	Refrigerator
4	Micropipettes
5	Permanent markers
6	Petri dishes
7	Eppendroff tubes
8	Ethanol
9	Tips and tips holder
10	Cotton swab
11	Test tubes
12	Laminar
13	Bacterial species
14	Cork borer
15	Dimethyl Sulphoxide
16	Neomycin

3.10.2 Preparation of media

- **Muller-Hinton Agar (MHA)**

Muller Hinton agar was boiled in a conical flask with continuous shaking after being added to distilled water at a 38 gm/liter ratio until it becomes a transparent solution. It

was autoclaved at 121 ℃ for 15 minutes, then poured into sterile petri plates with a 90 mm diameter at a rate of 25 mL for each plate. The plates were kept for setting.

- **Muller Hinton Broth (MHB)**

In a beaker, the required amount of nutrient broth was dissolved in distilled or autoclaved water at a ratio of 21 grams per liter and autoclaved at 121 ^{o}C for 15 minutes to sterilize. After cooling, 10 mL of it was kept in a sanitized test tube with a screw-top lid.

3.10.3 Collection of standard cultures

The standard cultures of microorganisms under investigation were gathered from the Central Department of Microbiology, TU. The test microorganisms were carpet-cultured onto MHA plates and they were then incubated. The organisms so obtained were examined for purity and compared to one another in terms of their physical, cultural, and biological traits. The species used in this investigation include *Escherichia coli* ATCC 25992, *Salmonella typhi* ATCC 14028, *Shigella sonnei* ATCC 25931, and *Staphylococcus aureus* ATCC 25923. 1 mg/mL Neomycin was used as the positive control.

3.10.4 Preparation of plant extract solution

To prepare the test solution with a concentration of 50 mg/mL in an Eppendorf tube, 50 mg of each plant extract was completely dissolved in 1 mL 50% DMSO using a vortex shaker. After that, this solution is kept in a refrigerator at 4 ℃ for later usage.

3.10.5 Screening and determination of antibacterial activity

Using the agar-well-diffusion technique, the antibacterial potential of plant extracts was examined. To match the turbidity to 0.5 McFarland standards (1.5×10^8 CFU/mL or 10^8 bacteria/mL), the test microorganisms were then subjected to 12 hours of incubation at 37 ℃ after inoculation in MHB. After that, bacteria were lawn cultured carefully using a sterile cotton swab all over the MHA plate. Using a cork borer of 6 mm diameter, five wells of about 4mm depth were bored into the lawn-cultured MHA plates. Then, 50 µL plant extract (50 mg/mL in 50% DMSO) was mixed in each well, and a separate well was used for the positive control (50 µL of 1 mg/mL neomycin) and the negative control (50 µL of 50% DMSO) respectively. The plate was then kept

in an incubator for 18 to 24 hours at 37 ℃ after 15 minutes at a normal temperature allowing it to diffuse over the plate. After incubation, the zone of inhibitions (ZoI) for each extract was measured in mm.

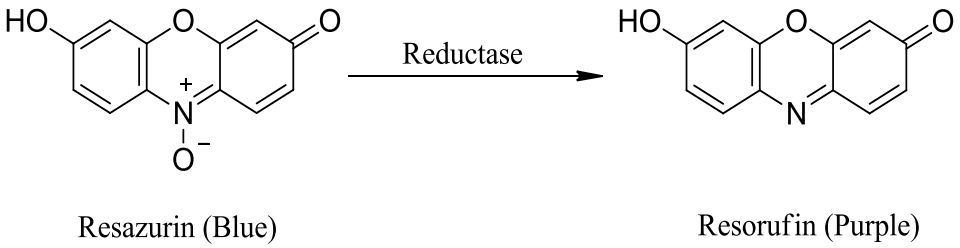
3.11 Determination of minimum inhibitory concentration (MIC)

The minimum concentration of a chemical required for the prevention of visible growth of any bacteria is called minimum inhibitory concentration (MIC) which was determined according to the previously reported procedure with minor modification (Sarker *et al*., 2007; Kowalska-Krochmal & Dudek-Wicher, 2021). It represents the potency of any chemicals against the specific bacteria which is expressed in terms of µg or mg/mL. The methanolic extracts or different solvent fractions of the plant extract showing significant antibacterial activity were subjected to the measurement of MIC.

Then, 100 µL of stock solutions (50 mg/mL) were serially diluted twofold in the 96-well plates to make the solutions of various concentrations up to 0.097 mg/mL. After that, every well except the negative control was administered with 5 µL (10^6 CFU/mL) of bacteria. After sealing with a lid it was let to remain at 37 ℃ for 18 to 24 hours for incubation. After the incubation period, 0.003% resazurin solution was mixed in each well and kept in an incubator for 3–4 hours at 37 ℃. The conversion of the blue color to pink due to the release of enzyme reductase indicates the growth of bacteria whereas the blue color indicated no bacterial growth. The minimum concentration with a blue-colored well was considered MIC.

3.12 Determination of minimum bactericidal concentration (MBC)

The lowest concentration of a chemical needed to eradicate or kill certain bacteria is known as the minimum bactericidal concentration. It is measured using the microdilution technique with serially diluted plant extracts according to the CLSI protocol (Heil & Johnson, 2016). MIC and higher concentrations were spread over MHA plates and subjected to incubation at 37 ℃ for 18 to 24 hrs. Then the bacterial growth on MHA plates was observed. MBC was the smallest concentration with zero bacterial growth



Resazurin (Blue) Resorufin (Purple)

Figure 33: Reaction for the conversion of resazurin to resorufin during MIC determination

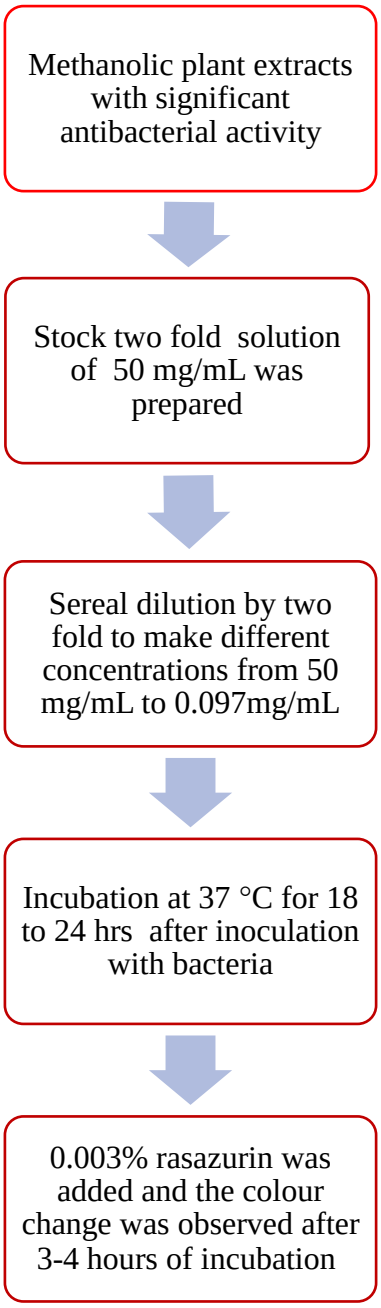


Figure 34*:* The procedure for the determination of the minimum inhibitory concentration

3.13 Mass spectrometry-based molecular annotation

The separation and characterization of phytochemicals cannot be accomplished by a single traditional approach, such as NMR, IR, or mass analysis. As a result, more advanced merging technology, such as LC-HRMS, which easily gives more structural information utilizing a small sample size, is commonly used to assign the various components in plant extracts (Xiao *et al*., 2012).

The analysis of metabolites in plant extract was carried out using LC-HRMS which offers data on the *m/z* ratio and the fine structure of the isotopes that could be employed to deduce the structure of the potent metabolites. When compared to other profiling techniques, high-resolution mass spectroscopy (HRMS) offers data with greater sensitivity, more reliability, and higher accuracy. At the Central Drug Research Institute (CSIR), Sophisticated Analytical Instrument Facility (SAIF), Lucknow, metabolites from an active fraction of EA and hexane were identified using LC-HRMS. Mass Spectrometer equipped with a G1329A autosampler, Accurate-Mass Q-TOF, an Agilent 6520, quaternary-pump (G1311A), and a diode-array detector (DAD) G1315D was used for the LC-HRMS study. The following settings were made for the source and scan: fragmentor: 175, skimmer 1: 65.0, nebulizer: 40 psi, VCap: 3500, gas flow: 141 11.01/min, gas temp: 30 ℃, RF Peak: 750, and octopole. For the elution of solvent, a 5 mM acetonitrile, water, and acetate buffer were used which was at a flow rate of 1.5 mL/min. 5% acetonitrile was used to start the elution gradient, Then, 30% acetonitrile for 10 minutes, 80% acetonitrile for thirty-two minutes, and finally returning to the original states. Throughout the process, a 30 ℃ temperature was maintained in the column. The diode array detector's flow cell processed the column elute during processing, and then transmitted to a Q-TOF HRMS using an electrospray interface. The mass spectrum ranging from 100-2000 Daltons was examined using positive electrospray ionization (ESI-positive mode) with a scan rate of 1.03 (MR, 2009).

3.14 Statistical analysis

Microsoft Excel was used to collect data and preliminary analysis. The experimental results were represented as ± mean standard error of the mean. Version 8 of GraphPad Prism software was used to calculate the IC_{50} values. In this study, the LC-HRMS of

the potent fraction of plant extract fractions was done to identify the metabolite compounds in the sample mixtures. Using retention duration, *m/z* value, and chemical formula, MestreNova 12.0 software was utilized to annotate the metabolites from the LC-HRMS raw data files. To search and assign the molecular formulae and structures of compounds, additional databases such as ChemDraw, PubChem, Dictionary of Natural Products, ChemSpider, and METLIN were also employed.

With mass ranges of 100–2000 Da, an ESI-positive ionization mode was employed for the MS analysis. Using MsConvert software (http://proteowizard.sourceforge.net/tools.s html) was used to convert the LC/HRMS raw data obtained in mzML format, and MestreNova 12.0 tool (MR, 2009) was used for peak alignment, peak detection, and then identification (target compound annotation) based on centroid data. (Holman *et al.,* 2014; Katajamaa & Orešič, 2005; Pluskal *et al.,* 2010). Compounds were annotated by comparing the spectrum data with the literature,

3.15 Molecular Docking

3.15.1 Preparation of ligand

PubChem search engine was used to retrieve the structures of selected compounds in sdf format (Kim *et al.,* 2016). The BIOVIA Discovery Studio Visualizer software was then used to convert ligands into .pdb format (https://discover.3ds.com/discovery-studio-visualizer).

3.15.2 Preparation of protein

The crystal structures of the target proteins were retrieved from the RCSB (Research Collaboratory for Structural Bioinformatics) Protein Data Bank (PDB) (Rose *et al.,* 2021). The structures of the three target proteins; *α*-amylase (PDB ID: 1HNY), *α*-glucosidase (PDB ID: 5NN8), and lipase (PDB ID: 1LPB) were downloaded in the 3D format using RCSB PDB. The downloaded protein structure was validated with the help of the online tool SAVES v6.0 (https://saves.mbi.ucla.edu/). The raw 3D structure was adjusted to fit and made accessible for docking investigation to produce a physiologically active protein. To optimize the protein, water molecules were removed and polar hydrogen and Kollman charges were added.

3.15.3 Protein-ligand docking

The AutoDockTools-1.5.6 version was used to perform the docking of the compounds acquired from the annotation of LC-HRMS data and prepared receptor enzymes. Before docking, the receptor was modified by deleting water molecules and adding Kollman- charges and polar hydrogens. The receptor and ligands were converted into the properly readable PDBQT format. A grid box (40×40×40 Å), and a spacing of 0.375 Å were set up in an auto grid to create a grid map for docking purposes. Then, a config.txt file was generated for each protein, containing information such as ligand = ligand.pdbqt, receptor = protein.pdbqt, and coordinates in the x-y-z dimension from the grid center with 8 exhaustiveness values and 4 energy ranges. A series of commands were input through the command prompt to execute docking, and as an output, log files with nine distinct poses and binding energy were produced. The docking result was visualized using BIOVIA Discovery Studio Visualizer (https://discover.3ds.com/discovery-studio-visualizer), which showed 2-D and 3-D output interaction with different amino acids along with their bond length.

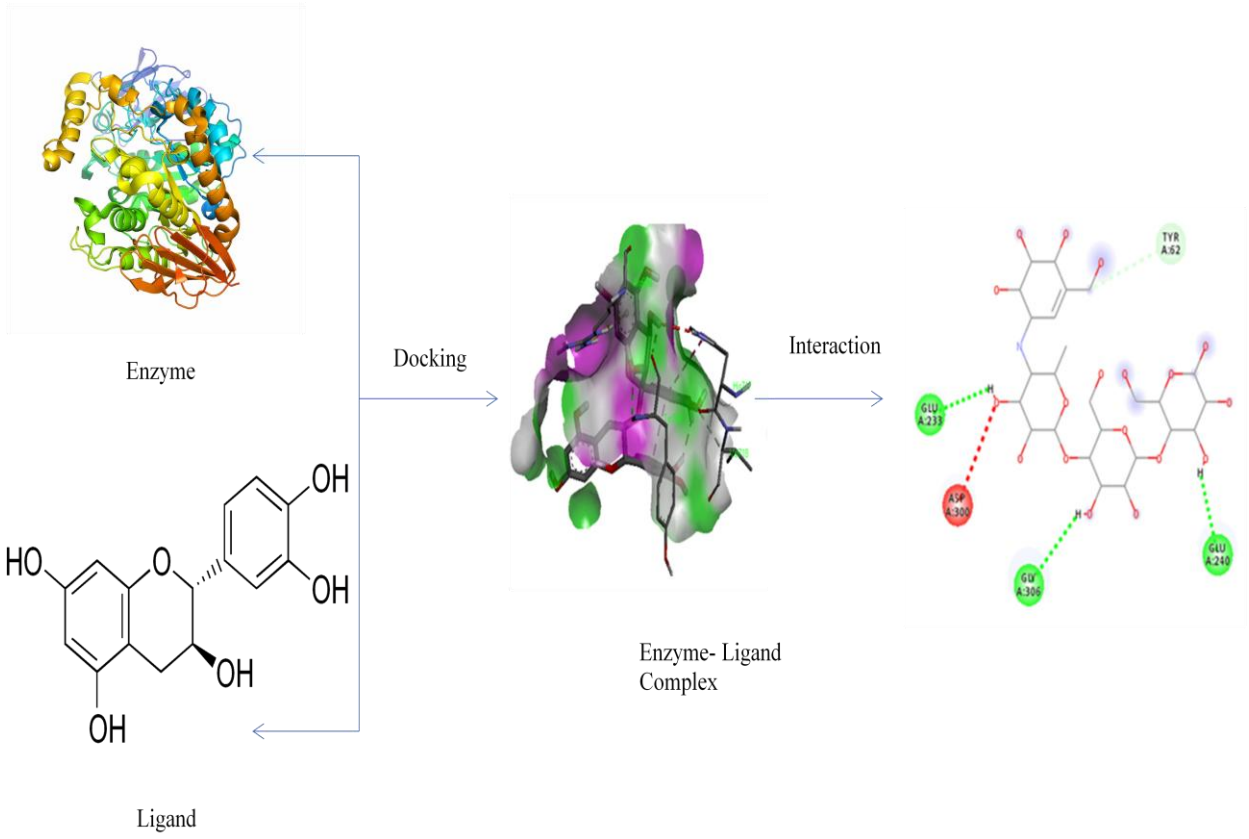


Figure 35: Molecular docking process

CHAPTER 4

4. RESULTS AND DISCUSSION

4.1 Phytochemical Analysis

4.1.1 Qualitative phytochemical screening

The percent yield of crude methanolic extract of *B. ciliata*, *M. pudica*, and *P. emblica* was found 15.86%, 17.6%, and 39.5% respectively. Among the three plants, the percentage yield is highest in *P. emblica* and the lowest in *B. ciliata*. Many factors might have an impact on the percentage extraction yield including the extraction technique, solvent, harvesting period, meteorological conditions, and sample-to-solvent ratio (Azwanida, 2015). It could also be a result of the diverse bioactive chemicals, which differ in their solubility profiles in the extraction solvent, that are found in various plant samples. The greater the solubility of the ingredients in the extraction solvent, the higher will be the value of percentage yield and vice versa (Truong *et al.,* 2019).

The unique biological activities of plants can be identified by their phytochemical properties. In this study, the crude (methanolic), hexane, EA, dichloromethane, and aqueous extracts of *M. pudica, B. ciliata,* and *P. emblica* obtained in Section 3.4.2 were used for the phytochemical screening of many bioactive chemicals following standard qualitative methods as described in section 3.5. The findings are shown in **Table 7** indicating positive test findings with a plus (+) sign and negative test results with a minus (-) sign.

The result of phytochemical identification revealed that the plant extracts included a wide range of chemicals that include flavonoids, terpenoids, alkaloids, saponins, tannins, steroids, carbohydrates, phenol, and essential oils. The common phytochemicals detected in every plant extract were phenolics, flavonoids, carbohydrates, terpenoids, tannins, and glycosides. The presence of steroids was detected only in EA and aqueous fractions of the plants. Saponin, tannins, and anthraquinones were not detected in DCM fractions of all plants

Table 7: Phytochemical screening of different fractions of plant extracts

Phytochemicals		***B. ciliate***					***M. pudica***					***P. emblica***				
	Performed test	**Me**	**H**	**D**	**E**	**A**	**Me**	**H**	**D**	**E**	**A**	**Me**	**H**	**D**	**E**	**A**
Alkaloids	Dragendorff's	+	+	-	+	+	+	-	-	+	+	+	+	+	+	+
Flavonoids	Alkaline reagent	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
Phenols	Ferric chloride	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Steroids	Steroid Test	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+
Terpenoids	Salkowski	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tannins	Braemer's	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+
Glycosides	Keller-Kiliani	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-
Saponins	Foam	+	+	-	+	+	+	-	-	+	-	+	+	-	+	-
Carbohydrates	Molisch's	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Anthraquinones	Anthraquinones	+	+	-	+	+	+	-	-	+	-	+	+	-	+	-

A: aqueous, **D**: dichloromethane, **E**: Ethyl acetate, **H**: hexane, **Me**: Methanol.

The fundamental phytochemicals present in nearly all plants were flavonoids and alkaloids. They are the most powerful antioxidants and free radical scavengers, defending cells from oxidative damage. In addition, they also have medically important qualities such as antimicrobial, anti-inflammatory, anti-tumor, and anti-diabetic functions (Eleazu *et al.*, 2012; Roy, 2017). Terpenoids display notable pharmacological activities like antiviral, anti-microbial, anti-inflammatory, anticarcinogenic, anti-cholesterol synthesis, anti-malarial, and anti-inflammation properties (Agidew, 2022). The plants selected for phytochemical study appeared to have the potential to both as a source of valuable pharmaceuticals and to enhance the health of consumers. This study, therefore, justifies the medical usage of these plant species.

4.1.2 Quantitative phytochemical analysis

4.1.2.1 Determination of TPC

A gallic acid calibration curve (10 µg/mL to 100 µg/mL) was generated, and the findings were computed using the regression equation ($y = 0.0271x$, $R^2 = 0.9655$).

Table 8: TPC values for various plant extracts

Medicinal Plants	TPC (mgGAE/g)				
	MeOH	Hexane	DCM	EA	Aqueous
B. ciliata	155.83 ± 1.51	128.24 ± 1.22	87.86 ± 2.45	168.24 ± 1.17	172.58 ± 2.37
M. pudica	131.78 ± 1.53	41.45 ± 2.6	66.81 ± 0.54	164.21 ± 1.81	152.01 ± 0.53
P. emblica	171.73 ± 1.22	48.14 ± 2.57	74.78 ± 5.30	172.26 ± 3.61	154.62 ± 3.29

Measurements were taken in triplicates. (n = 3)

The TPC values were represented as milligrams of gallic acid equivalent per gram mass of dry extract (mg GAE/gm).

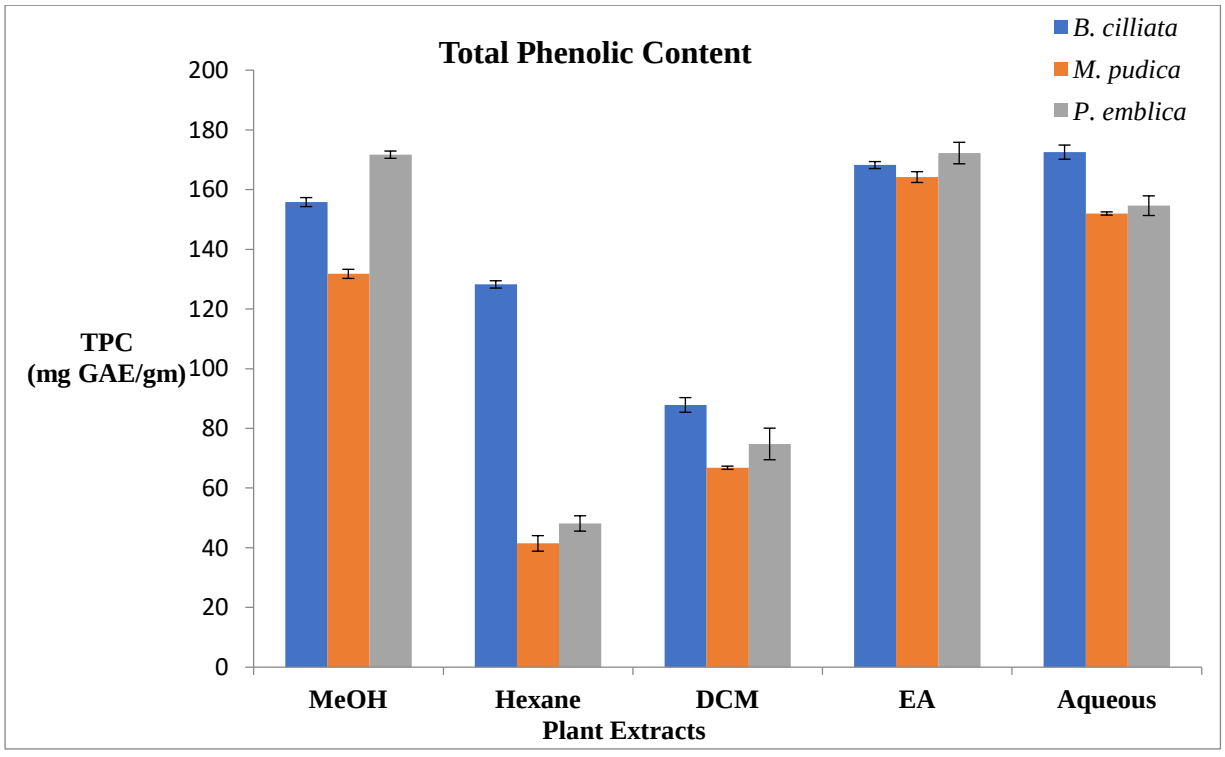


Figure 36: Comparision of TPC for different plant extracts

The TPC (mean ± standard error) for methanolic and various solvent extracts of plant ranges from 172.58 ± 2.37 to 41.45 ± 2.6 mg GAE/gm dry weight. Among all the extracts, the aqueous fraction of the *B. ciliata* extracts had the greatest TPC value (172.58 ± 2.37 mg GAE/gm) while the DCM fraction had the lowest (87.86 ± 2.45 mg GAE/gm). Similarly, in the instance of *M. pudica*, the hexane fraction had the lowest (41.45 ± 2.6 mg GAE/gm) phenolic content while the EA fraction had the greatest (164.21 ± 1.81 mg GAE/gm). EA extract of *P. emblica* exhibited the highest (172.26 ± 3.61 mg GAE/gm) and the hexane fraction showed the lowest (48.14 ± 2.57 mg GAE/gm) TPC **(Table 8).**

The findings demonstrated that the solvents had a significant impact on polyphenol content. More phenolics were present in polar solvent fractions than in non-polar solvent fractions. Methanol and ethanol were found to be the most efficient among the tested solvents and to produce the highest amount of total phenolics detected from grape seeds (Jayaprakasha *et al.,* 2001). In another research, the TPC values in mg GAE/gm were found for the methanolic extracts of *B. ciliata* (145.85 ± 0.15) and *P. emblica* (154.15 ± 0.85) (Sharma *et al.,* 2015). *B. ciliata* (357.079 ± 11.946 mg GAE/gm) and *P. emblica* (250.420 ± 11.540 mg GAE/gm) had greater TPC values in their 70% acetone extracts (Genwali *et al.,* 2013). The TPC of *B. ciliata* was previously reported to be 473.4 ± 15.1 mg GAE/g and 249.7 ± 1.3 mg GAE/g from methanolic and EA fractions respectively (Singh *et al.,* 2017). Contrary to this, our study showed 155.83 ± 1.51 mg GAE/g of TPC from methanol and 168.24 ± 1.17 mg GAE/g of TPC from EA fraction.

Likewise, the TPC findings for methanol, hexane, and EA extracts of *M. pudica* were reported to have 57.431 ± 1.096, 28.523 ± 5.296, and 42.550 ± 2.228 mg GAE/g, respectively, as opposed to our research outcome of 131.78 ± 1.53, 41.45 ± 2.6, and 164.21 ± 1.81 mg GAE/g (Tunna *et al.,* 2015). The investigation carried out on *P. emblica* resulted in 439.9 ± 1.3 mg/g TPC from EA fraction and 62.5 ± 0.7 mg GAE/g TPC from an aqueous fraction (Liu *et al.,* 2008) but in our investigation, the EA and water fraction showed 172.26 ± 3.61 mg GAE/g and 154.62 ± 3.29 mg GAE/g TPC, respectively. In our previous study, the TPC values of methanolic crude extracts of *B. ciliata, P.emblica,* and *M. pudica* measured were 159.43 ± 1.29, 135.52 ± 19.74

and 123.62 ± 8.91 mg GAE/gm, respectively (Sapkota *et al.,* 2021) which are very comparable to the values obtained in this study.

The differences in the content of bioactive phytochemicals may also be due to climate, harvesting time, type (cultivated or wild), storage conditions, type of solvent used, method of extraction, and genetic factors (Klepacka *et al.,* 2011; Zhao *et al.,* 2015) (Klepacka *et al.,* 2011; Liu *et al.*, 2008). The higher TPC levels in EA and aqueous solvent extracts indicated the abundance of polar phenolic compounds in these extracts. Methanolic extracts had a number of phenolic compounds exhibiting high TPC values due to their accumulated effect.

4.1.2.2 Determination of TFC

The TFC values for various fractions of all plant extracts are shown in **Table 9.** Total flavonoid content (TFC) was detected with the help of a calibration curve of standard quercetin ($y = 0.024x + 0.004$, $R^2 = 0.971$) and represented as quercetin equivalent (mg QE/gm dry mass of the extract). The TFC (mean ± standard error) ranges from 5.86 ± 0.55 mg to 64.89 ± 4.70 mg QE/gm dry mass. The EA extract of *M. pudica* showed the highest 64.89 ± 4.70 mg QE/gm TFC while the DCM extract of *P. emblica* exhibited the lowest 5.86 ± 0.55 mg QE/gm TFC. The DCM fractions of all the extracts showed the lowest flavonoid contents.

Table 9: TFC values for various fractions of plant extracts

Medicinal Plants	**Total flavonoid Contant (mg QE/g)**				
	MeOH	**Hexane**	**DCM**	**EA**	**Aqueous**
B. ciliata	47.26 ± 1.21	26.25 ± 1.63	11.28 ± 0.10	28.49 ± 0.67	19.60 ± 3.10
M. pudica	20.11 ± 0.75	11.77 ± 1.18	10.64 ± 0.32	64.89 ± 4.70	16.46 ± 2.42
P. emblica	13.12 ± 0.29	18.07 ± 0.52	5.86 ± 0.55	48.04 ± 0.91	20.98 ± 3.36

Measurements were taken in triplicates. (n = 3)

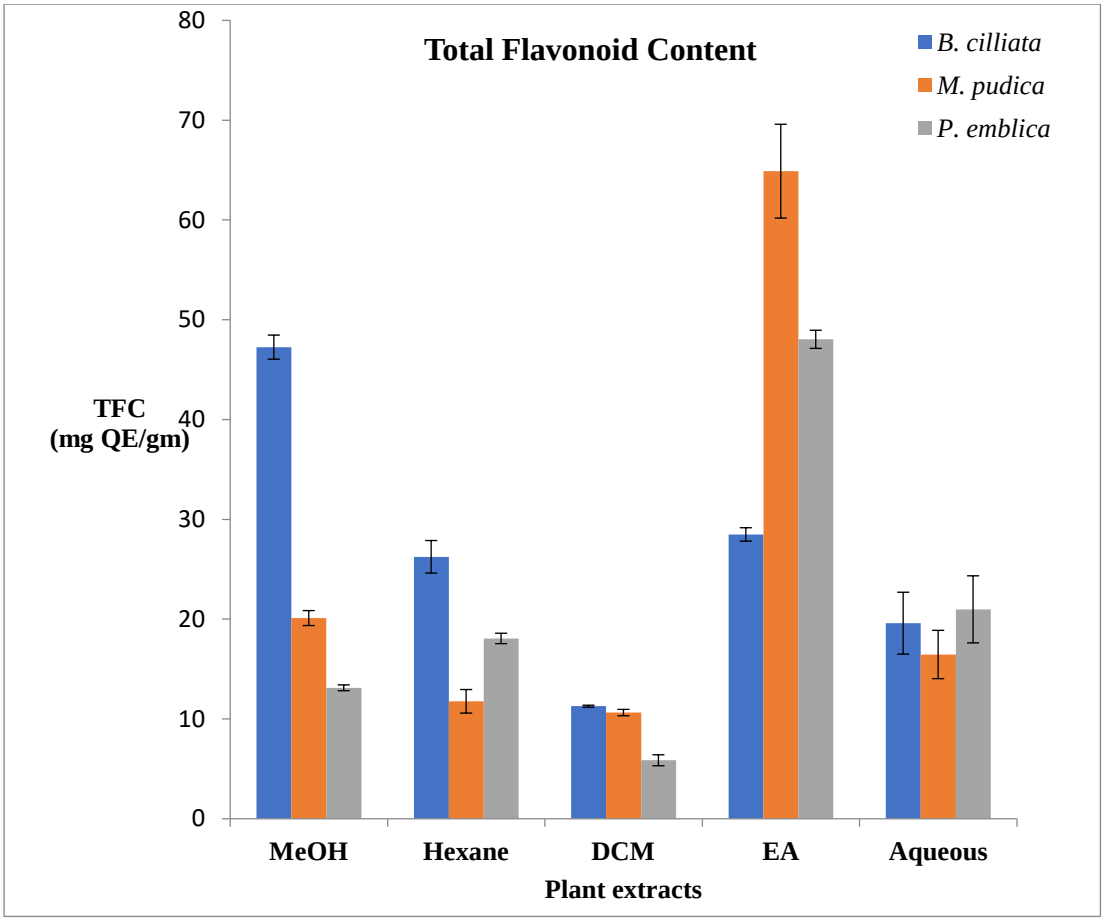


Figure 37: Comparision of TFC for different plant extracts

Our results revealed that in the cases of *M. pudica* and *P. emblica*, the EA fractions had the highest content of flavonoids whereas in *B. ciliata*, the crude methanolic extract showed high TFC. The research has shown that the majority of the flavonoids and phenolic compounds are efficiently extracted during the extraction and fractionation procedures using semi-polar solvents; EA, acetone, and more polar solvents like methanol (Umar *et al.,* 2010). The concentration and the nature of the solvent and the flavonoids, the pH, the extraction temperature and duration, the pressure, the material's sizes, and the harvesting time of the plant are other factors that may vary the TFC in the plant extracts (Juntachote *et al.,* 2006). The crude methanolic fraction of *B. ciliata* showed the greatest value of TFC which is attributed to the synergistic effect of the presence of various constituents in the crude methanolic extract.

A prior study on *B. ciliata* reported TFC of 89.9 ± 0.1 mg QE/g from methanol and 208.4 ± 0.6 mg QE/g from EA as compared to 47.26 ± 1.21 mg QE/g from methanol

and 28.49 ± 0.67 mg QE/g from EA fractions in our investigation (Singh *et al.,* 2017)**.** Likewise, *M. pudica* was reported to have TFC values of 16.97 ± 1.472, 0.927 ± 0.461, and 3.90 ± 0.059 mg QE/g, for methanol, hexane, and EA extract, respectively, (Tunna *et al.,* 2015) whereas our results revealed TFC of 20.11 ± 0.75, 11.77 ± 1.18, and 64.89 ± 4.70 mg QE/g. The flavonoid concentration of methanolic fruit extracts of *P. emblica* from various parts of China ranged from 38.7 mg QE/g to 20.3 mg QE/g. (Liu *et al.,* 2008) The Chutney made from *P. emblica* mostly used in Nepal was found to have TFC ranging from 153.47 to 39.67 mg QE/g (KC *et al.,* 2020). In another investigation, the TFC values reported for EA, butanol, and aqueous extract of *P. emblica* fruit were found 215.45 ± 16.5 mg QE/g, 20.45 ± 1.5 mg QE/g and 127.67 ± 8.64 mg QE/g respectively (Jhaumeer *et al.,* 2018).

4.2 *In vitro* assays

4.2.1 DPPH assay for antioxidant activity

The antioxidant efficacies of methanolic extracts and various solvent fractions were measured via DPPH assay. Before determining the IC_{50} (the lowest concentration of antioxidant needed to scavenge 50% of the DPPH) the percentage of scavenging activity was first calculated for each fraction. The results were compared with standard quercetin with an IC_{50} value of 2.86 ± 0.51μg/mL. In comparison with standard quercetin, EA, crude, and aqueous fractions showed strong radical scavenging activity. Moreover, the results revealed moderate to strong IC_{50} values of *M. pudica* varying from 11.98 ± 0.36 μg/mL in EA to 141.53 ± 10.73 μg/mL in hexane fraction **(Table 10).**

Phenolic and flavonoid molecules present within the extract have a significant effect on the ability to scavenge free radicals that depends upon the number and location of hydroxyl groups to provide hydrogen atoms or electrons to free radicals (Amarowicz *et al.,* 2004). Due to their redox characteristics, which include functioning as hydrogen atom donors or reducing agents, phenolics, and flavonoids both contribute appreciably to antioxidant activity (Keshari *et al.,* 2016). The DCM fractions were determined with comparatively lower activity. The IC_{50} values of crude and various solvent extracts are represented in tabulated form with the standard quercetin in **Table 10**.

Table 10: Antioxidant activity of various fractions of selected plants

Medicinal plants	DPPH radical scavenging (IC_{50} values in µg/mL)				
	MeOH	**Hexane**	**DCM**	**EA**	**Aqueous**
B. ciliata	52.60 ± 3.63	60.24 ± 2.19	108.20 ± 2.73	18.42 ± 1.29	16.99 ± 2.56
M. pudica	34.35 ± 5.11	81.28 ± 8.23	118.10 ± 0.76	21.39 ± 3.76	95.06 ± 3.03
P. emblica	22.36 ± 1.95	141.53 ± 10.73	88.85 ± 10.59	11.98 ± 0.36	22.34 ± 2.71
Quercetin (Control)	2.86 ± 0.51				

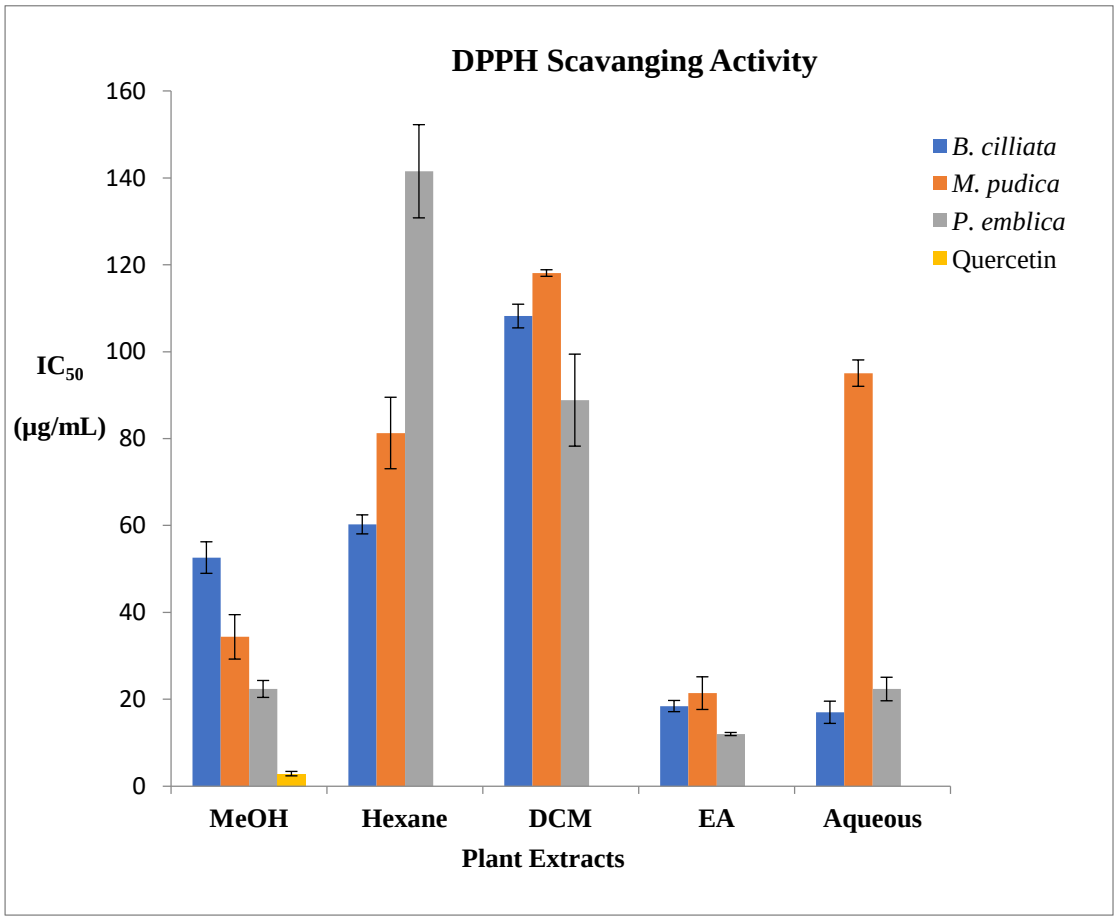


Figure 38: Comparision of IC_{50} values for an antioxidant activity for different extracts

According to IC_{50} values from our study the antioxidant activities of *M. pudica* 34.35 ± 5.11 μg/mL (methanol), 81.28 ± 8.23 μg/mL(hexane), and 21.39± 3.76 μg/mL (EA), which were also found to be 92.302± 0.0077, 7.18± 0.0005, and 49.59 ± 0.0024 μg/mL, respectively (Tunna *et al.,* 2015). In our previous work, the antioxidant activity of methanolic extract of *M.pudica, B. ciliata,* and *P.emblica* was reported to have 26.5 ± 1.1, 23.7 ± 0.4 and 13.2 ± 0.1 μg/mL respectively (Sapkota *et al.,* 2021).

The antioxidant activity of flavonoids was found to depend on the number, position, and configuration of hydroxyl groups in their structures. In our study, most of the flavonoids in the EA fraction of the plant extracts contained catechol moiety with a number of -OH groups in their structures which is crucial for high antioxidant activity. The hydroxylations in ring A and B (particularly in 4′ and 5' position) was found to enhance the antioxidant activity whereas methylation and acetylation of phenolic groups at positions C-5, C-7, and C-8 were found to decrease the antioxidant activity (Sarian *et al.,* 2017).

In our investigation on *P. emblica,* the EA fraction (11.98 ± 0.36 μg/mL) showed the maximum DPPH scavenging activity and almost similar activity of crude (22.36 ± 1.95 μg/mL) and aqueous (22.34 ± 2.71 μg/mL) fractions. Following our study, the IC_{50} of EA and aqueous extracts were found to be 12.6 μg/mL and 142.6 ± 5.3 μg/mL, respectively (Liu, *et al.,* 2008). The presence of phenolic compounds in *P. emblica* fruit is responsible for its antioxidant potential. (Kumar *et al.,* 2006). The lower values of IC_{50} and higher contents of phenolics in these extracts indicate the presence of higher concentrations of phytochemicals responsible for DPPH scavenging activity. Higher antioxidant activity in EA fractions of the plant extract was probably attributed due to the abundance of the polar antioxidant compounds in this fraction. The methanolic solvent pulls out the wide range of compounds to show higher scavanging activity in crude (methanolic) extract due to synergistic effect of various compounds present.

4.2.3 *α*-amylase inhibition activity

The *α*-amylase inhibitory activity exhibited by various plant extracts and standard acarbose is shown in **Table 11**. The EA fraction displayed significant anti-*α*-amylase activity following our study (Afham *et al.,* 2022; Bhandari *et al.,* 2008; Fauzi *et al.,* 2018; Tunna *et al.,* 2015). The IC_{50} of the standard compound acarbose was found to

be 3.13 ± 0.14 µg/mL. The EA extract of *B. ciliata* demonstrated the strongest *α*-amylase inhibition, with an IC_{50} of 38.50 ± 1.32 µg/mL. Apart from DCM, other *B. ciliata* fractions, such as methanol (IC_{50} = 59.68 ± 0.69 µg/mL), hexane (IC_{50} = 50.84 ± 2.17 µg/mL), and aqueous (IC_{50} = 74.26 ± 1.66 µg/mL), also shown excellent potential for this enzyme. The extracts from *P. emblica* (except for the EA fraction) showed lower than 50% inhibition. Throughout the assay, all of the plant's DCM fractions demonstrated lower enzyme inhibition.

By mitigating the progressive deterioration of the function of pancreatic β-cell brought on by oxidative stress, compounds like (-)-3-O-galloylcatechin and (-)-3-O-galloylepicatechin, which were isolated from the EA fraction of *B. ciliata*, function as a porcine pancreatic α-amylase inhibitor (Tunna *et al.*, 2015). Condensed and hydrolyzed tannins have been implicated in the regulation of postprandial blood glucose levels in diabetes through the inhibition of pancreatic and salivary *α*-amylases as well as starch absorption from the intestine, according to previous *in-vivo* and *in-vitro* research (Kato *et al.*, 2017).

Table 11: IC50 values for α-amylase inhibition of various plant extracts and their fractions

Medicinal plants	**IC_{50} (µg/mL)**				
	MeOH	**Hexane**	**DCM**	**EA**	**Aqueous**
B. ciliate	59.68 ± 0.69	50.84 ± 2.17	11.10 ± 0.3 3%*	38.50 ±1.32	74.26 ± 1.66
M. pudica	114.83 ± 5.15	337.60 ± 10.33	29.79 ± 1.09%*	110.90± 1.61	40.54 ± 0.63%*
P. emblica	9.69 ± 1.28 %*	9.08 ± 0.94 %*	3.49 ± 0.86%*	306.20 ± 18.5	8.22 ± 1.13 %*
Acarbose	3.13 ± 0.14 (mg/mL)				

*Percentage inhibition at 500 µg/mL, Measurements were taken in triplicates. (n = 3)

4.2.4 *α*-Glucosidase inhibition activity

All the plant extracts were screened at a 500 µg/mL concentration for both α-amylase enzymes and α-glucosidase for the determination of percentage inhibition. Only those extracts that demonstrated more than 50% inhibition against both enzymes were subjected to further testing to determine their IC_{50} values. The inhibitory activity of various plant extracts along with standard acarbose against *α*-glucosidase is illustrated in **Table 12.** The findings of the *α*-glucosidase assay demonstrated that the various fractions of plant extracts had IC_{50} values ranging from 3.4 ± 0.04 µg/mL to 292.97 ± 0.55 µg/mL and even less than 50% inhibition in DCM fraction indicating strong to moderate inhibitory activity.The variation may be due to the presence of the wide range of inhibitor compounds of diverse nature and activity. All the extracts from *M. pudica* showed good inhibition activity showing the presence of various metabolites with diverse polarity at different levels. Among the various solvent fractions (except *P. emblica*) EA, crude, and water fractions showed strong inhibitory potentials against *α*-glucosidase. Acarbose was utilized as a positive control with IC_{50} of 2.06 ± 0.07 mg/mL for *α*-glucosidase.

Our findings concur with a study that found considerable *α*-glucosidase inhibitory activity in methanol, EA, and aqueous fractions of *M. pudica* (Afham *et al.,* 2022; M Bhandari *et al.,* 2008; Fauzi *et al.,* 2018). With an IC_{50} value of 75.16 ± 0.92 µg/mL, quercetin extracted from the EA fraction of *M. pudica* exhibited *α*-glucosidase activity. By enhancing GLUT4 translocation and activation in 3T3-L1 adipocytes and the PI3K/p-Akt-dependent pathway, gallic acid lowers blood sugar levels (Afham *et al.,* 2022; Gandhi *et al.,* 2014). It also acts as an insulin secretagogue and prevents the death of pancreatic beta cells (Sameermahmood *et al.,* 2010). Similarly, quercetin, the main component present in the fruits of this species, is regarded as a possible anti-diabetic medication because of its activity through the interaction of PPAR-γ and glycogen phosphorylase (Srinivasan *et al.,* 2018).

Table 12: IC_{50} values for α-glucosidase inhibition of various plant extracts and their fractions

Medicinal Plants	IC_{50} (µg/mL)				
	MeOH	**Hexane**	**DCM**	**EA**	**Aqueous**
B. ciliate	26.30 ± 0.56	40.74 ± 1.16	292.97 ± 0.55	3.41 ± 0.04	8.09 ± 0.28
M. pudica	13.50 ± 0.56	14.17 ± 0.26	17.59 ± 0.43%	21.02 ± 0.78	16.62 ± 0.32
P. emblica	80.62 ± 6.45	282.60 ± 4.98	14.79 ± 0.06%	11.48 ± 0.77	70.52 ± 3.65
Acarbose	2.06 ± 0.07 (mg/mL)				

% inhibition at 500 µg/mL, Measurements were taken in triplicates. (n = 3)

4.2.5 Lipase inhibition activity

At various dosages, the lipase inhibitory potential of certain medicinal herbs was investigated. The activity of specific medicinal herbs for lipase inhibition was examined at various doses. Based on the screening results, further dilution of various concentrations was carried out after lipase inhibition was tested at 5 mg/mL. The results demonstrated moderate to low action (IC_{50} values: 0.49 ± 0.02 to 5.37 ± 0.07 mg/L) compared to the IC_{50} value of a positive control orlistat (IC_{50}: 179.70 ±3.60 µg/mL). Methanolic, hexane, and EA fractions demonstrated more activity across all fractions than DCM and aqueous fractions **(Table 13)**.

Table 13: IC_{50} values for pancreatic lipase inhibition of various plant extracts and their fractions

Medicinal plants	IC_{50} (mg/mL)				
	MeOH	**Hexane**	**DCM**	**EA**	**Aqueous**
B. ciliate	1.07 ± 0.03	1.55 ± 0.02	3.11 ± 0.10	2.01 ± 0.08	1.99 ± 0.17
M. pudica	1.33 ± 0.05	0.49 ± 0.02	5.37 ± 0.07	0.82 ± 0.05	1.84 ± 0.09
P. emblica	-	2.45 ± 0.03	4.19 ± 0.09	3.64 ± 0.12	-
Orlistat	179.70 ± 3.60 µg/mL				

Percentage inhibition at 500 µg/mL, Measurements were taken in triplicates. (n = 3)

The plant has several components like saponins, polyphenols, flavonoids, tannins, and terpenes that inhibit lipase enzymes (Garza *et al.,* 2011). These substances, which were mentioned in our prior work, may also be accountable for the anti-lipase activity

of our extracts (Sapkota *et al.,* 2022). Pancreatic lipase was synergistically inhibited by orlistat and kaempferol at relatively small dosages. The results revealed a synergistic effect when the resultant concentrations of orlistat and kaempferol were below 114.60 µM and 30.24 µM; however, kaempferol might largely substitute orlistat to achieve the same antiobesity benefits (Tanemura *et al.,* 2020). The three medications' combination (ECG-EGCG-orlistat) showed strong synergy in preventing pancreatic lipase (George *et al.,* 2020). Ellagic acid, chebulagic acid, and gallic acid each had an IC_{50} value of 90 µg/mL, 57.4 µg/mL, and 5192 µg/mL for inhibiting pancreatic lipase in *P. emblica*, according to a prior study (Patil *et al.,* 2012). As a result of decreased triglyceride buildup and downregulation of adiponectin, FABP4, PPARγ, and cEBPα, respectively, the ethanolic fruit extract of *P. emblica* exhibited anti-lipase action (Balusamy *et al.,* 2020).

4.3 Antimicrobial activity test

The antibacterial efficacy of plant extract fractions against ATCC strains of four bacteria *Escherichia coli* ATCC 2591, *Staphylococcus aureus* ATCC 25923, *Shigella sonnei* ATCC 25931, and *Salmonella typhi* ATCC 14028, were tested. T**able 14** summarizes the antibacterial activity with ZoI.

Table 14: ZoI of different fractions against different test microorganisms

Microorganism	**Zone of inhibition (mm)**																
	B. ciliata					***M. pudica***					***P. emblica***					**Neomycin**	**50% DMSO**
	M	**H**	**D**	**E**	**A**	**M**	**H**	**D**	**E**	**A**	**M**	**H**	**D**	**E**	**A**		
S. aureus	20	13	13	21	20	19	8	-	27	12	18	15	19	28	17	27	-
E. coli	18	13	9	21	18	8	-	-	12	-	-	9	-	11	-	17	-
S. typhi	13	10	9	14	11	12	-	-	17	12	11	7	14	14	8	23	-
S. sonnei	23	15	10	25	22	23	12	-	30	21	23	17	21	28	21	30	-

Note: **A** : Aqueous. **E** : Ethyl Acetate **D** : DCM, **H** : Hexane, and **M** : Methanol

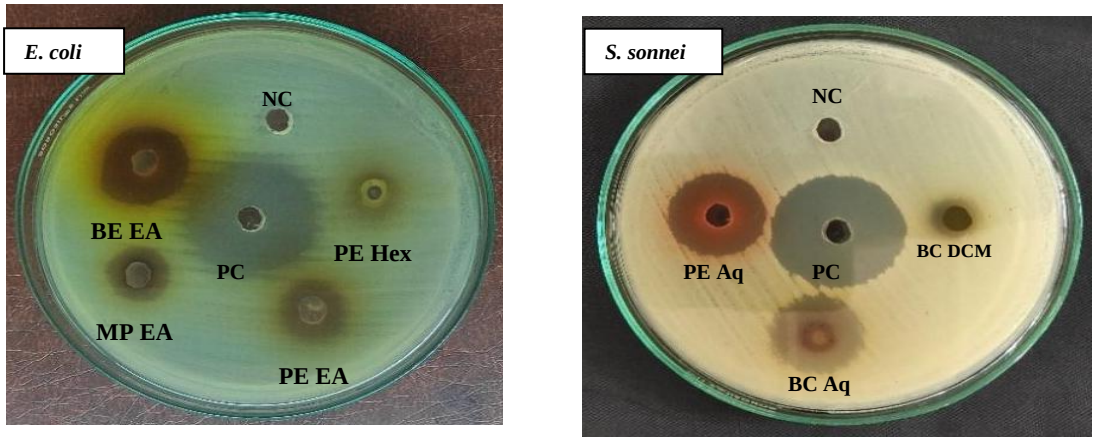
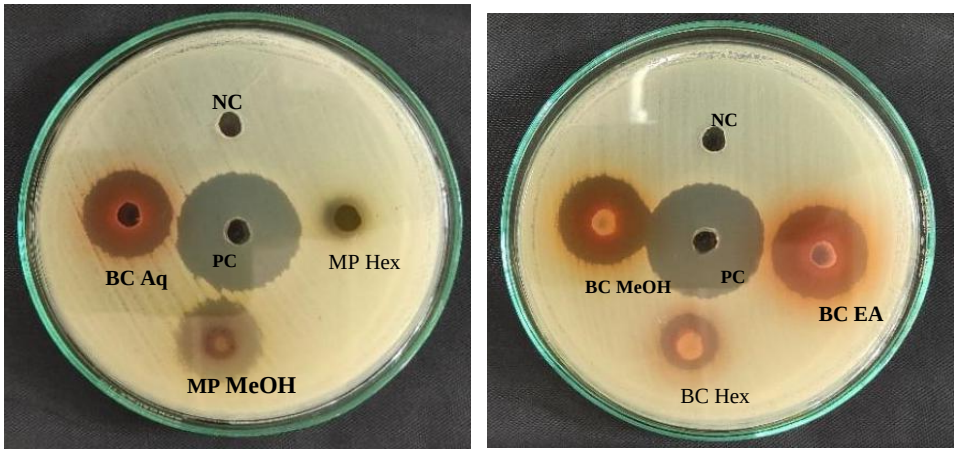


Figure 39: Antibacterial tests against *Escherichia coli,* and *Shigella sonnei*



Note: NC = 50% DMSO; PC= Neomycin; BC Aq = *B.cilliata* (Aquous); BC Hex = *B.cilliata* (Hexane); BC MeOH = *B.cilliata* (Methanol); BC EA = *B.cilliata* (EA); MP Hex =*M. pudica* (Hexane) and MP MeOH = *M. pudica* (Methanol)

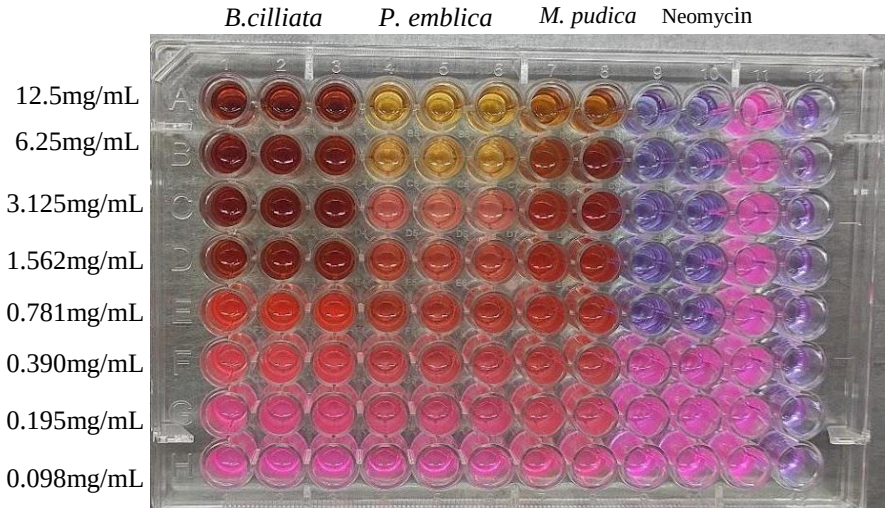
Figure 40*:* Antibacterial activity tests against *Staphylococcus aureus* ATCC 25923

In all tested bacteria, the EA fraction had the highest zone of inhibition. According to earlier reports, the maximum inhibition against *S. aureus* was found in EA extracts of *B. ciliata* (ZoI = 21 mm) and *E. coli*. (ZOI = 11 mm). Another investigation utilizing 50 mg/mL ethanolic extracts discovered that ZoI in antibacterial efficacy against *E. coli*, *S. aureus*, and *S. typhi* was found at 23.7 ± 0.25 mm, 24.0 ± 0.10 mm, and 22.8 ± 0.15 mm respectively (Khan *et al.*, 2014). Using 50 mg/mL methanolic extracts of *M. pudica*, we observed the following ZoI results: *E. coli* (8 mm), *S. aureus* (19 mm),

S. sonnei (23 mm), and *S. typhi* (12 mm). In the previous study, ZoI for the antibacterial activity of *M. pudica* using 5 mg/mL methanolic extract was reported to be *K. pneumoniae* (20 mm), *E. coli* (12 mm), *S. aureus* (15 mm), and *S. typhi* (14.5 mm) (Arokiyaraj *et al.*, 2012).

4.3.1 Determination of minimum inhibitory concentration (MIC)

The EA fraction of selected plants was tested to determine the MIC. The MIC value varies between 1562.5 and 6250 µg/mL. Details about MIC are displayed in **Table 15**



Note: 9, 10 (A-H): Neomycin 250–1.95 µg/mL, 11 (A–H): Media + bacteria, 12 (A–H): Media only (Negative control)

Figure 41: Resazurin microtiter assay to determine MIC against *E. coli*

Table 15: MIC values of EA fractions of plant extracts

Microorganisms	MIC (µg/mL)			
	B. ciliate	***M. pudica***	***P. emblica***	**Neomycin**
S. aureus	1562.5	3125	6250	1.56
E. coli	1562.5	1562.5	6250	15.63
S. typhi	3125	1562.5	3125	1.56
S. sonnei	1562.5	3125	3125	1.56

4.3.2 Determination of minimum bactericidal concentration (MBC)

The EA fraction from all plants had the maximum ZoI against the tested microorganisms which was then subjected to MBC determination. The MBC ranges between 6250 to 12,500 µg/mL. The antibiotic drug neomycin (positive control) had shown significant efficacy against the test microorganisms. Details about MBC are listed in **Table 16.**

Table 16: MBC values of EA fractions of plant extracts

Microorganism	**MBC (µg/mL)**			
	B. ciliata	***M. pudica***	***P. emblica***	**Neomycin**
S. aureus	12,500	12,500	12,500	12.5
E. coli	6250	12,500	12,500	62.5
S. typhi	6250	12,500	12,500	12.5
S. sonnei	12,500	12,500	12,500	6.25

Our observation on the EA fraction of *P. emblica* found the MIC values of 6250 µg/mL and 3125 µg/mL against *S. aureus* and *S. typhi* respectively. Similarly, the MBC values against both *S. aureus* and *S. typhi* was found to be 12500 µg/mL. In contrast to the values, the MIC and MBC determination on methanolic extract of *P. emblica* against *S. aureus* and *S. typhi* showed (50 µg/mL, 65 µg/mL) and (35 µg/mL, 45 µg/mL) respectively (Goud *et al.*, 2008).

Our analysis revealed that the EA extract has the greatest potential for antimicrobial activity, then after the methanolic (crude) extract. The metabolites terpenoids, phenols, flavonoids, tannins, saponins, steroids, and glycosides are all found in methanolic extracts. These phenolic and flavonoid compound's antimicrobial activity is ultimately mediated by a variety of mechanisms, including suppression of nucleic acid synthesis, adhesion, prevention of biofilm development, inhibition of cytoplasmic membrane function, and modification of membrane permeability, all of which result in cell death and pathogenicity attenuation (Cowan, 1999). The EA

fractions possess significant phenolic and flavonoid contents, as previously described, these phytocompounds could develop a synergistic impact on antimicrobial activity.

4.4 LC-HRMS-based molecular annotation

The compounds that have been identified are listed in **Table 17** below, along with information about their theoretical and observed mass-to-charge ratios (*m/z*), molecular formula, absolute errors in parts per million (ppm), and double bond equivalence (DBE), and retention time (Rt) in minutes measured in the positive ion mode of ESI. The chemicals were identified based on the measured mass spectrum, and the results were evaluated by comparing them to data from the literature.

P. emblica EA extracts indicated the occurrence of phenolic compounds such as gallic acid with *m*/*z* = 171.02, methyl gallate with *m*/*z* = 185.05, quercetin, a flavonoid with *m*/*z* = 303.05), an isoflavone irisflorentin with *m*/*z* = 387.1, O-Caffeoylhydroxycitric acid with *m*/*z* = 371.06, galloyl-hexahydroxydiphenoyl (HHDP)-glucose with *m*/*z* = 483.07, 2- 3,4,8,9,10-pentahydroxydibenzo [b,d]pyran-6-one with *m*/*z* = 277.06, prodelphinidin B3 with *m*/*z* = 595.14, isoquercetin with *m*/*z* = 465.1, cassiaoccidentalin B with *m*/*z* = 576.15, kaemferol with *m*/*z* = 287.05, trihydroxy dimethoxy flavone with *m*/*z* = 331.08, aflotaxin B1 with *m*/*z* = 329.06, isorhamnetin with *m*/*z* = 317.06, emodin with *m*/*z* = 271.06, and trigalloyllevoglucosan with *m*/*z* = 619.09. Also, *P. emblica* extracts with [M + H^+], *m*/*z* = 185.05, DBE 5, and fragment ions at 170.97 and 127.03 molecular formula $C_8H_8O_5$, is annotated as methyl gallate (Abu-Reidah *et al*., 2015).

Table 17: List of Secondary metabolites detected in *B. ciliata*, *P. emblica*, and *M. pudica* using LC-HRMS

Annotated compounds	Calculated mass	Observed mass (*m/z*)	Formula	DBE	Absolute error (ppm)	Rt (min)	Fragment peak	Source	Reference
Bergenin	328.08	329.08	$C_{14}H_{16}O_9$	7.0	2.84	11.20	314.78;251.05; 237.07; 194.40	*B. ciliata*	(Li *et al.,* 2013)
Afzelechin	274.08	275.08	$C_{15}H_{14}O_5$	9.0	0.29	13.28	257.17, 233.08	*B. ciliata*	(D. Zhang *et al.,* 2020)
Epiafzelechin	274.08	275.08	$C_{15}H_{14}O_5$	9.0	0.29	13.28	257.17, 233.08	*B. ciliata*	(Brito *et al.,* 2014; Y. Lin *et al.,* 2012; Mittal *et al.*,)
Orientin	448.10	449.10	$C_{21}H_{20}O_{11}$	12.0	3.18	16.34	329.36; 299.30	*B. ciliata*	(J. Wang *et al.,* 2012)
Catechin	290.07	291.08	$C_{15}H_{14}O_6$	9	1.25	12.22	313.07 $[M+Na]^+$, and 139.03	*M. pudica*	(Ibrahim *et al.,* 2019; Shen *et al.,* 2006)
Epicatechin	290.07	291.08	$C_{15}H_{14}O_6$	9	1.25	12.22	313.07 $[M+Na]^+$, and 139.03	*M. pudica*	(Ibrahim *et al.,* 2019; Shen *et al.,* 2006; Shi *et al.,* 2016)
Trihydroxydimethoxyflavone	330.07	331.08	$C_{17}H_{14}0_7$	11	0 .90	15.83	301.08, and 315.09	*B. ciliata*	(F. Zhang *et al.,* 2020b)
Gallocatechin	306.07	307.08	$C_{15}H_{14}O_7$	9	0.77	10.16	329.07 $[M+Na]^+$, 289.07, 139.03	*M. pudica*	(Shen *et al.,* 2006b)
Epigallocatechin	306.07	307.08	$C_{15}H_{14}O_7$	9	2.26	7.15	329.07 $[M+Na]^+$, 289.07, 139.03	*M. pudica*	(Shen *et al.,* 2006; Shi *et al.,* 2016; Yuan *et al.,* 2014)
Procyanidin B1	578.15	579.15	$C_{30}H_{26}O_{12}$	18	0.01	11.82	427.10 [M + H -152] +, 289.07 (kampferol)	*M. pudica*	(Friedrich *et al.,* 2000; Shen *et al.,* 2006)

Procyanidin B3	578.15	579.15	$C_{30}H_{26}O_{12}$	18	0.01	11.82	427.10 [M + H -152] +, 289.07 (kampferol)	*M. pudica*	(Klausen *et al.*, 2010; Shen *et al.*, 2006)
Chlorogenic acid	354.09	355.10	$C_{16}H_{18}O_9$	8.0	0.68	11.97	193.02	*M. pudica*	(Ijaz *et al.*, 2019; Ncube *et al.*, 2014)
Vitexin	432.11	433.11	$C_{21}H_{20}O_{10}$	12.0	1.81	14.30	343.04; 313.07; 285.14	*M. pudica*	(J. Zhang *et al.*, 2011)
Myricetin	318.03	319.04	$C_{15}H_{10}O_8$	11	4.58	14.51	181.05; 153.01	*M. pudica*	(Cai *et al.*, 2016; Saldanha *et al.*, 2013)
Isoquercetin	464.09	465.1	$C_{21}H_{20}O_{12}$	12	3.59	14.72	303.05 (Quercetin), 289.07 (Kampferol)	*P. emblica*	(W. Liu *et al.*, 2021)
Prodelphinidin B3	594.13	595.14	$C_{30}H_{26}O_{13}$	18	3.23	14.79	427.08, 169.07, 291.09, 305.07	*P. emblica*	(Friedrich *et al.*, 2000; Pinto *et al.*, 2021)
Cassiaoccidentalin B	576.15	577.15	$C_{27}H_{28}O_{14}$	14.0	3.66	15.33	-	*P. emblica*	(A. Lobstein *et al.*, 2002)
Aflotaxin B1	328.06	329.06	$C_{17}H_{12}O_7$	12.0	0.18	16.20	-	*P. emblica*	(Hernandez *et al.*, 2021)
Kaempferol	286.04	287.05	$C_{15}H_{10}O_6$	11	0.90	18.33	259.13, 165.09, 153.12	*P. emblica*	(March & Miao, 2004)
Emodin	270.05	271.06	$C_{15}H_{10}O_5$	11	1.73	19.28	253.16, 243.17, 229.14, 225.13 and 197.08	*P. emblica*	(Zhan *et al.*, 2016)
Isorhamnetin	316.05	317.06	$C_{16}H_{12}O_7$	11	3.86	18.72	303.21, 274.20, 153.12	*P. emblica*	(Chen *et al.*, 2015)
Methyl gallate	184.04	185.05	$C_8H_8O_5$	5.0	2.38	12.43	170.97; 127.03	*P. emblica*	(J. Zhang *et al.*, 2011)
Quercetin	302.04	303.05	$C_{15}H_{10}O_7$	11.0	4.82	15.28	273.12, 257.13	*P. emblica*	(Scigelova *et al.*, 2011)

Irisflorentin	386.09	387.1	$C_{20}H_{18}O_8$	12	1.59	11.09	357.09 [M + H - CH_3×2] $^+$, 372.07 [M + H - CH3] $^+$	*P. emblica*	(Roger *et al.*, 2012; Tamfu *et al.*, 2021; Y.-Y. Zhang *et al.*, 2011)
Gallic acid	170.02	171.02	$C_7H_6O_5$	5.0	0.62	7.30	127.03 [M+H-CO_2] $^+$	*P. emblica*	(Sawant *et al.*, 2010; M. Singh *et al.*, 2017)
HHDP-glglucose	482.07	483.07	$C_{20}H_{18}O_{14}$	12.0	2.12	12.07	251.21; 277.03; 303.20;	*P. emblica*	(M. Singh *et al.*, 2017)
2-O-Caffeoylhydroxy citric acid	370.05	371.06	$C_{15}H_{14}O_{11}$	9.0	3.23	9.30	-	*P. emblica*	(M. Wu *et al.*, 2022)
3,4,8,9,10-Penta hydroxydibenzo[b,d]pyran6-one	276.04	277.06	$C_{13}H_8O_7$	10.0	1.03	13.54	-	*P. emblica*	(J. Zhang *et al.*, 2011b)
Trigalloyllevoglucosan IX	618.09	619.09	$C_{20}H_{26}O_{22}$	8.0	4.33	13.76	-	*P. emblica*	(Abu-Reidah *et al.*, 2015)

Base peak *m/z* 303.05, molecular formula $C_{15}H_{10}O_{7,}$ DBE 11, and fragment ions at 257.13 and 273.12 and caused by the loss of $[CO + H_2O]^+$ and $[Y\text{-}CHO]^+$ is considered as quercetin (Scigelova *et al.,* 2011). The base peak (*m/z* 387) with the fragment ions at 357.09 $[M + H - CH_3 \times 2]^+$, 372.07 $[M + H - CH_3]^+$ estimated to be irisflorentin (Roger *et al.,* 2012). Additionally, gallic acid, having the chemical formula $C_7H_6O_5$ (base peak m/z 171.02), DBE 5, and the fragment ions peak $[M + H\text{-}CO_2]^+$ at 127.03 is another annotated molecule (M. Singh *et al.,* 2017). Moreover, $[M + H]^+$ (*m/z* 371.06) with chemical formula $C_{15}H_{14}O_{11,}$ and DBE 9 is supposed to be 2-O-Caffeoylhydroxycitric acid (Wu *et al.,* 2022). Base peak *m/z* 277.06, DBE 10 with the molecular formula $C_{13}H_8O_{7,}$ annotated as 3,4,8,9,10-pentahydroxydibenzo [b, d] pyran-6-one (Singh *et al.,* 2016). Similarly, molecular formula $C_{20}H_{26}O_{22}$, the base peak with *m/z* 619.09, and DBE 8 are annotated as trigalloyllevoglucosan IX (Abu-Reidah *et al.,* 2015).

The phytochemicals catechin/epicatechin with *m/z* = 291.08, procyanidin B1/ procyanidin B3 with *m/z* = 578.15, gallocatechin/epigallocatechin with *m/z* = 307.8, vitexin with *m/z* = 433.11, chlorogenic acid with *m/z* = 355.10, and myricetin with *m/z* = 319.04 were present in the ethyl extract of *M. pudica*. Furthermore, afzelechin/epiafzelechin (*m/z* = 275.08), bergenin (*m/z* = 329.08), diosmetin (*m/z* = 301.07) and orientin (*m/z* = 449.10) were found in the *B. ciliata* fraction.

The compounds found in the *M. pudica* extract with the molecular formula $C_{15}H_{14}O_{6,}$ base peak (*m/z* 291.08), DBE 9, and peaks of the fragment at 313.07 $[M + Na]^+$ and 139.03 could be catechin/epicatechin (Shi *et al.,* 2016). Gallocatechin and epigallocatechin were correlated and analyzed based on fragment peaks at $[M + Na]^+$ 329.07, 139.03, and 289.07 (Shen *et al.,* 2006; Zhang *et al.,* 2020). Other annotated compounds with characteristic fragment ions are kaempferol 427.10 $[M + H - 152]$, procyanidin B1/procyanidin B3 with molecular formula $C_{30}H_{26}O_{12,}$ 289.07 and base peak $[M + H]^+$ at *m/z* 579.15, and DBE 18 (Friedrich *et al.,* 2000; Shen *et al.,* 2006). The molecular ion (*m/z* 355.10) with molecular formula $C_{16}H_{18}O_{9,}$ DBE 8, and fragment ions at 193.02 is annotated as chlorogenic acid (Ijaz *et al.,* 2019; Klausen *et al.,* 2010). The base peak (*m/z* 433.11) with the molecular formula $C_{21}H_{20}O_{10,}$ and DBE 12 with fragment ion peaks of 313.07; 343.04; and 285.14 is considered as vitexin. As a result of the hexose unit's crosswise cleavage and the loss of $C_3H_6O_3$ (90

Da) and $C_4H_8O_4$ (120 Da) from the [M + H] + ion, respectively, the fragment peaks with *m/z* 343.04 and 313.07 were formed. The loss of CO (28 Da) from the ion at *m/z* 313.07 resulted in the production of the product ions at *m/z* 285.14 (Monica *et al.,* 2020). The fragments peak at 181.05 and 153.01 and the base peak at *m*/*z* 319.04 with DBE 11, and the chemical formula $C_{15}H_{10}O_8$ is assigned as myricetin (Lin *et al.,* 2012).

Among the characteristic phytochemicals in *B. ciliata* bergenin, *m*/*z* =329.08, orientin, *m*/*z* = 449.10, afzelechin/epiafzelechin, *m*/*z* = 275.08, and diosmetin, *m*/*z* = 301.07 were identified. In a previous study, the compound bergenin was annotated in the *B. ciliata* EA extract with the base peak at *m*/*z* 329.08, fragment peaks at 194.40, 237.07, 251.05, and 314.78, DBE 7, and molecular formula $C_{14}H_{16}O_9$ (Li *et al.,* 2013). Similarly, *m/z* 275.08 (base peak), chemical formula $C_{15}H_{14}O_5$, DBE 9, the fragment peaks at 257.17 (water loss) and 233.08 are thought to be afzelechin/epiafzelechin. The compound with the *m/z* 449.10 base peak, DBE 12, the chemical formula $C_{21}H_{20}O_{11}$, and the fragment ions $[M + H\text{-}H_2O]^+$ peaks at 431.12 correlating to the losses of the water molecule between the sugar's 2″-hydroxyl and the aglycone's 5 or 7-hydroxyl group. Additionally, the base peak at *m*/*z* = 301.07, DBE 11 molecular formula $C_{16}H_{12}O_6$, and with similar fragment ions peaks at 258.12, 153.31, and 149.09 is under the molecule diosmetin (Wang *et al.,* 2012).

4.5 Molecular docking

The molecular docking of the compounds annotated from the plant extracts of three medicinal plants *M. pudica, P. emblica, and B. ciliata* with the digestive enzymes *α*-glucosidase, *α*-amylase, and lipase was performed to find a compound that inhibits these diabetic proteins.

These annotated compounds showed binding affinity in the range of -5.9 to - 8.6 kcal/mol, -6.2 to -9.6 kcal/mol, and -5.9 to -9.8 kcal/mol for glucosidase, lipase, and amylase respectively as depicted in **Table 18**. The compounds cassiaoccidentalin B, procyanidin B1**,** procyanidin B3, and prodelphinidin B3 showed the maximum binding affinity of -8.6, -8.4, -8.5, and -8.5 kcal/mol towards glucosidase. Although procyanidin B3 was absent in the extracts of *P. emblica* and *B. ciliata*, it was present in the EA extract of *M. pudica* and showed good binding affinity when docked with

all three of the target enzymes which was supported by TPC (164.21 ± 1.81 mg GAE/g), TFC (64.89 ± 4.70 mg QE/g) and DPPH scavenging activity (IC_{50}: 21.39 ± 3.76 µg/mL). The catechol system in the B ring of these flavonoids contains the electron cloud that may enable hydrogen atoms to create hydrogen bonds with the enzymes' active site residue, which is essential for inhibiting the activity of the enzyme (Sarian *et al.*, 2017).

Table 18: The binding energy (in kcal/mol) of commercial drugs (taken as standard) and the annotated compounds with various digestive enzymes.

S.N.	Compounds	*α*-Glucosidase (5NN8)	Lipase (1LPB)	*α*-Amylase (1HNY)
1.	3,4,8,9,10-Pentahydroxydibenzo [b, d]pyran-6-one	-7.8	-8.8	-7.5
2.	Afzelechin	-6.8	-8.8	-8.2
3.	Bergenin	-7.3	-8.4	7.1
4.	caffenoylhydroxycitric acid	-6.3	-7.9	-7.4
5.	cassiaoccidentalin B	-8.6	-8.5	-8.6
6.	Catechin	-6.7	-9.2	-8.6
7.	Chlorogenic acid	-7.2	-8.6	-7.7
8.	Diosmetin	-7.0	-9.7	-7.7
9.	Emodin	-7.8	-9.8	-8.2
10.	Epiafzelechin	-6.5	-9.2	-8.3
11.	Epicatechin	-6.8	-9.3	-8.6
12.	Epigallocatechin	-7.2	-9.0	-8.6
13.	Gallic acid	-5.9	-6.1	-5.9
14.	Gallocatechin	-7.2	-9.0	-8.5
15.	Irisflorentin	-6.1	-8.4	-7.4
16.	Isoquercetin	-7.3	-8.9	-8.3
17.	Isorhamnetin	-6.8	-9.3	-8.6

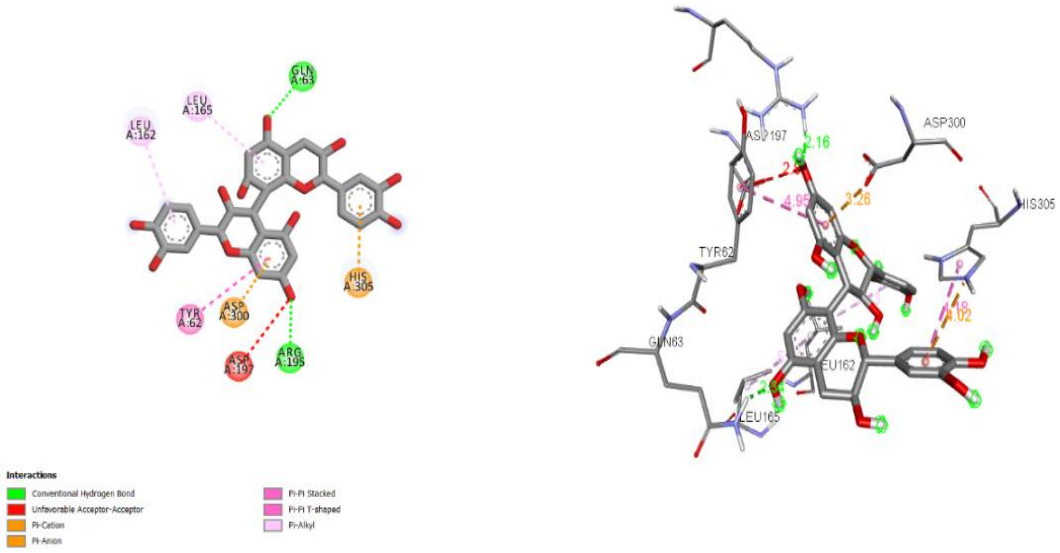
18.	Kaempferol	-6.8	-9.5	-8.4
19.	Methyl gallate	-5.9	-6.2	-5.9
20.	Myricetin	-7.1	-9.5	-8.7
21.	Orientin	-7.6	-8.5	-8.4
22.	Procyanidin B1	-8.4	-8.7	-9.8
23.	Procyanidin B3	-8.5	-9.1	-9.5
24.	Prodelphinidin B3	-8.5	-8.5	-9.3
25.	Quercetin	-6.9	-9.8	-8.6
26.	Vitexin	-7.5	-8.6	-8.5
27.	Acarbose	-7.1	-	-7.2
28.	Orlistat	-	-6.7	-

Molecular docking of annotated compounds with lipase indicates that the quercetin, emodin, procyanidin B3, myricetin, kaempferol, epicatechin, diosmetin, and catechin have a higher binding affinity towards lipase. Among them, quercetin and emodin show similar binding affinity with a binding energy of -9.8 kcal/mol, as well as myricetin and kaempferol with a binding affinity of -9.5 kcal/mol.

The compounds cassiaoccidentalin B, catechin, epicatechin, epigallocatechin, Isorhamnetin, myricetin, procyanidin B1, procyanidin B3, and prodelphinidin B3 have a higher binding affinity towards the enzyme amylase. Among them, cassiaoccidentalin B, catechin, epicatechin, epigallocatechin, and isorhamnetin show similar affinity towards amylase with a binding affinity of -8.6 kcal/mol. While the other compounds like myricetin, procyanidin, procyanidin B3, and prodelphinidin B3 have binding affinity -8.7, -9.8, -9.5, and -9.3 kcal/mol respectively. Among all these compounds procyanidin B3 has the highest binding affinity for all enzymes i.e., α-glucosidase, lipase, and α-amylase with a binding affinity of -8.5, -9.1, and -9.5 kcal/mol.

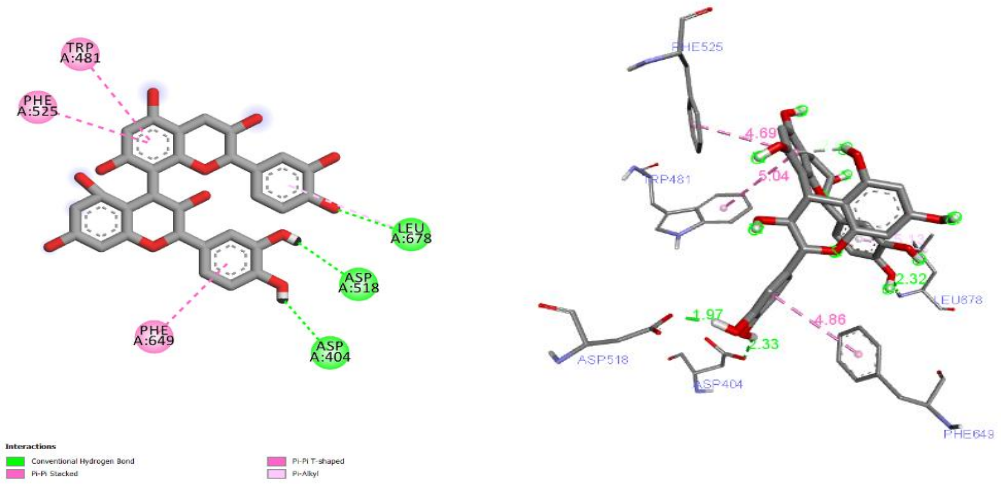
The human α-glucosidase enzyme (PDB ID: 5NN8) of resolution 2.45 Å is an asymmetric monomer with active site residing in the catalytic domain of residue

P123, TRP 376, ASP 282, ASP 404, TRP 481, ILE 441, MET 519ASP 518, ASP 616, TRP 613, ARG 600, GLY 896 PHE 649, TRP 618, HIS 674, and GLU 945. Along with the active site, GLY 123, ASP 91, CYS 127, ALA 93, GLN 124, HIS 432, GLY 435, ARG 437, HIS 742, TRP 126, LEU 756, GLY 434, and GLN 757 are reported to be residues linked to a secondary substrate binding site close to the C-terminal ends of the strands of the catalytic (*β/α*) (Adinortey *et al.*, 2022). On docking with *α*-glucosidase, Procyanidin B3 showed the Hydrogen bond interaction with amino acid residues ASP 518, ASP 404, and LEU 678 with bond lengths 1.97, 2.33, and 2.32 Å respectively. This compound also showed the *π*-alkyl interaction with amino acid residue LEU 678. Amino acid residues PHE 649, TRP 481, and PHE 525 interacted through the *π*-*π* stacked bonds with this compound. As the procyanidin B3 has interacted with the active amino acid, ASP 518 through the H-bond interaction, this could inhibit the glucosidase effectively.



a. 2D- image **b**. 3D- image

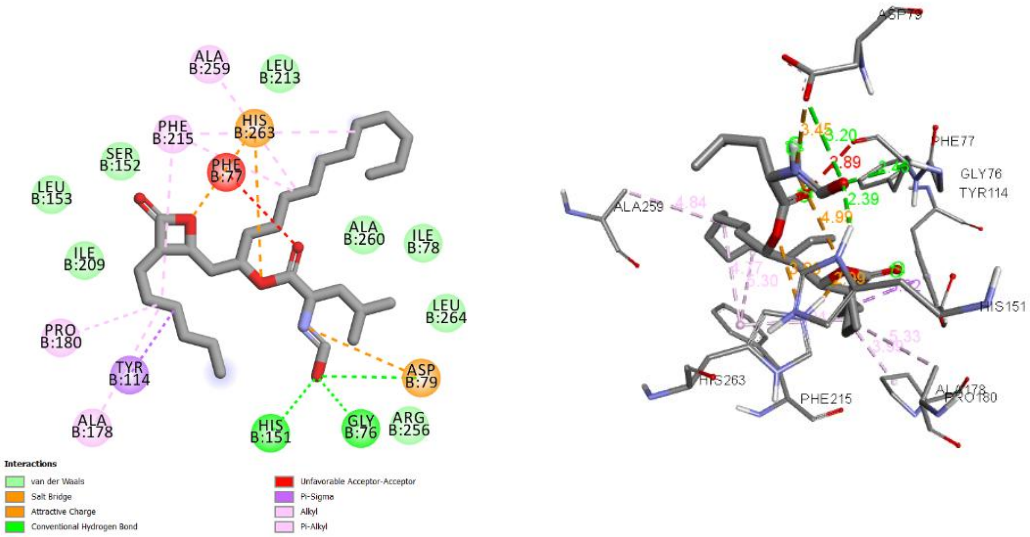
Figure 42: Interaction of acarbose with *α*-glucosidase



a.2D- image **b**. 3D- image

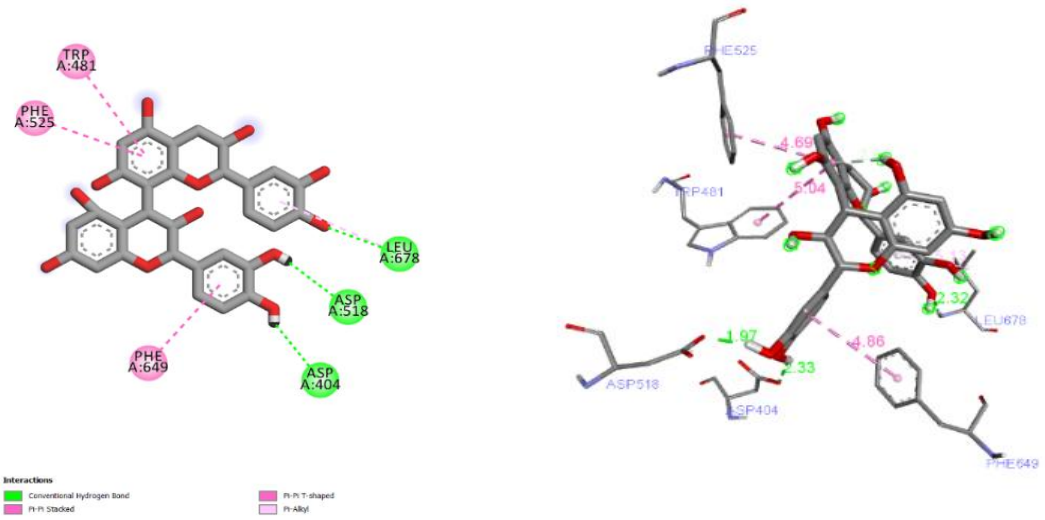
Figure 43: Interaction of procyanidin B3 with *α*-glucosidase

Likewise, human pancreatic lipase (PDB ID: 1LPB) of resolution 2.46 Å has active site residue PHE 77, SER 152, LEU 153, HIS 263, and ASP 176, (Nguyen *et al.*, 2020). Amino acid residues, HIS 263, ASP 205, ALA 259, ASN 212, GLN 244, and LYS 239 of lipase showed the hydrogen bond interaction with the procyanidin B3, while LYS 238, GLU 233 interacted through π-anion bonding. procyanidin B3 also interacted with ALA 260 and PHE 215 through π-alkyl and π-π stacked bonding respectively.



a. 2D- image **b**. 3D- image

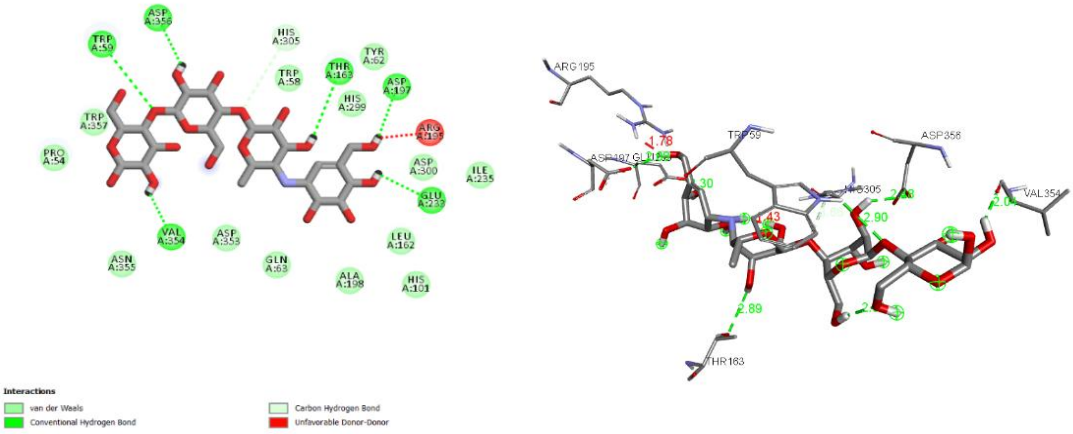
Figure 44: Interaction of orlistat with pancreatic lipase



a. 2D- image **b.** 3D- image

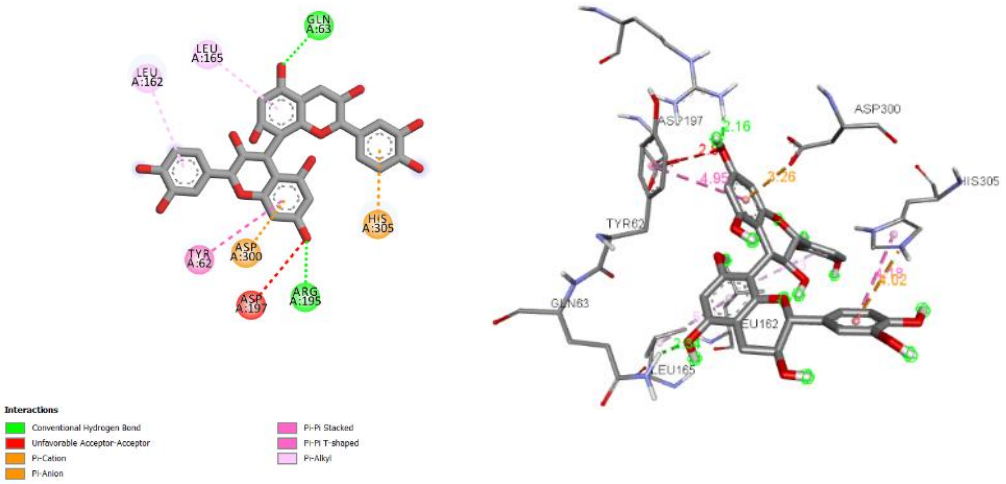
Figure 45: Interaction of procyanidin B3 with pancreatic lipase

Human α-amylase enzyme (PDB ID: 1HNY) has a resolution of 1.80 Å. The amino acid residues ASP 165, ARG 61, LYS 200, ASP 197, and ASP 300 are among those found in the α-amylase active site. In addition, a variety of aromatic and nonpolar residues, including TYR 62, HIS 101, TRP 58, PRO 163, TRP 59, ILE 235, HIS 299, TYR 258, ALA 307, and HIS 305, are present (Bohara *et al.*, 2022). A molecular docking analysis showed that the residues GLN 63, and ARG 195 of *α*-amylase interacted with procyanidin B3 with hydrogen bond, While LEU 162, and LEU 165 showed *π*-*π* stacked interactions. Amino acid residues ASP 300, and HIS 305 interacted with the *π*-anion bonding. Whereas amino acid residue ASP 197 interacted with unfavorable acceptor-acceptor bonding.



a. 2D- image **b.** 3D- image

Figure 46: Interaction of acarbose with *α*-amylase



a. 2D- image **b.** 3D- image

Figure 47: Interaction of procyanidin B3 with α-amylase

The annotated compounds procyanidin B3 interacted with an active amino residue of α-glucosidase and lipase i.e. ASP 518, HIS 263 through hydrogen bonding while it interacted with active amino acid residues i.e, ASP 300, and ASP 197 through π-anion bonding and unfavorable acceptor-acceptor bonding. As this compound has interacted with active amino acid residues of various digestive enzymes through different types of bonds, this could be a potent compound for the treatment of diabetes**.** Based on the results of our study, procyanidin B3 is concluded as one of the effective metabolites to inhibit various enzymes of diabetic targets

CHAPTER 5

5. CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The current study focuses on observable evidence of the inhibitory action of plant metabolites with α-glucosidase, α-amylase, and pancreatic lipase, followed by antimicrobial activities. According to the study, EA fractions of each plant extract showed high antibacterial activity followed by methanolic extract than other fractions and had the highest ZoI against the tested microorganism The EA fractions from all plants were tested to determine the MIC. The MIC value varies between 1562.5 to 6250 μg/mL. The EA fraction of each plant contained the highest value of ZoI against the tested bacteria, and the MBC value was then determined. The MBC ranges between 6250 to 12,500 μg/mL. In the EA fraction, all three plants exhibited substantial inhibitory activity against α-glucosidase, α-amylase, and pancreatic lipase. The presence of several potentially bioactive phytochemicals, as determined by LC-HRMS analysis, accounts for a plant's antidiabetic efficacy.

Additionally, molecular docking research revealed that the various phytochemicals found in these medicinal plants have a strong binding energy for the enzymes of the diabetic target. The compounds cassiaoccidentalin B, procyanidin B1**,** procyanidin B3, and prodelphinidin B3 demonstrated the maximum binding affinity to glucosidase with the binding energy of 8.6, 8.4, 8.5, and 8.5 kcal/mol respectively. The compounds cassiaoccidentalin B, catechin, epicatechin, epigallocatechin, Isorhamnetin, myricetin, procyanidin B1, procyanidin B3, and prodelphinidin B3 have a higher binding affinity towards the enzyme amylase.

Among these compounds, Procyanidin B3 has the highest binding energy i.e., -9.5, -8.5, and -9.1 kcal/mol for α-glucosidase, α-amylase, and lipase, respectively demonstrating it as a potent anti-diabetic phytoconstituent. Procyanidin B3 also engaged in a variety of interactions with the active amino acid residues of these enzymes, thereby enhancing its anti-diabetic effects. Therefore, the findings of our study support the indigenous uses of *M. pudica, B. ciliata,* and *P. emblica* as

medicinal plants as well as the rationale for their potential efficacy as a major inhibitor of lipase, *α*-glucosidase, *α*-amylase, and for the management of diabetes.

5.2 Recommendation

The medicinal plants included in this study have been traditionally used in various herbal formulations as well as by ethnic communities. The time of harvest, the choice of solvent, the temperature, and the altitude at which the plant was grown can affect the quantitative analysis of the chemical components in plants. This study showed that the principal phytochemical components of the plants *B.cilliata*, *M. pudica*, and *P. emblica* include polyphenols, tannins, saponins, glycosides, anthraquinones, and flavonoids. It was discovered via several *in-vitro* investigations that the plant contains beneficial secondary metabolites that are responsible for antioxidant, antibacterial., anti-inflammatory, hypoglycemic, analgesic, and antiobesity activities. Therefore, it is strongly recommended to do further research to extract bioactive chemicals in their pure form from plant material and conduct *in vivo* studies to assess their efficacy.

Additionally, a variety of bioactive substances from the plants' EA extracts have been identified utilizing LC-HRMS technology. According to *in silico* analysis, Procyanidin B1, procyanidin B3, prodelphinidin B3, catechin, epicatechin, epigallocatechin, isorhamnetin, and myricetin have a good binding affinity towards carbohydrate degrading enzymes such as α-amylase and *α*-glucosidase, which may be the cause of the inhibition of carbohydrate degradation. According to molecular docking of annotated chemicals with lipase quercetin, emodin, procyanidin B3, myricetin, kaempferol, epicatechin, diosmetin, and catechin have a greater binding affinity towards lipase. Therefore, the chemicals listed above may be potential therapeutic candidates for the treatment of hyperglycemia. Procyanidin B3 has the strongest binding affinities for the target enzymes, including pancreatic lipase, *α*-glucosidase, and pancreatic *α*-amylase. This compound may be the most effective possible drug candidate for the management of diabetes and obesity, while a more pharmacological study of this compound is still necessary.

CHAPTER 6

6. SUMMARY

This research work has been divided into three parts which include phytochemical analysis, quantitative *in vitro* enzyme inhibition assays, molecular annotation of secondary metabolites from LC-HRMS data, and molecular docking of annotated compounds against enzymes of diabetic targets ie., α-amylase, α-glucosidase, and pancreatic lipase.

The initial part describes the phytochemical evaluation of the variety of the selected plant extracts i.e., *M. pudica*, *P. emblica*, and *B. ciliata*. through the qualitative screening for the identification of various phytoconstituents, quantitative measurement of TPC and TFC, and evaluation of antioxidant activities with their IC_{50} values. There were flavonoids, alkaloids, saponins, tannins, anthocyanins, and glycosides present, according to the phytochemical examination.

This study also includes the antimicrobial evaluation of different extract fractions against four bacteria *E.coli*, *S. typhi*, *S. aureus*, and *S. sonnei*. via the agar well diffusion method. The extracts showed good inhibition against the tested microorganisms with reference to the positive control neomycin. Since the plants have been used for the treatment of obesity and diabetes in traditional practice, the *in vitro* study was carried out to explore the percentage inhibition and IC_{50} values for the inhibition of carbohydrates and lipid hydrolyzing enzymes α-glucosidase, α-amylase, and pancreatic lipase.

Furthermore, the LC-HRMS analysis for the potent EA extracts was conducted to identify the secondary metabolites. Using MestreNova software, the spectra were analyzed for the determination of *m/z* values and molecular masses with the comparison of the literature library. Ultimately, all the annotated compounds were subjected to *in silico* analysis through molecular docking with the target enzymes to investigate the most possible drug candidate compound. The potent bioactive compounds were explored based on various ligand-enzyme interactions like hydrogen bonding, $\sigma - \pi$ interactions, van der Waal's interactions, and binding energy values. Among the various compounds annotated Procyanidin B3 was seen to be the most

potent compound against all three target enzymes which could be employed as a potential drug candidate for the control of hyperglycemia.

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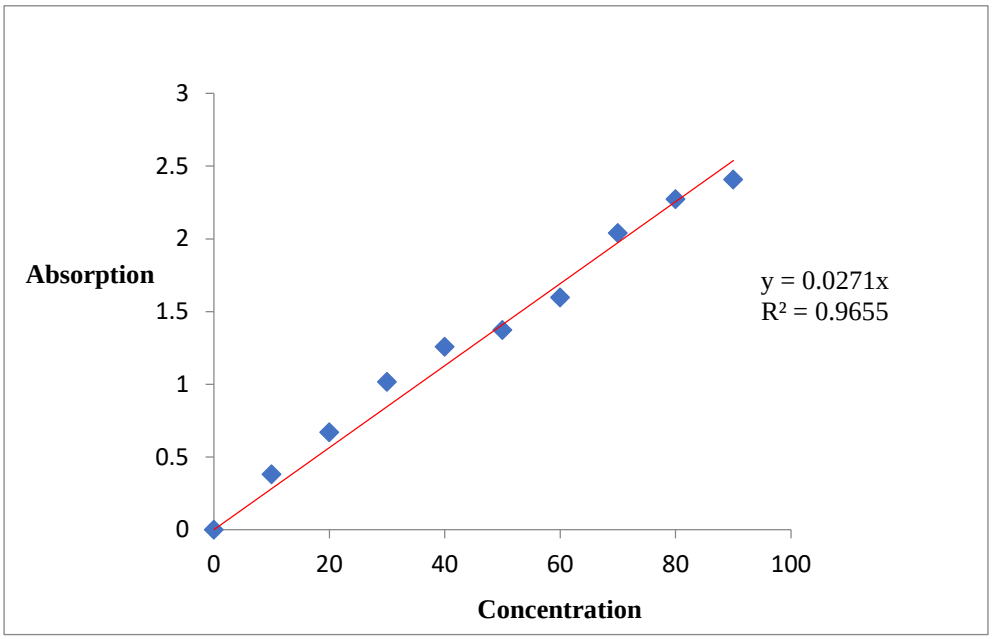
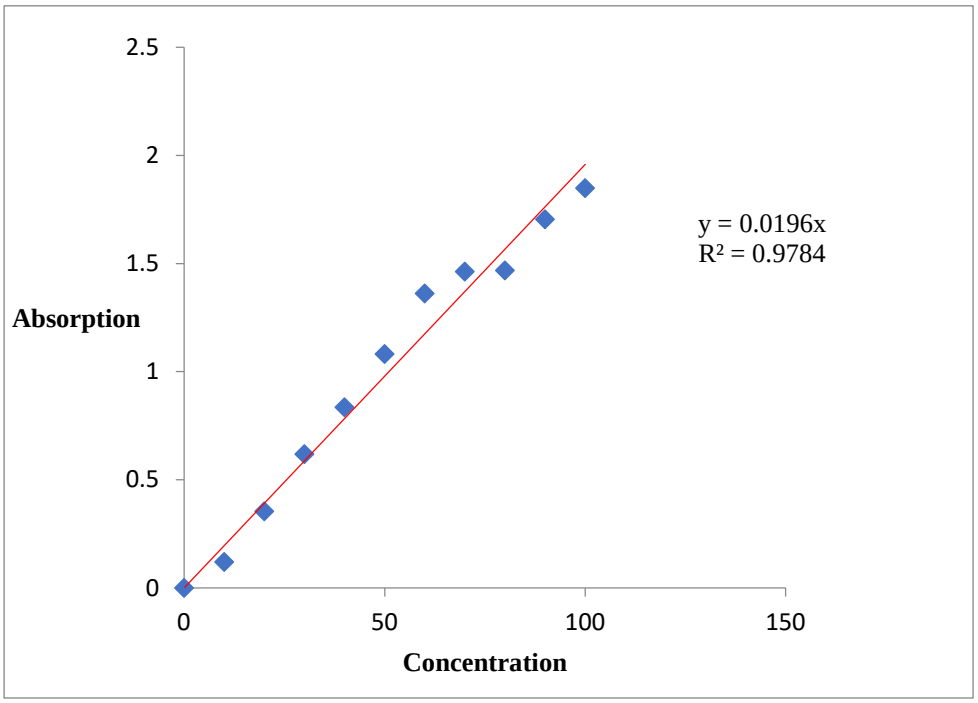
APPENDICES

Appendix A: Calibration curves for the determination of TPC and TFC

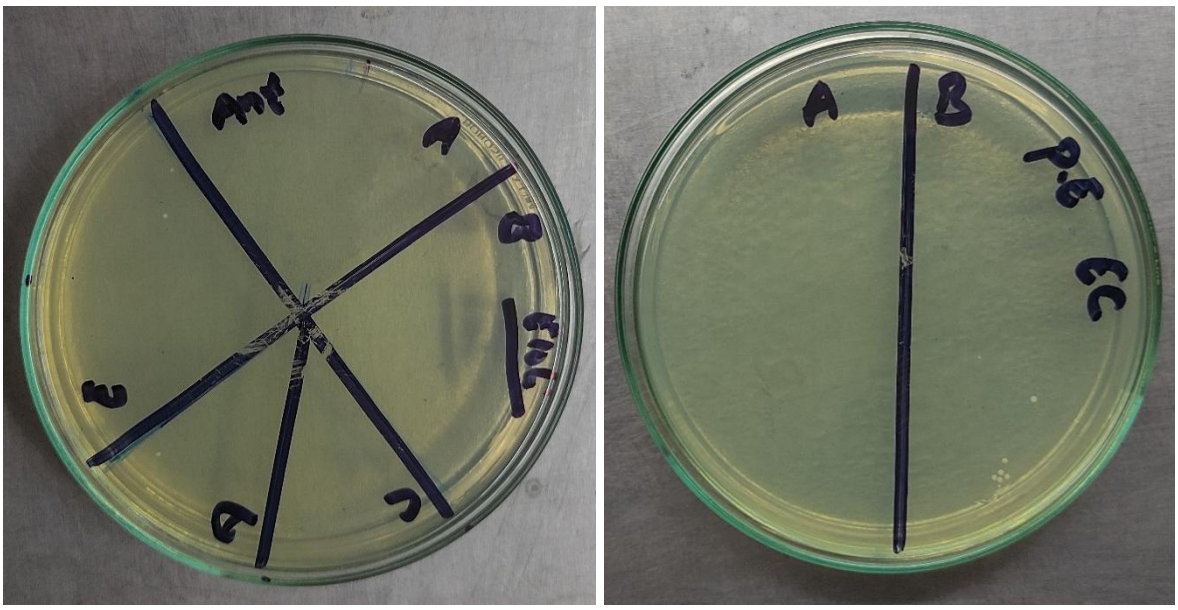


Appendix A1: Calibration Curve of Gallic Acid



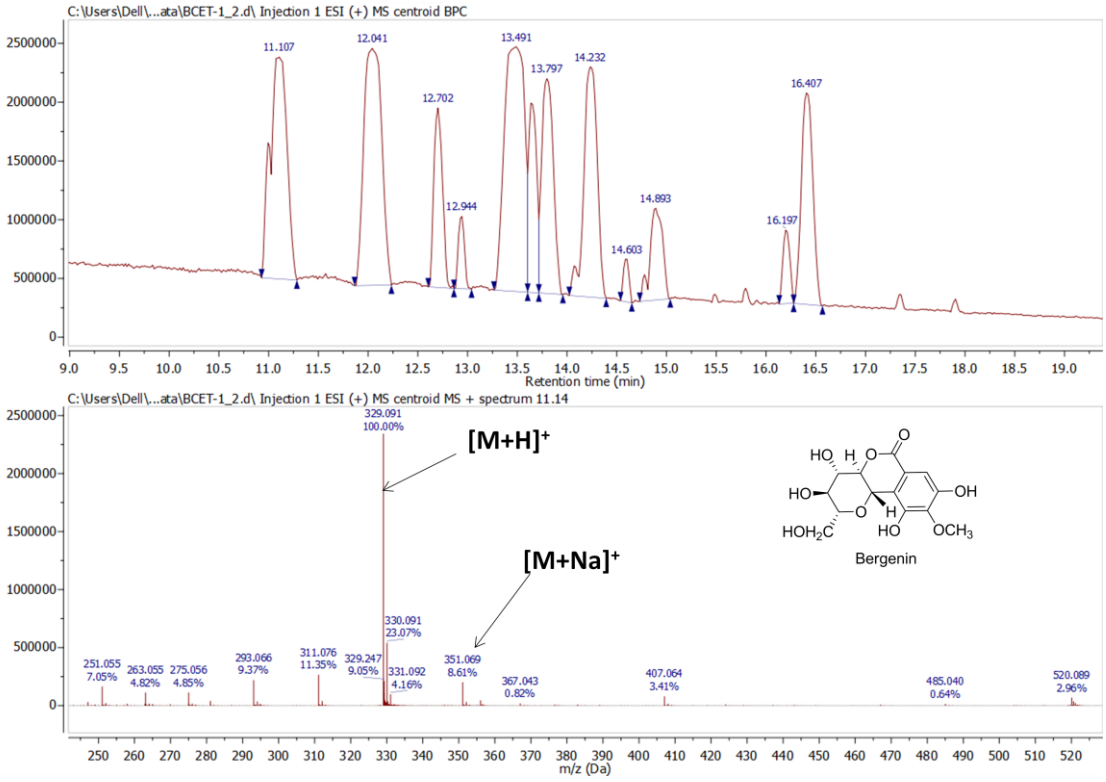
Appendix A2: Calibration Curve of Quercetin

Appendix B: Bacterial sub-cultures on MHA media

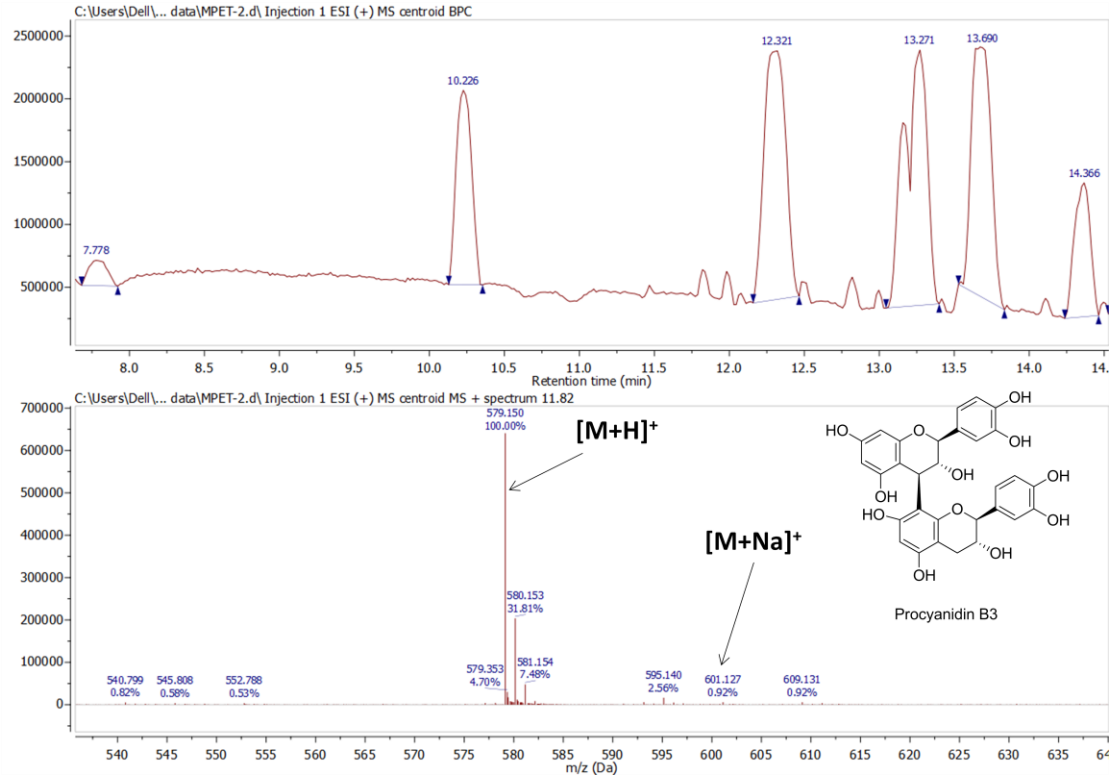


Appendix B: Determination of MBC of Neomycin and *P. emblica* against *E.coli*

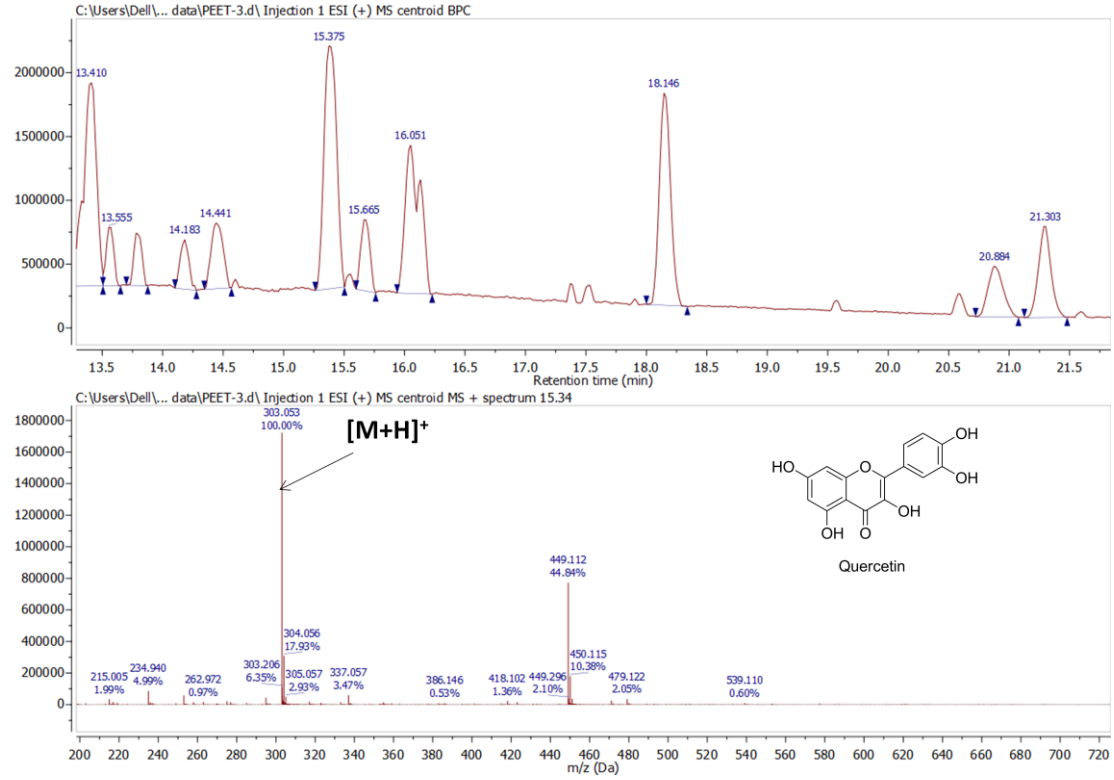
Appendix C: Mass spectra of various plant extracts obtained from LC-HRMS



Appendix C1: Mass spectrum of Bergenin (*Bergenia ciliata*)

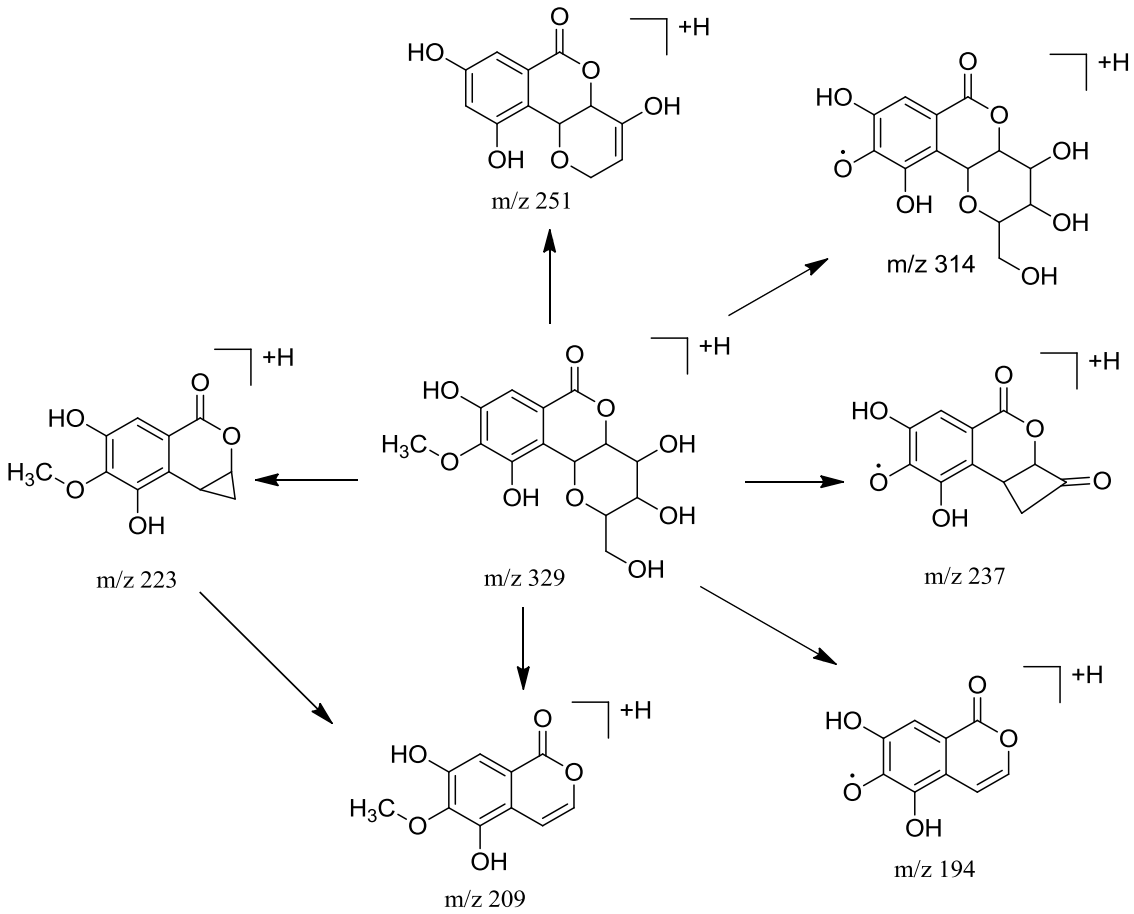


Appendix C2: Mass spectrum of Procyanidin B3 (*Mimosa pudica*)

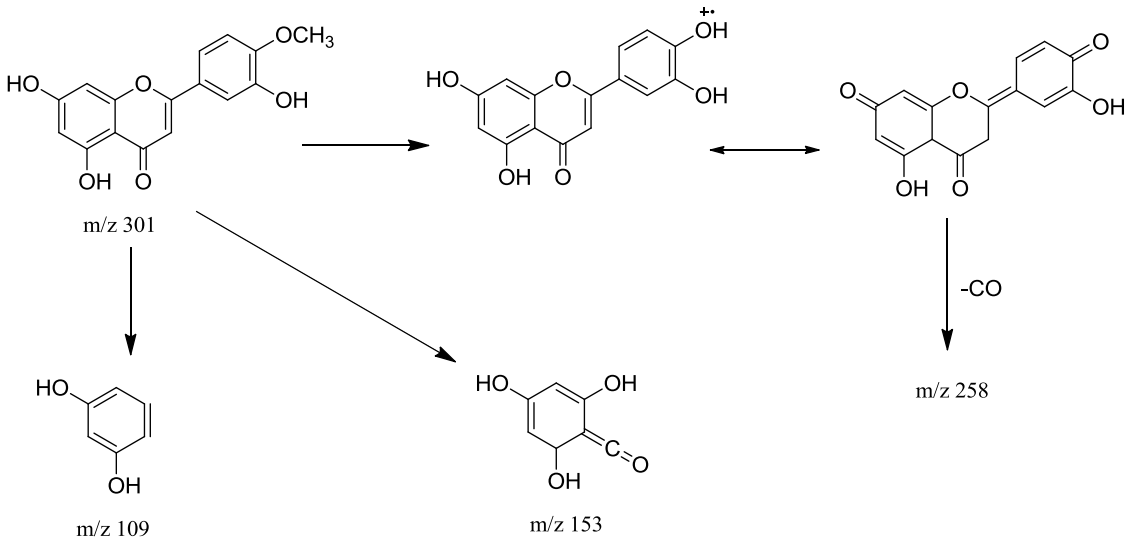


Appendix C3: Mass spectrum of Quercetin (*Phyllanthus emblica*)

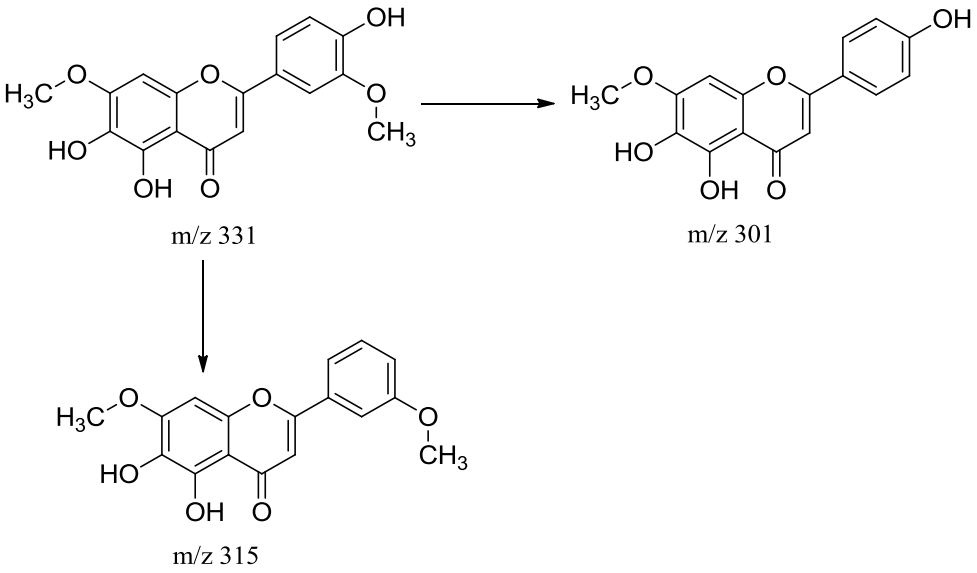
Appendix D: Fragmentation pattern of identified plant metabolites during LC-HRMS



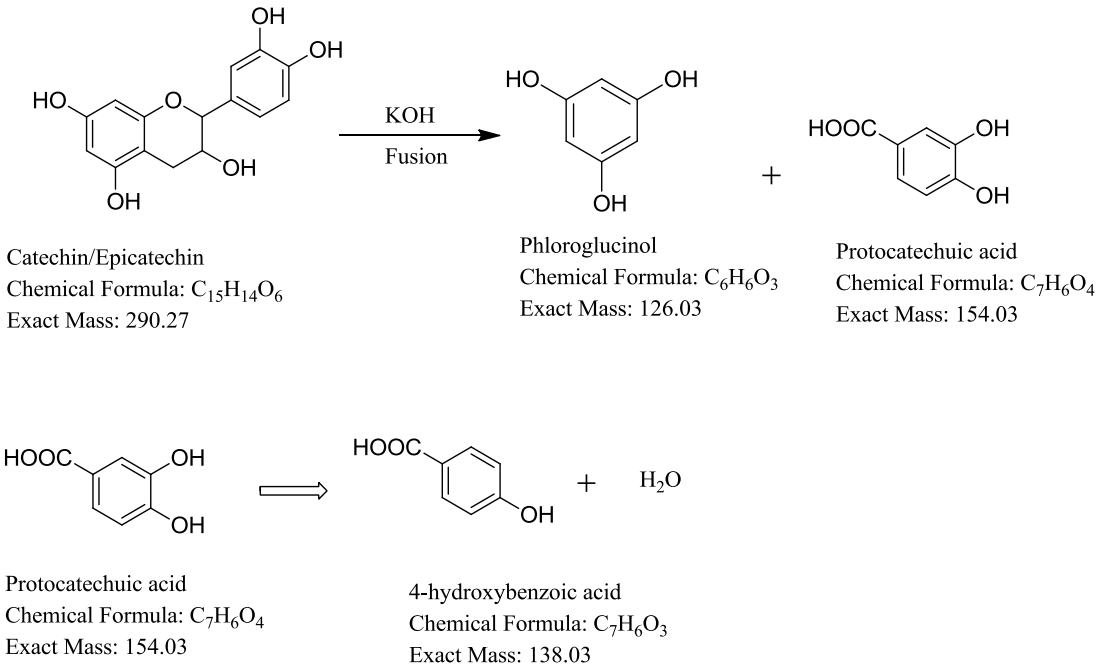
Appendix D 1: Fragmentation pattern of Bergenin



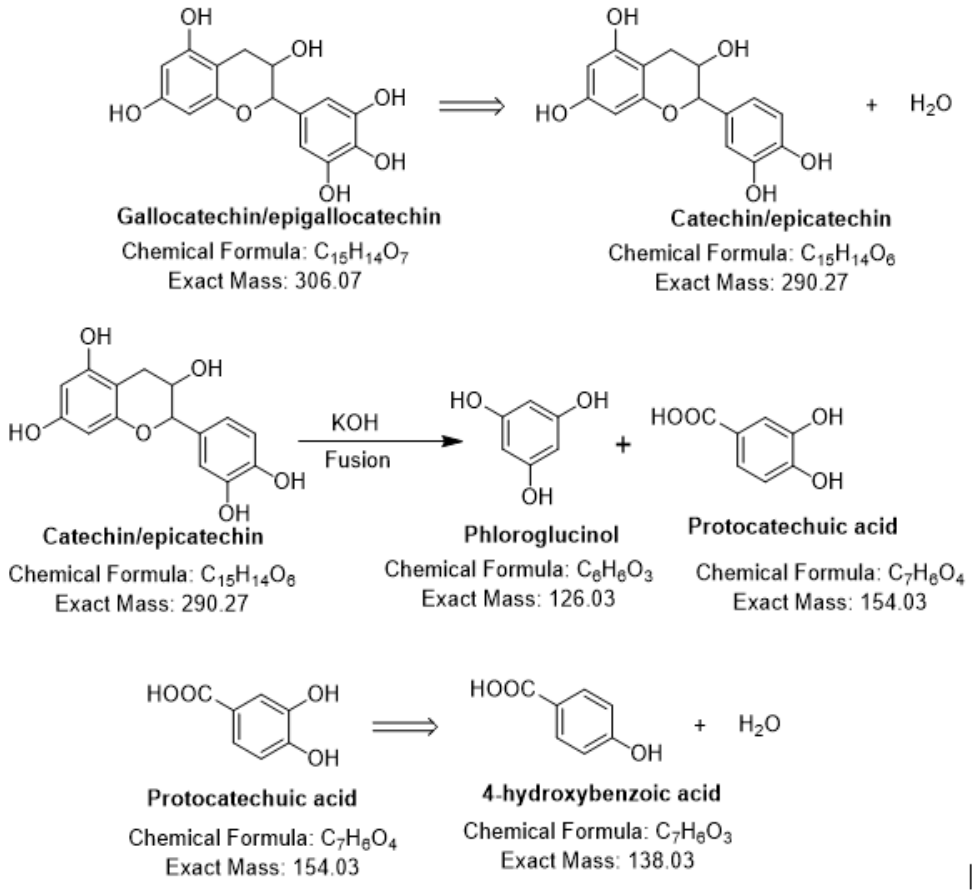
Appendix D 2: Fragmentation pattern of Diosmetin



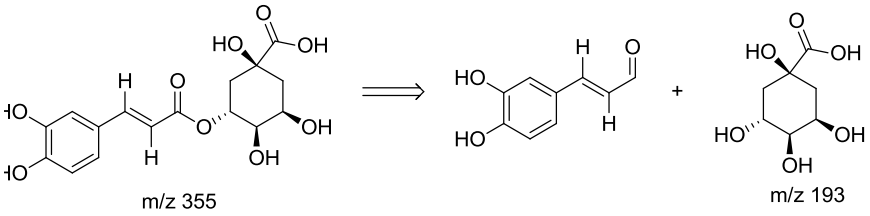
Appendix D 3: Fragmentation pattern of Trihydroxy-dimethoxyflavone



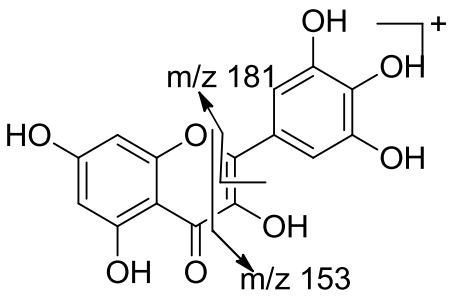
Appendix D 4: Fragmentation pattern of Catechin/Epicatechin



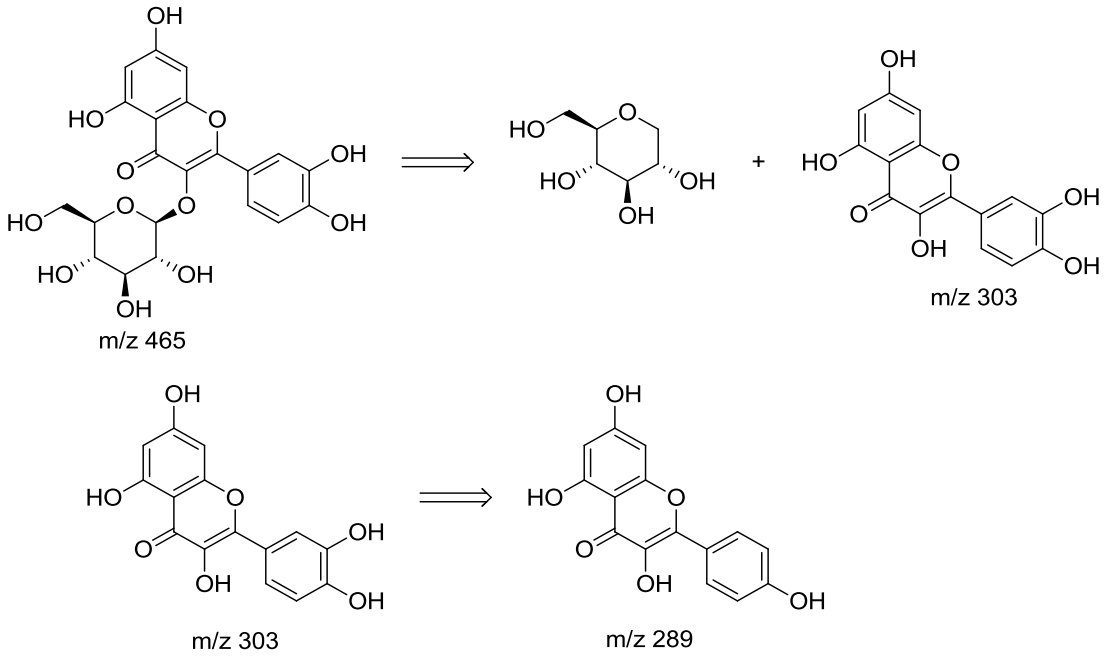
Appendix D 5: Fragmentation pattern of Gallocatechin/Epigallocatechin



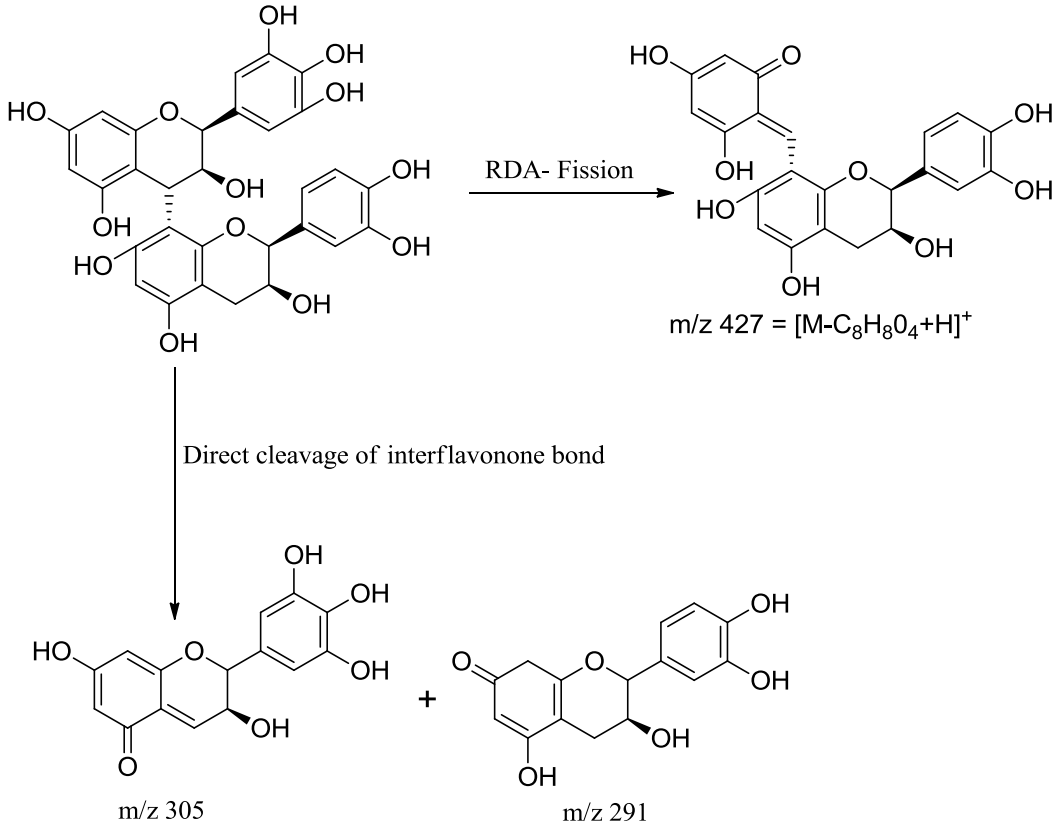
AppendixD6: Fragmentation pattern of Chlorogenic acid



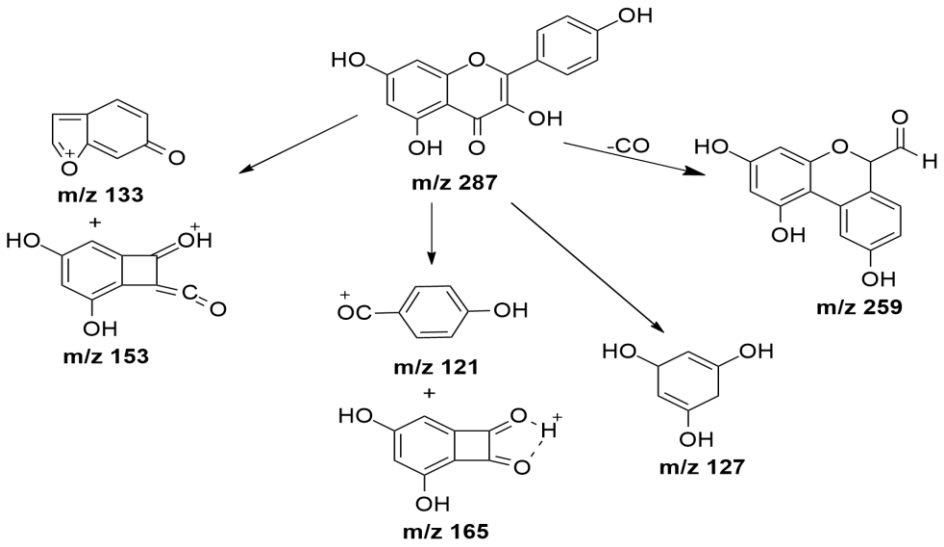
Appendix D 7: Fragmentation pattern of Myricetin



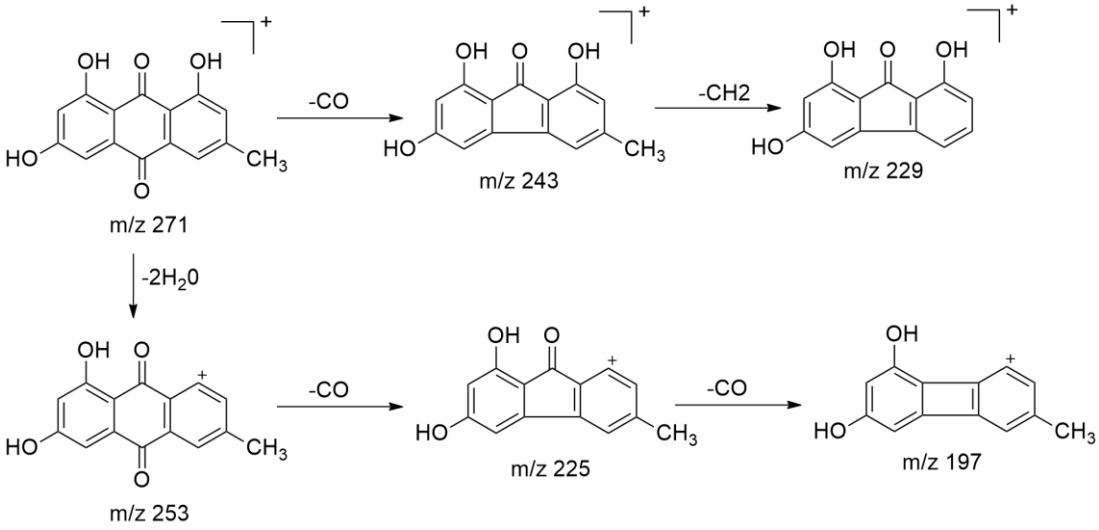
Appendix D 8: Fragmentation pattern of Isoquercetin



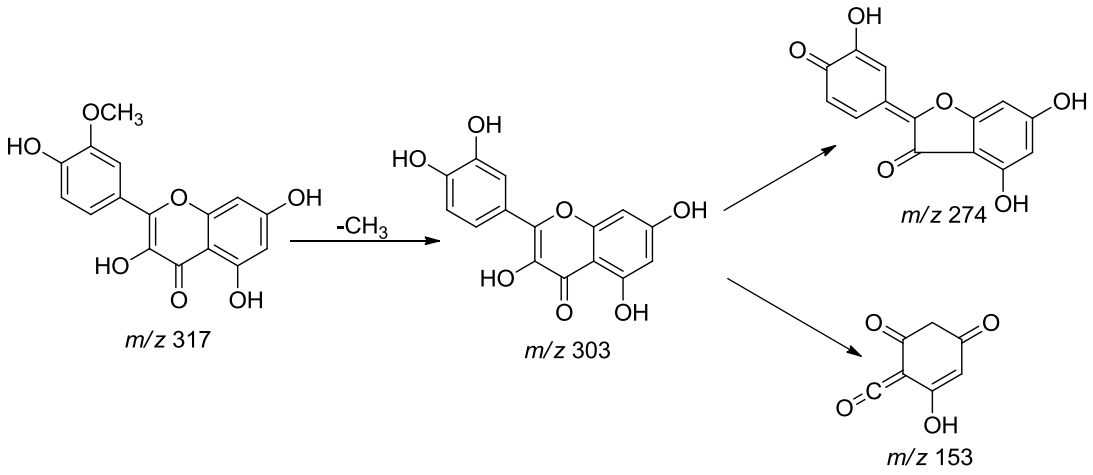
Appendix D 9: Fragmentation pattern of Prodelphinidin B3



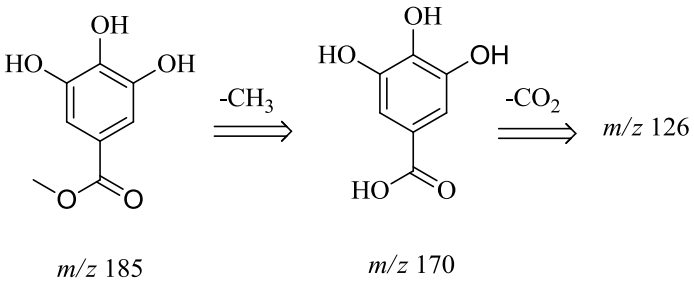
Appendix D 10: Fragmentation pattern of Kaempferol



Appendix D 11: Fragmentation pattern of Emodin

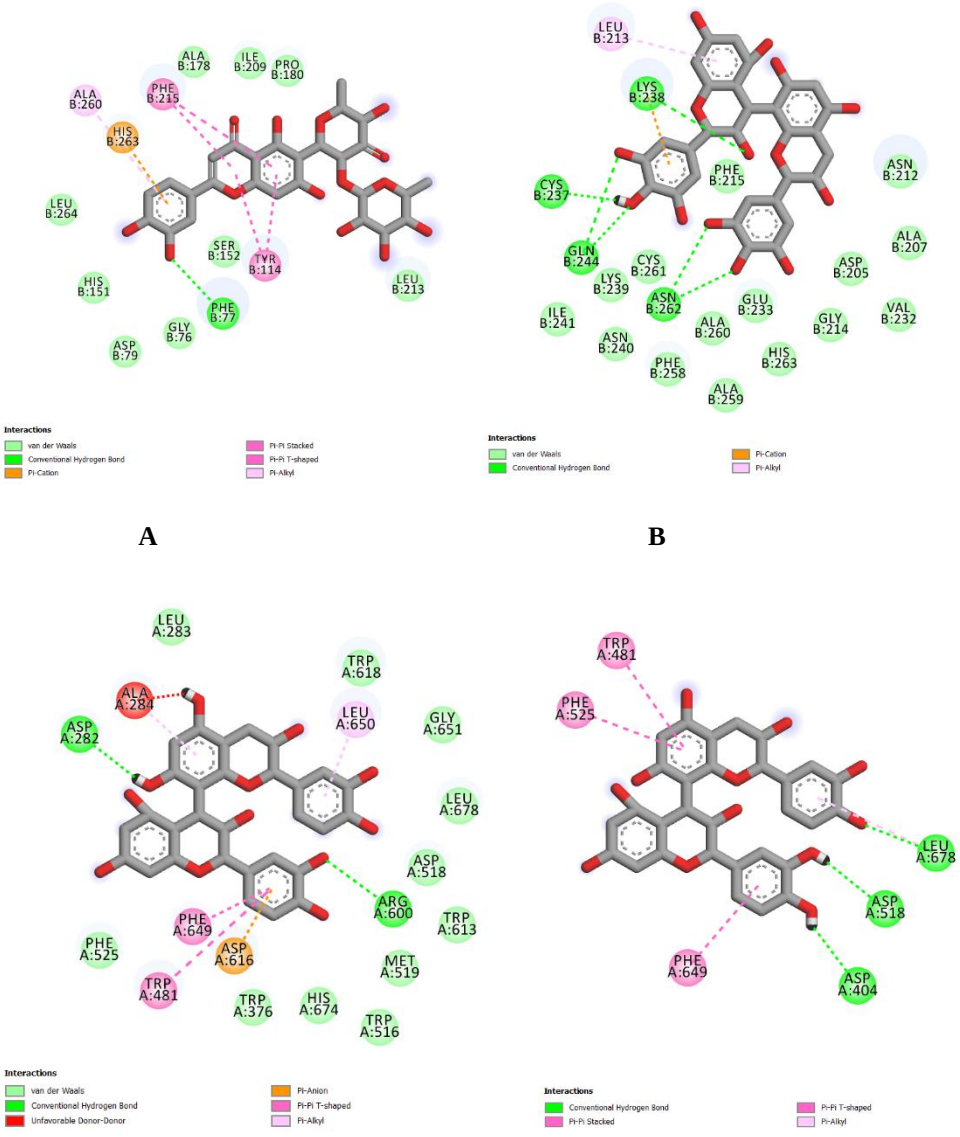


Appendix D 12: Fragmentation pattern of Isorhamnetin



Appendix D 13: Fragmentation pattern of Methyl Gallate

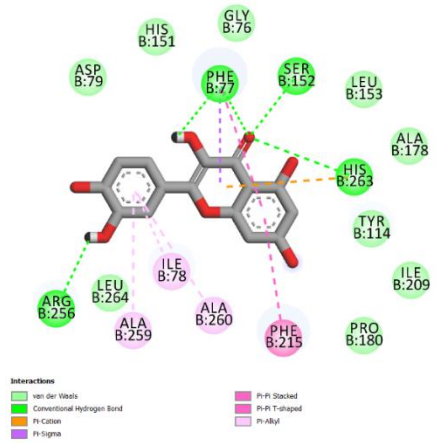
Appendix E: Molecular docking images showing interactions between the enzymes of diabetic target and identified compounds



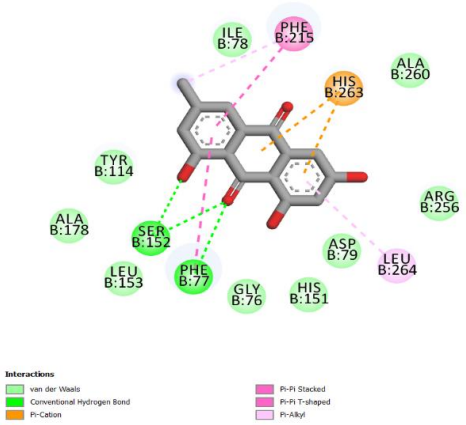
A **B**

C **D**

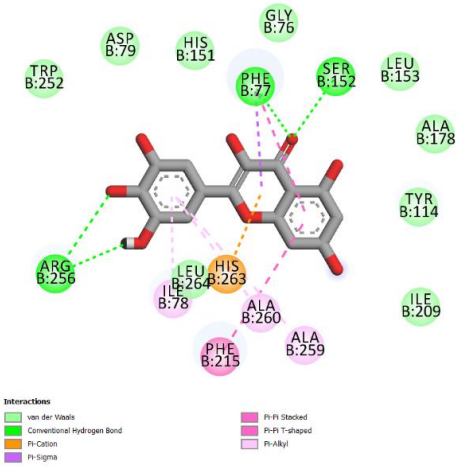
Appendix E1 : Interaction of α-glucosidase With **A.** Cassiaoccidentalin B, **B.** Prodelphinidin B3 **C.** Procyanidin B1 **D.** Procyanidin B3,


GLY B:76
HIS B:151
ASP B:79
PHE B:77
SER B:152
LEU B:153
ALA B:178
HIS B:263
TYR B:114
ILE B:78
ILE B:209
LEU B:264
ARG B:256
ALA B:259
ALA B:260
PHE B:215
PRO B:180
Interactions
van der Waals
Conventional Hydrogen Bond
Pi-Cation
Pi-Sigma
Pi-Pi Stacked
Pi-Pi T-shaped
Pi-Alkyl

A


ILE B:78
PHE B:215
ALA B:260
HIS B:263
TYR B:114
ARG B:256
ALA B:178
SER B:152
ASP B:79
LEU B:264
LEU B:153
PHE B:77
GLY B:76
HIS B:151
Interactions
van der Waals
Conventional Hydrogen Bond
Pi-Cation
Pi-Pi Stacked
Pi-Pi T-shaped
Pi-Alkyl

B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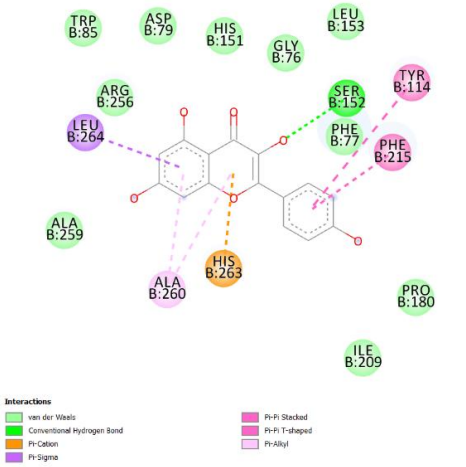
ASP B:79
HIS B:151
TRP B:252
PHE B:77
SER B:152
LEU B:153
ALA B:178
TYR B:114
ARG B:256
LEU B:264
HIS B:263
ILE B:78
ILE B:209
ALA B:260
PHE B:215
ALA B:259
Interactions
van der Waals
Conventional Hydrogen Bond
Pi-Cation
Pi-Sigma
Pi-Pi Stacked
Pi-Pi T-shaped
Pi-Alkyl

C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ASP B:79
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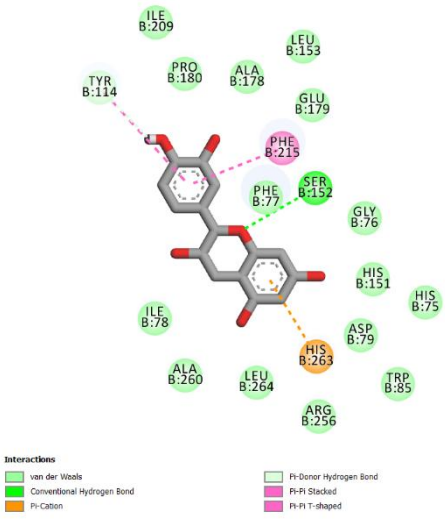
SER B:152
LEU B:264
PHE B:77
PHE B:215
ALA B:259
HIS B:263
ALA B:260
PRO B:180
ILE B:209
Interactions
van der Waals
Conventional Hydrogen Bond
Pi-Cation
Pi-Sigma
Pi-Pi Stacked
Pi-Pi T-shaped
Pi-Alkyl

D


ILE B:209
LEU B:153
PRO B:180
ALA B:178
TYR B:114
GLU B:179
PHE B:215
SER B:152
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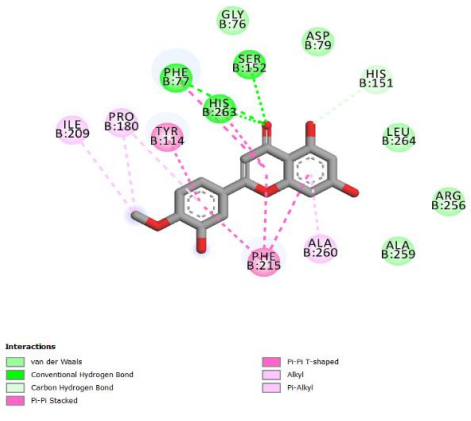
GLY B:76
HIS B:151
HIS B:75
ILE B:78
ASP B:79
HIS B:263
ALA B:260
LEU B:264
TRP B:85
ARG B:256
Interactions
van der Waals
Conventional Hydrogen Bond
Pi-Cation
Pi-Donor Hydrogen Bond
Pi-Pi Stacked
Pi-Pi T-shaped

E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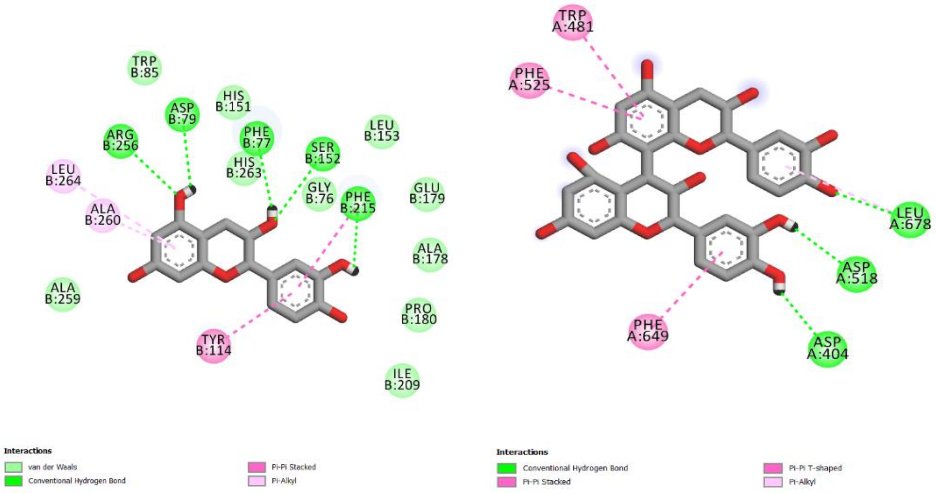
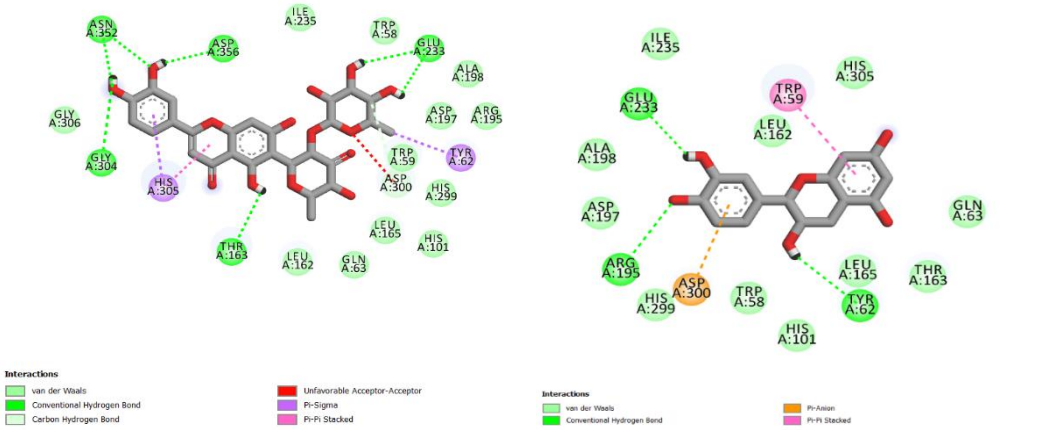
HIS B:263
ILE B:209
PRO B:180
TYR B:114
LEU B:264
ARG B:256
PHE B:215
ALA B:260
ALA B:259
Interactions
van der Waals
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Pi-Pi Stacked
Pi-Pi T-shaped
Alkyl
Pi-Alkyl

F

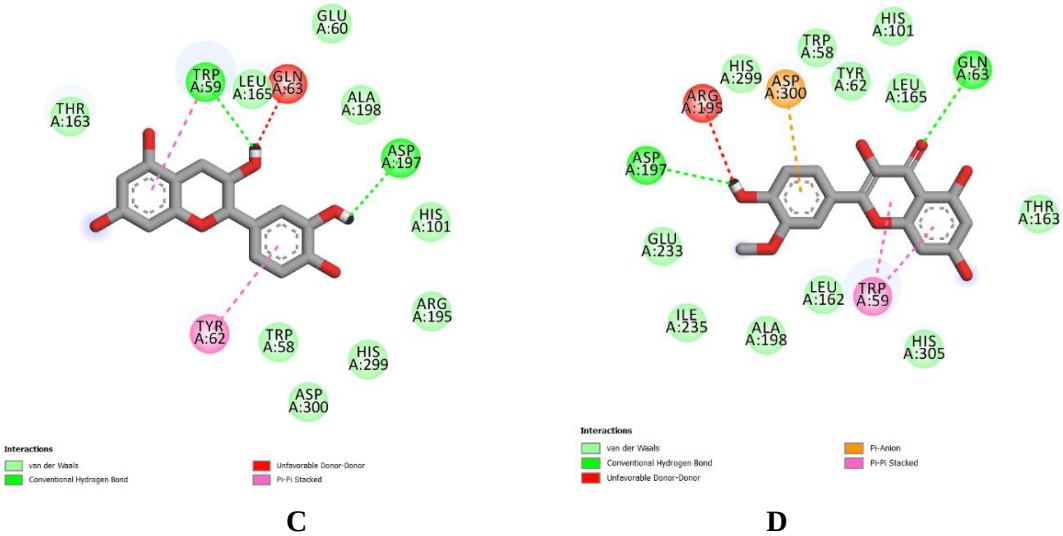


G **H**

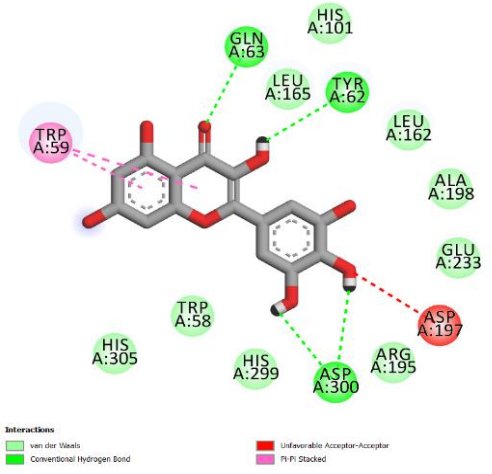
Appendix E2: Interaction of *α*-amylase with **A.** Quercetin, **B.** Emodin, **C.** Myricetin, **D.** Kaempferol, **E.** Epicatechin, **F.** Diosmetin **G.** Catechin **H.** Procyanidin B3,



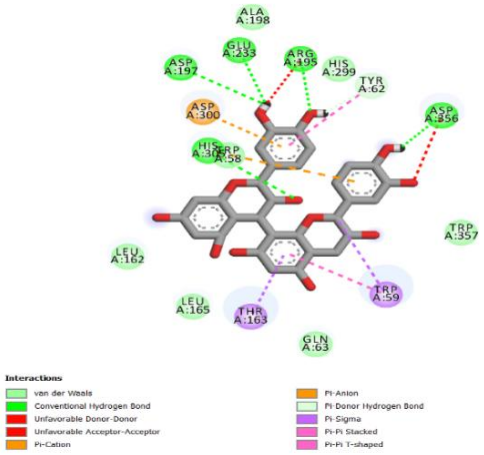
A **B**



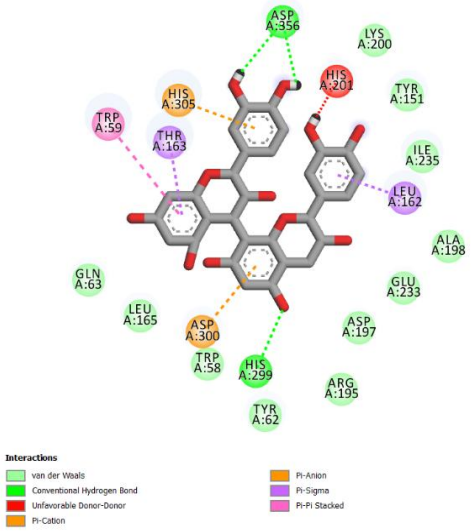
C **D**



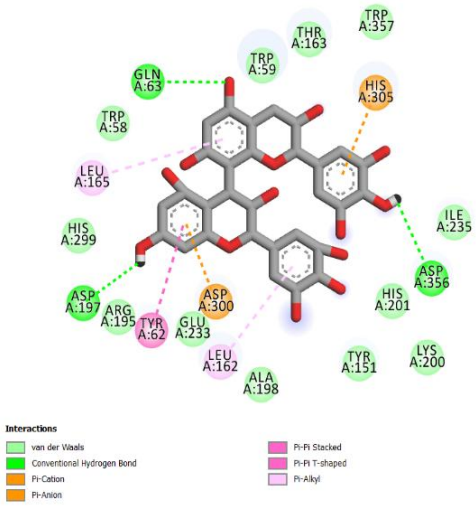
E



F



G



H

Appendix E3: Interaction of α-amylase with **A.** Cassiaoccidentalin B, **B.** Catechin, **C.** Epicatechin, **D.** Epigallocatechin, **E.** Isorhamnetin, **F.** Myricetin, **G.** Procyanidin B1, **H.** Prodelphinidin B3

Appendix F: Questionnaire for the ethnobotanical survey of medicinal plants

General Information:

Name …… Age …

Ruralmunicipality/Municipality:…… Ward No ……

Caste/Ethnic group……. Altitude:……

Specific Information:

(1) What are the plants that you frequently used to cure diabetic disorders?

(2) What is its local name in your language?

(3) Which parts of the plant do you use, root, stem, leaf, fruit, or any others?

(4) How do you prepare the drugs: in the form of juice, paste, infusion, tablets, decoction, or other forms?

(5) How do you take the drugs: oral or external and what is the dose amount?

(5) How long do you take the drugs for antidiabetic disorder and what times per day?

(6) Do these drugs have any side effects during medication: nausea, diarrhea, headache, or any others?

(7) How long do you store these drugs for medication: one/two/three weeks or cannot be stored?

(8) Do you use any antidote during medication?

(9) Do you use these drugs with allopathic drugs at a time?

(10) How do you know the effects of these drugs in your body: blood sugar test or any other test?

(11) What is the availability of these medicinal plants in your locality: common/rare/endangered?

विरुवाको लोकवानस्पतिक सर्वेक्षण

(Ethnobotanical Survey of the Plant)

(प्रश्नावली)

व्यक्तिगत विवरण मितिः.....

प्रयोगकर्ताको नाम /थरः..... उमेरः.....

गाउपालिका नगरपालिकाः..... वार्ड नं.: .....

जाति जनजातिः ..... भौगोलिक अवस्थिति.:.....

अन्य विवरण

१) मधुमेह (चिनी रोग) को उपचारमा प्राय तपाईले प्रयोग गर्ने विरुवा कुन हो ?

२) यो बिरुवाको स्थानीय भाषामा नाम के हो ?

३) तपाईले यस बिरुवाको कुन भाग (पात, जरा, काण्ड , फल वा पूरा बिरुवा) औषधिको रुपमा प्रयोग गर्नुहुन्छ ?

४) यो बिरुवाबाट तपाई कसरि औषधि तयार गर्नुहुन्छ ? (रस, पेष्ट, भिजाएको रस, काढा, भष्म, चूर्ण, टेब्लेट वा अन्य)

५) तपर्ष्ई यो औषधि कति मात्रामा र कसरि लिनुहुन्छ ?

६) यो औषधि संकलन गरिसकेपछि कति समयसम्म भण्डारण गरेर घरमा राख्नुहुन्छ ? वा भण्डारण गरेर राख्न मिल्दैन ?

७) यो औषधि प्रयोग गर्दा यसको असर रोक्ने अन्य कुनै दवाई प्रयोग गर्नुहुन्छ ?

८) के यो औषधि सग अन्य कुनै आधुनिक औषधि पनि प्रयोग गर्नुहुन्छ ?

९) यो औषधिको सेवनले शरीरमा सुगर घटेको वा यसले असर गरेको कसरि थाहष पाउनुहुन्छ ?

१०) यी औषधीय बिरुवाहरु स्थानीय रुपमा कत्तिको उपलब्ध छन ? प्रशस्त पाईन्छन् वा बिरलै वा लोपोन्मुख अवस्थामा छन् ?

नेपाल सरकार
वन तथा वातावरण मन्त्रालय
वनस्पति विभाग

राष्ट्रिय हर्बेरियम तथा वनस्पति प्रयोगशाला

पत्र संख्या: ०७९/८०
च.नं. ९९२

गोदावरी, ललितपुर

मिति २०७९/०८/२३

2023/8/8 18:00

विषय: नमूना पहिचान सम्बन्धमा।

श्री बसन्त कुमार सापकोटा
Central Department of Chemistry
Kirtipur, Kathmandu

प्रस्तुत विषयमा तहाँको २०७९/०८/२० को निवेदन साथ वनस्पतिको नमूना प्राप्त भई व्यहोरा अवगत भयो। निवेदन मार्फत ल्याइएको नमूनाको पहिचान गरी प्राविधिक विशेषज्ञको प्रतिवेदन (पाना १) यसै पत्रसाथ संलग्न गरी पठाइएको व्यहोरा अनुरोध छ।

२०७९/८/२३

हेमराज पौडेल
अनुसन्धान अधिकृत
(१८२५६१)
नि. कार्यालय प्रमुख

विधिक विशेषज्ञको प्रतिवेदन

१. नमुना परीक्षण गर्न पठाउने व्यक्ति/निकायः- श्री रसायन शास्त्र केन्द्रीय विभाग कीर्तिपुर काठमाडौं।

१(क) विद्यार्थीको नामः- श्री बसन्त कुमार सापकोटा

२. प्राप्त नमुनाको विवरणः- हर्बेरियम नमुना थान ६

३. यस कार्यालयमा प्राप्त मितिः- २०७७/१२/१२

४. परीक्षणका आधारहरुः- (क) हर्बेरियममा भएको नमुनाहरु संगको तुलनात्मक अध्ययन ।
(ख) सन्दर्भ सामग्रीहरुको अध्ययन ।

५. पहिचान प्रतिवेदनः- प्राप्त नमुनाहरुको Morphological अध्ययन र यस राष्ट्रिय हर्बेरियम तथा वनस्पति प्रयोगशालाको हर्बेरियममा राखिएका नमुनाहरु संगको तुलनात्मक अध्ययन गर्दा उक्त नमुनाहरु निम्नानुसार भएको प्रमाणित हुन गएको ।

S.No.	Collection NO.	Scientific Name	Family	Remarks
1	BS-05	*Calotropis gigantea* (L.) W. T. Aiton	Asclepiadaceae	
2	BS-02	*Asparagus racemosus* Wild.	Asparagaceae	
3	BS-03	*Phyllanthus emblica* L.	Euphorbiaceae	
4	BS-04	*Solanum nigrum* L.	Solanaceae	
5	BS-01	*Mimosa pudica* L.	Fabaceae	
6	Bs-06	*Bergenia ciliata* (Haw.) Stens.	Saxifragaceae	

२०७७/१२/१२

६. परीक्षण गर्ने अधिकारीः-

हेर्म राज पौडेल
अनुसन्धान अधिकृत
(१८२५६१)

Appendix G: Scientific Publications and Conference/Seminar Participation

Appendix G1: List of published articles

1. **Basanta Kumar Sapkota,** Karan Khadayat, Kabita Sharma, Bimal Kumar Raut, Dipa Aryal., Bijaya Bahadur Thapa, Niranjan Parajuli, "Phytochemical Analysis and Antioxidant and Antidiabetic Activities of Extracts from *Bergenia ciliata Mimosa pudica, and Phyllanthus emblica", Advances in Pharmacological and Pharmaceutical Sciences*, vol. 2022, Article ID 4929824, **11** pages, 2022. https://doi.org/10.1155/2022/4929824

2. **Sapkota, B.K.;** Khadayat, K.; Aryal., B.; Bashyal., J.; Jaisi, S.; Parajuli, N. LC-HRMS-Based Profiling: Antibacterial and Lipase Inhibitory Activities of Some Medicinal Plants for the Remedy of Obesity. *Sci. Pharm*. 2022, ***90,*** 55. https://doi.org/10.3390/scipharm90030055

3.Sapkota, B. K., Khadayat, K., Adhikari, B., Poudel, D. K., Niraula, P., Budhathoki, P., & Parajuli, N. (2021). Phytochemical analysis, antidiabetic potential, and in-silico evaluation of some medicinal plants. *Pharmacognosy Research*, ***13***(3).

4. Aryal., B., Niraula, P., Khadayat, K., Adhikari, B., Khatri Chhetri, D., **Sapkota, B. K.,** & Parajuli, N. (2021). Antidiabetic, antimicrobial., and molecular profiling of selected medicinal plants. *Evidence-based complementary and alternative medicine*, *2021*, 1-15.

5. Shrestha, D. K., **Sapkota, B. K.,** & Sharma, K. P. (2022). Assessment of Phytochemicals, Antioxidant Activity, Total Phenolic and Flavonoid Contents of Selected Nepalese Medicinal Plants. *Nepal Journal of Biotechnology*, ***10***(2), 45-51.

Appendix G2: Participation in Conference/Seminar

1. "Eighth Graduate Conference Himalayan Knowledge Conclave 2022" held on April 4-5, 2022 organized by Mid-West University, Birendranagar, Surkhet (As an oral presenter)

2."9th Conference on Science and Technology" held on June 26-28, 2022 organized by Nepal Academy of Science and Technology (NAST), Khumaltar, Lalitpur Nepal (As an oral presenter)

3. Participated as an oral presenter in the **"Research Project Dissemination Seminar"** held on December 27, 2022, organized by the Central Department of Chemistry, T.U., Kirtipur, Kathmandu

4. Participated in three days workshop on **"Tools and Techniques in Chemistry"** held on April 2-4, 2019 organized by Nepal Chemical Society and Central Department of Chemistry, Kirtipur, Kathmandu

5. Participated in a two-day workshop on **"Tools and Techniques and Advanced Data Analysis in Chemistry"** held on August 30-31, 2019 organized by Nepal Chemical Society and Central Department of Chemistry, Kirtipur, Kathmandu

6. Participated in a one-day seminar on **"COVID-19 Research Project"** held on December 28, 2021organised by the Central Department of Chemistry, T.U., Kirtipur, Kathmandu

Hindawi
Advances in Pharmacological and Pharmaceutical Sciences
Volume 2022, Article ID 4929824, 11 pages
https://doi.org/10.1155/2022/4929824

Research Article

Phytochemical Analysis and Antioxidant and Antidiabetic Activities of Extracts from *Bergenia ciliata*, *Mimosa pudica*, and *Phyllanthus emblica*

Basanta Kumar Sapkota, Karan Khadayat, Kabita Sharma, Bimal Kumar Raut, Dipa Aryal, Bijaya Bahadur Thapa, and Niranjan Parajuli

Biological Chemistry Lab, Central Department of Chemistry, Tribhuvan University, Kirtipur, Kathmandu 44618, Nepal

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Received 30 April 2022; Revised 28 May 2022; Accepted 17 June 2022; Published 7 July 2022

Academic Editor: Heng Yen Khong

Diabetes is a metabolic disorder of high blood sugar levels which leads to various chronic health-related complications. The digestive enzymes α-amylase and α-glucosidase play a major role in the hydrolysis of starch to glucose; hence, inhibiting these enzymes is considered an important strategy for the treatment of diabetes. Medicinal plants such as *Bergenia ciliata*, *Mimosa pudica*, and *Phyllanthus emblica* are commonly used in traditional remedies due to their numerous health benefits. This study aimed to determine the phytochemicals as well as TPC and TFC contents in these plant extracts along with their antioxidant and enzyme inhibitory activity against α-glucosidase and α-amylase. The ethyl acetate extracts of selected plants have shown higher TPC and TFC contents. The aqueous extract of *B. ciliata* (IC_{50}: $16.99 \pm 2.56\,\mu$g/mL) and ethyl acetate extract of *P. emblica* (IC_{50}: $11.98 \pm 0.36\,\mu$g/mL) and *M. pudica* (IC_{50}: $21.39 \pm 3.76\,\mu$g/mL) showed effective antioxidant activities. Furthermore, ethyl acetate extract of *B. ciliata* showed significant inhibitory activity against α-amylase and α-glucosidase with IC_{50} values of $38.50 \pm 1.32\,\mu$g/mL and $3.41 \pm 0.04\,\mu$g/mL, respectively. Thus, secondary metabolites of these medicinal plants can be repurposed as effective inhibitors of digestive enzymes.

1. Introduction

Diabetes, a global health problem, is a chronic metabolic disorder due to dysfunction in the production and/or utilization of insulin [1]. Diabetes has become a subject of concern worldwide due to its chronic health complications such as cardiovascular diseases, nephropathy, neuropathy, lower-limb amputations, retinopathy, and others, leading to complicated lifestyle and mortality [2]. There is an increased risk of infection in diabetic patients due to the disruption in the immune system. Secondary metabolites in natural products are the source of major lead compounds for the optimization of pharmacological activities due to their less toxicity and moderate side effects [3]. The metabolites such as flavonoids, alkaloids, and polyphenols have a wide range of biological activities such as antioxidant, antimicrobial, anti-inflammatory, anticancer, antidiabetic, and other activities [4]. Besides, polyphenols can reduce oxidative stress and can inhibit enzymes of carbohydrate digestion and play a significant role in preventing hyperglycemia [5, 6].

B. ciliata (family Saxifragaceae), known as Pakhanbhed in Nepali, is traditionally used for the treatment of diabetes in local communities [7]. Besides that, crude extracts of rhizomes and leaves of this species were studied for anti-inflammatory, antimicrobial, anticancer, antidiabetic, antioxidant, and others activities [8–12], and its major phytoconstituents include bergenin, gallic acid, gallicin, tannic acid, catechin, (-)-3-0-galloylepicatechin, (-)-3-0-galloylcatechin, stigmasterol, B-sitosterol, galloylated leucoanthocyanidin-4-glucoside, allantoin, and afzelechin [13]. *P. emblica*, commonly known as Amla of Euphorbiaceae

family, possesses several pharmacological properties such as antioxidant, antimicrobial, antifungal, anticancer, antidiabetic, and other properties [14]. Some of the major chemical constituents of this species are chebulagic acid, chebulic acid, corilagin, phyllanemblinin A, gallic acid, ellagic acid, malic acid, mucic acid, (-)epicatechin, and mallonin [15]. *M. pudica* of the family Fabaceae commonly found in South Asian countries possesses antidiabetic, antibacterial, wound-healing, antivenom, and anticancer properties and is traditionally used for treatment of fever and dyspepsia [16]. Phytochemical analysis revealed the presence of flavonoids C-glycosides, sterols, terpenoids, tannins, fatty acids, p-coumaric acid, mimopudine, and mimosine in *M. pudica* [17]. This study aims to evaluate antidiabetic activity based on α-amylase and α-glucosidase as well as antioxidant activities of three medicinal plants found in Nepal.

2. Material and Methods

2.1. Chemicals. Methanol, ethanol, ethyl acetate, dichloromethane, and hexane were purchased from Thermo Fischer Scientific (India). Gallic acid and quercetin were purchased from HiMedia (India). α-Glucosidase (*Saccharomyces cerevisiae*), 4-nitrophenyl-α-D-glucopyranoside (pNPG), α-amylase from porcine pancreases, 2-chloro-4-nitrophenyl-α-D-maltotrioside (CNPG3), acarbose, and neomycin were obtained from Sigma-Aldrich (Germany).

2.2. Medicinal Plant Extracts. B. ciliata, *M. pudica, and P. emblica* were collected from the different geographical regions in Nepal and were botanically identified by the National Herbarium and Plant Laboratories (NHPL)/KATH, Godawari-3, Lalitpur, and their voucher specimen were assigned (Table 1). The plant materials were shade-dried at room temperature and then pulverized by the mixture and soaked into methanol for 24 hrs following the cold percolation protocol. Then they were filtered through Whatman Filter Paper 1 and collected in a conical flask, and the same process was repeated thrice. The collected methanol was evaporated from primary extracts using a rotary evaporator under reduced pressure at 40°C. Then, the percentage yield was calculated using the dry weight of extract and the dry weight of powder soaked in methanol. The secondary extracts were prepared by dissolving the primary extract in water and then fractionated with different solvents such as hexane, dichloromethane, and ethyl acetate based on increased polarity [18]. The percentage yield was calculated by the given formula:

$$\%\text{Yield} = \frac{\text{Dry weight of extract}}{\text{Dry weight of a plant}} \times 100. \quad (1)$$

2.3. Phytochemical Identification. The chemical method was used to identify phytochemicals present in the extracts. Different tests such as steroids, alkaloids, phenols, terpenoids, tannins, and glycosides were performed as previously described methods [19–21]

2.4. Determination of Total Phenolic and Flavonoid Contents. The total phenolic and flavonoid contents (TPC and TFC) were determined using the method described earlier. For the determination of TPC, Folin-Ciocalteau's, and for the determination of TFC, the aluminum trichloride method was used [22–24]. Both assays were performed in 200 μL, and absorbance was taken using a microplate reader (Synergy LX, BioTek, Instruments, Inc., USA). Gallic acid and quercetin were used as the standard to generate calibration curves at various concentrations and expressed as gallic acid and quercetin equivalent (mg GAE/g and mg QE/g), respectively.

2.5. Antioxidant Assay. The antioxidant activity of different extracts was evaluated against DPPH radicals according to the method described earlier [25, 26]. Exactly an equal volume of samples with different concentrations was mixed with 0.1 mM DPPH reagent to maintain a final volume of 200 μL. Then, it was incubated in dark at room temperature for 30 min, and then absorbance was recorded at 517 nm. Quercetin was used as the standard to compare the antioxidant efficacy of plant extracts. The given formula determined the percentage scavenging:

$$\text{Scavenging DPPH radical} = \frac{\text{OD}_{\text{control}} - \text{OD}_{\text{test sample}}}{\text{OD}_{\text{control}}} \times 100. \quad (2)$$

2.6. In Vitro α-Glucosidase Inhibition Assay. The α-glucosidase inhibition was determined following the method described earlier. The test samples in 30% DMSO were mixed with enzyme (0.2 units/mL) in 100 mM phosphate-buffered saline (pH 6.8) and then preincubated at 37°C for 10 min. The reaction was started by adding pNPG as substrate (0.7 mM) and incubated for 15 min at the same temperature [27]. The absorbance was measured at 405 nm using a microplate reader and inhibitory activity was calculated using the following formula:

$$\%\,\text{Inhibition} = \frac{\text{OD}_{\text{control}} - \text{OD}_{\text{test sample}}}{\text{OD}_{\text{control}}} \times 100. \quad (3)$$

2.7. In Vitro α-Amylase Inhibition Assay. The α-amylase inhibition was determined using a literature method. The test samples in 30% DMSO were mixed with enzyme (1.5 units/mL) in 50 mM phosphate-buffered saline (pH 7.0, 0.9% NaCl) and then preincubated at 37°C for 10 min. The reaction was started by adding CNPG3 as substrate (0.5 mM) and left for 15 min for incubation at the same temperature [28]. The absorbance was measured at a 405 nm microplate reader and inhibitory activity was calculated using the given formula mentioned earlier (equation (1)).

2.8. Statistical Analysis. The Gen5 Microplate Data Collection and Analysis software was used for result processing, followed by Microsoft Excel. The data were expressed as

Table 1: Details of medicinal plants under study, their collection sites, parts used, and percentage yield.

Voucher specimen	Medicinal plants	Local name	Parts used in research	% yield (w/w)	Collection site	Altitude (m)	Geographical coordinates
BS-02	*B. ciliata*	Pakhanbhed	Stem	15.86	Shantipur, Gulmi, Nepal	1490 m	27°39′48.3″ N 83°28′52.3″ E
BS-04	*M. pudica*	Lajawati	Whole part	17.6	Shankar Nagar, Butwal, Nepal	158 m	28°11′24″ N 82°13′48″ E
BS-05	*P. emblica*	Amla	Fruit	39.5			

mean ± standard error of the mean. The IC_{50} values were determined using GraphPad Prism (Version 8) software. The XLSTAT software (Addinsoft, USA, NY) was used to perform principal component analysis and correlation analysis.

3. Results

3.1. Percentage Yield and Phytochemical Identification. The percentage yield of selected plants was calculated with highest being obtained in *P. emblica* (39.5%) followed by *M. pudica* (17.6%) and *B. ciliata* (15.86%). Our study indicated that the highest yield is obtained from fruit, whole plant, and stem, respectively. The phytochemical identification indicated the presence of alkaloids, phenols, flavonoids, terpenoids, tannins, and glycosides as shown in Table 2.

3.2. Analysis of Total Phenolic and Flavonoid Contents. The TPC and TFC of methanolic primary extracts and their partitioned fractions (secondary extracts) are mentioned in Table 3. Among all fractions, the EA fraction had the highest TPC, while, in TFC, crude and EA fractions had the highest contents. The TPC and TFC were expressed as mg GAE/g and mg QE/g of extracts using calibration curves of gallic acid and quercetin, respectively.

3.3. Antioxidant Assay. The antioxidant activities of different fractions were determined using the DPPH assay. The IC_{50} value of various solvent fractions showed medium-to-strong DPPH scavenging activity ranging from 11.98 to 141.53 μg/mL. The potent activity was shown by ethyl acetate, as well as crude and aqueous fractions. The quercetin was used as standard and the IC_{50} value was found to be 2.86 ± 0.51 μg/mL. The details about the IC_{50} values of each fraction are shown in Table 4.

3.4. Inhibition of Digestive Enzymes. The results revealed that the IC_{50} value of various solvent fractions showed medium-to-strong inhibitory activity. However, the ethyl acetate fraction showed strong activity against α-amylase, while, in the case of α-glucosidase, ethyl acetate, crude, and aqueous fractions showed significant activities. For both enzymes, acarbose was used as a positive control with IC_{50} values of 3.13 ± 0.14 μg/mL and 2.06 ± 0.07 mg/mL for α-amylase and α-glucosidase, respectively. The details are given in Table 5.

3.5. Correlation Analysis between TPC, TFC, DPPH, α-Amylase, and α-Glucosidase. The Pearson correlation analysis was performed between TPC, TFC, DPPH, α-amylase, and α-glucosidase as shown in Table 6.

3.6. Principal Component Analysis. Principal component analysis (PCA) was performed on five different variabilities among varied solvent fractions to obtain information about the interrelationship among variables. Among the five components, PC1 and PC2 showed eigenvalues >1, while the remaining principal components had eigenvalues <1 and so have not been discussed further (Figure 1).

The principal axis 1 (PC1) accounted for 55.807% of the variance, whereas principal axis 2 (PC2) accounted for 24.226% and they altogether accounted for 80.033% of the total variance of the data matrix, with PC1 being the prominent one (Figure 2).

The eigenvalues of two principal component axes among five were found to be more than one with 2.79 and 1.21, respectively. The principal component score plot produced from PC1 revealed that all variables were positively associated and, hence, showed a good correlation between them. PC1 showed positive factor loadings for all variables. TFC showed the highest factor loading at 0.834, followed by TPC with a 0.831 factor loading value depicting that TFC could be the best individual factor loadings selection. PC2 confirmed positive factor loadings for three variables, that is, TPC, TFC, and DPPH, while DPPH could be the best selection for individual factor loadings with a maximum value of 0.828 followed by TPC (Table 7). Figure 2 revealed the loading plot of phytochemical contents and pharmacological parameters. TPC, TFC, α-amylase, and α-glucosidase were found to have significant effects on PC1, while DPPH had strong influences on PC2.

4. Discussion

Diabetes patients are treated by controlling the blood glucose to a normal level, in both the fasting and postprandial states. α-Amylase is responsible for the hydrolysis of a 1,4-glucosidic linkage of starch, glycogen, and oligosaccharides. Then, α-glucosidase found on the brush border interface membrane of intestinal cells further breaks down the disaccharides into glucose, readily available for intestinal absorptions. One of the strategies to control diabetes is to inhibit these two enzymes and reduce the glucose absorption resulting from the breakdown of starch by these enzymes [29]. Therefore, an effective and nontoxic inhibitor of digestive enzymes from medicinal plants has been investigated for a long time [30].

Table 2: Phytochemical identification of extracts.

		B. ciliata					*M. pudica*					*P. emblica*				
Phytochemical	Test performed	C	H	D	E	A	C	H	D	E	A	C	H	D	E	A
Alkaloids	Dragendorff's	+	+	−	+	+	+	−	−	+	+	+	+	+	+	+
Flavonoids	Alkaline reagent	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+
Phenols	Ferric chloride	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Steroids	Steroid test	−	−	−	+	+	−	−	−	+	+	−	−	−	+	+
Terpenoids	Salkowski	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tannins	Braemer's	+	+	−	+	+	+	+	−	+	+	+	+	−	+	+
Glycosides	Keller-Kiliani	+	+	+	+	+	+	−	+	+	+	+	+	+	+	−
Saponins	Foam	+	+	−	+	+	+	−	−	−	+	−	−	−	−	+
Carbohydrate	Molisch's	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Anthraquinones	Anthraquinones	+	+	−	+	+	+	−	−	+	−	+	+	−	+	−

C: crude, H: hexane, D: dichloromethane, E: ethyl acetate, and A: aqueous.

This study is focused on the investigation of the antidiabetic potential of Nepalese medicinal plants, namely, *B. ciliata*, *M. pudica*, and *P. emblica*, which have been used in the formulation of traditional medicine for the treatment of several diseases [7, 8, 12, 14]. Here, phenolic and flavonoid contents of each solvent fraction of individual plants under study were determined. Our findings revealed that among methanolic extracts and their different fraction such as hexane, DCM, EA, and water-based on polarity, methanolic extracts, and EA fractions had shown the highest phenolic and flavonoid contents. Both phenolics and flavonoids play an important role in the antioxidant activity of their redox properties, acting as a reducing agent, as well as donors of hydrogen atoms [31]. Terpenoids are considered primary antioxidants as these compounds can donate hydrogen atoms to radicals, ultimately slowing down the lipid oxidation process [32]. Phytochemical identification revealed the presence of alkaloids, tannins, terpenoids, glycosides, steroids, saponins, and anthocyanin that might inherent the antioxidant activity. For the solvent fraction, ethyl acetate showed strong antioxidant activity with IC_{50} values of $28.15 \pm 0.17\,\mu$g/mL, $46.06\,\mu$g/mL, and $11.98 \pm 0.36\,\mu$g/mL for *B. ciliata, M. pudica,* and *P. emblica*, respectively, followed by medium scavenging activity of methanol and aqueous fractions, which are comparable to the finding of previous reports [33–36]. Similarly, our result resembles the study that showed significant α-glucosidase activity in ethyl acetate, methanol, and aqueous fraction, with strong activity against α-amylase enzyme in ethyl acetate fractions [7, 36–38].

A previous study on *B. ciliata* showed TPC of 473.4 ± 15.1 mg GAE/g and TFC of 89.9 ± 0.1 mg QE/g from methanol and TPC of 249.7 ± 1.3 mg GAE/g and TFC of 208.4 ± 0.6 mg QE/g from ethyl acetate, while in our study the TPC and TFC were reported as 155.83 ± 1.51 mg GAE/g and 47.26 ± 1.21 mg QE/g from methanol and 168.24 ± 1.17 mg GAE/g and 28.49 ± 0.67 mg QE/g from EA fraction. The antioxidant was reported as $53.5\,\mu$g/mL from methanolic leaf extract, $2593.3\,\mu$g/mL from ethyl acetate, and $3026.7\,\mu$g/mL from hexane extract [39], while in our study we found $52.6 \pm 3.63\,\mu$g/mL from methanol, $18.42 \pm 1.29\,\mu$g/mL from EA, and $60.24 \pm 2.19\,\mu$g/mL from hexane. A previous study had shown $84.3 \pm 13.2\%$ inhibition in EA fraction and $65.3 \pm 2.7\%$ inhibition in water fraction partitioned from the methanolic extract. Two phenolic compounds, namely (-)-3-O-galloylcatechin and (-)-3-O-galloylepicatechin, were isolated from ethyl acetate fraction of *B. ciliate* [7]. Both compounds, largely present in tea, act as a strong rat intestinal α-glucosidase and porcine pancreatic α-amylase inhibitor via prohibition of the progressive deterioration of pancreatic beta-cell function as a result of oxidative stress [40].

M. pudica was reported to have TPC values of 28.523 ± 5.296, 57.431 ± 1.096, and 42.550 ± 2.228 mg GAE/g, respectively, for hexane, methanol, and ethyl acetate extract as compared to 41.45 ± 2.6, 131.78 ± 1.53, and 164.21 ± 1.81 mg GAE/g of our study. Likewise, TFC value was found to be 0.927 ± 0.461, 16.97 ± 1.472, and 3.90 ± 0.059 mg QE/g, respectively, whereas our results showed flavonoid content of 11.77 ± 1.18, 20.11 ± 0.75, and 64.89 ± 4.70 mg QE/g. In accordance with IC_{50} values ($81.28 \pm 8.23\,\mu$g/mL for hexane, $34.35 \pm 5.11\,\mu$g/mL for methanol, and $21.39 \pm 3.76\,\mu$g/mL for ethyl acetate) of our research, antioxidant activities for these extracts were also found to be 92.302 ± 0.0077, 7.18 ± 0.0005, and $49.59 \pm 0.0024\,\mu$g/mL, respectively [37]. The study revealed that ethyl acetate extract exhibits better DPPH scavenging activity with a minimum IC_{50} value of $46.06\,\mu$g/mL and higher TPC and TFC values of 15.64 ± 1.31 mg of GAE/100 g and 1.97 ± 0.47 mg of QUE/100 g, respectively, which are following our study. The synergistic effect of bioactive components like flavonoids and phenolic components found in them may be responsible for the antioxidant and biological activity of further inhibiting the progression of oxidative stress-induced disorders [35]. Stigmasterol operates as a metal chelator, peroxide, and lipid peroxide scavenger due to the unsaturation of the rings, which attributes to conjugation [41]. The mechanism of action of quercetin, an antioxidant compound, is due to the combined impact of possessing 3′,4′-dihroxy group coupled with 5′–OH and 3″ substitution, while that of avicularin may be due to sugar moiety linked to the quercetin structure leading to a considerable reduction in molecule's scavenging ability [42, 43]. Similarly, stigmasterol, quercetin, and avicularin isolated from ethyl acetate fraction of *M. pudica* showed

Table 3: Total phenolic and total flavonoid contents of methanolic extracts and their fraction.

	TPC (mg GAE/g)					TFC (mg QE/g)				
Medicinal plants	Crude	Hexane	DCM	EA	Aqueous	Crude	Hexane	DCM	EA	Aqueous
B. ciliata	155.83 ± 1.51	128.24 ± 1.22	87.86 ± 2.45	168.24 ± 1.17	172.58 ± 2.37	47.26 ± 1.21	26.25 ± 1.63	11.28 ± 0.10	28.49 ± 0.67	19.60 ± 3.10
M. pudica	131.78 ± 1.53	41.45 ± 2.6	66.81 ± 0.54	164.21 ± 1.81	152.01 ± 0.53	20.11 ± 0.75	11.77 ± 1.18	10.64 ± 0.32	64.89 ± 4.70	16.46 ± 2.42
P. emblica	171.73 ± 1.22	48.14 ± 2.57	74.78 ± 5.30	172.26 ± 3.61	154.62 ± 3.29	13.12 ± 0.29	18.07 ± 0.52	5.86 ± 0.55	48.04 ± 0.91	20.98 ± 3.36

DCM: dichloromethane; EA: ethyl acetate.

Table 4: Antioxidant activity of different fractions of selected plants.

Medicinal plants	DPPH radical scavenging (IC_{50} value, μg/mL)				
	Crude	Hexane	DCM	EA	Aqueous
B. ciliata	52.60 ± 3.63	60.24 ± 2.19	108.20 ± 2.73	18.42 ± 1.29	16.99 ± 2.56
M. pudica	34.35 ± 5.11	81.28 ± 8.23	118.10 ± 0.76	21.39 ± 3.76	95.06 ± 3.03
P. emblica	22.36 ± 1.95	141.53 ± 10.73	88.85 ± 10.59	11.98 ± 0.36	22.34 ± 2.71
Quercetin (control)			2.86 ± 0.51		

α-glucosidase activity with an IC_{50} value of 91.08 ± 1.54, 75.16 ± 0.92, and 481.7 ± 0.703 μg/mL, respectively [44].

The study done on *P. emblica* showed 439.9 ± 1.3 mg/g TPC and 12.6 ± 0.2 μg/mL IC_{50} values for antioxidant wandhile 62.5 ± 0.7 mg/g TPC and 142.6 ± 5.3 μg/mL IC_{50} values for antioxidant from an aqueous fraction [45]. In our study, 172.26 ± 3.61 mg GAE/g and 154.62 ± 3.29 mg GAE/g TPC were found from EA and water fraction. The IC_{50} values of 11.98 ± 0.36 and 22.34 ± 2.71 μg/mL antioxidant were reported from EA and water fraction. Likewise, IC_{50} values of 5.68% *w*/*v* (56.8 mg/mL) and 0.87% *w*/*v* (8.7 mg/mL) were reported for α-amylase and α-glucosidase in aqueous fractions [36] as compared to 8.22% inhibition at 500 μg/mL and IC_{50} value of 70.52 ± 3.65 μg/mL in our study, respectively. Studies have shown that the major constituents like gallic acid, ellagic acid, and quercetin, along with other natural compounds, are responsible for a strong antioxidant effect as well as an antidiabetic effect [46–48]. Antioxidative stress activity of these compounds is due to their capability to inhibit the release of malondialdehyde (MDA) from RIN cells along with the reduction in the level of nitric oxide (NO) and glutathione (GSH) that are responsible for mitigating inflammatory responses [49, 50]. Gallic acid on improving the translocation and activation of GLUT4 in 3T3-L1 adipocytes [51] and PI3K/p-Akt-dependent pathway [52] exhibits its blood glucose-lowering activity. Besides that, gallic acid also prevents the apoptosis of pancreatic β-cells and acts as an insulin secretagogue [53]. Ellagic acid has antidiabetic properties due to its effect on pancreatic β-cells, which promote insulin production and reduce glucose intolerance [54]. Likewise, quercetin, one of the major constituents found in fruits of this species, is considered a potential antidiabetic drug due to its action via the combined effect of PPAR-γ with glycogen phosphorylase [55]. Similarly, compounds like chebulagic acid and corilagin act as a α-glucosidase inhibitor by inhibiting the glucose absorption [56, 57]. Previously, *in vitro* and *in vivo* study had shown the role of condensed and hydrolyzed tannins to control postprandial blood sugar levels in diabetes via inhibiting salivary and pancreatic α-amylases along with intestinal absorption of starch [58].

Correlation, a statistical analysis, is used to measure the relationship between different variables, with changes in one variable associated with changes in another, in either the same direction (positive correlation) or the opposite direction (negative correlation) [59]. In this study, significant positive correlations were observed between TPC with TFC, DPPH, and α-glucosidase inhibition. However, TFC and α-glucosidase inhibition have a significant correlation with α-amylase inhibition. The phenolic content and antioxidant activity were found to have a significant linear correlation, indicating that phenolic compounds could be responsible for antioxidant activity [35]. An increase in oxidative stress can cause insulin resistance, impaired insulin secretion, and late diabetic complication. Antioxidants, by inhibiting lipid peroxidation, can play an important role in the management of type 1 and type 2 diabetes mellitus [60]. Studies showed that there is a positive correlation between TPC, TFC, and antioxidant activities [61]. As a result, our findings can be correlated to the prior study demonstrating the positive role of phenolic and flavonoid content in free radical scavenging activity [62]. The positive correlation between TPC, TFC, α-amylase inhibition, and α-glucosidase inhibition is also consistent with a previous study which exhibited that TPC, TFC, and digestive enzyme inhibitory activities are positively correlated demonstrating TPC and TFC as the contributors to the inhibition of digestive enzymes [63, 64].

Principal component analysis (PCA) is a multivariate statistical technique used for analyzing the description of large datasets and retrieving the most useful statistics [65]. The PCA was carried out on all variables simultaneously to divide the pattern of variation. In this study, 2 principal components accounted for 80.03% of total variation with 55.81% and 24.23%, respectively. The correlation between original variables and the factors derived from PCA is called factor loading, which ranges from −1 to 1, where the value of −1 or 1 represents a strong correlation between both, while a value close to 0 represents a weak correlation. An absolute value of more than 0.4 represents 16% total variation and should only be interpreted according to Field (2005). All the variables had positive factor loading in PC1 and one variable from the recognized parameter was chosen based on individual loading [66].

The factor score can be used for multivariate classification of different fractions of three mentioned plant species by plotting in the two dimensions with PC1 scores (*x*-axis) against PC2 scores (*y*-axis). The classification based on factor score is shown in Figure 3. The different fraction by variable biplot effectively revealed the visual comparison among all fractions based on multiple variables and also showed interrelationships among the variables. The angles between the vectors and the distance of the fractions from the origin of the biplot were used to extract important information. If the angle between two variables vectors is <90°, then the correlation between the traits is positive; if the angle is >90°, then variables show a negative correlation, while if the angle is equivalent to 90°, then variables show no dependency on each other [67]. The angle between two variables, DPPH and α-glucosidase, was >90°, so there is a negative correlation between them, while the remaining variables have <90° angle

Table 5: α-Amylase and α-glucosidase inhibition and their IC_{50} values of different fractions from medicinal plants.

Medicinal plants	IC_{50} value (μg/mL)									
	α-Amylase					α-Glucosidase				
	Crude	Hexane	DCM	EA	Aqueous	Crude	Hexane	DCM	EA	Aqueous
B. ciliata	59.68 ± 0.69	50.84 ± 2.17	11.10 ± 0.33%*	38.50 ± 1.32	74.26 ± 1.66	26.30 ± 0.56	40.74 ± 1.16	292.97 ± 0.55	3.41 ± 0.04	8.09 ± 0.28
M. pudica	114.83 ± 5.15	337.60 ± 10.33	29.79 ± 1.09%*	110.90 ± 1.61	40.54 ± 0.63%*	13.50 ± 0.56	14.17 ± 0.26	17.59 ± 0.43%*	21.02 ± 0.78	16.62 ± 0.32
P. emblica	9.69 ± 1.28%*	9.08 ± 0.94%*	3.49 ± 0.86%*	306.20 ± 18.5	8.22 ± 1.13%*	80.62 ± 6.45	282.60 ± 4.98	14.79 ± 0.06%*	11.48 ± 0.77	70.52 ± 3.65
Acarbose	3.13 ± 0.14 (μg/mL)					2.06 ± 0.07 (mg/mL)				

*Percentage inhibition at 500 μg/mL.

Table 6: Correlation analysis of phenolics, flavonoids, antioxidants, and enzyme inhibitory effect.

	TPC	TFC	% DPPH scavenging	% α-amylase inhibition	% α-glucosidase inhibition
TPC	1				
TFC	0.553*	1			
% DPPH scavenging	0.611*	0.375	1		
% α-amylase inhibition	0.436	0.652**	0.087	1	
% α-glucosidase inhibition	0.522*	0.456	−0.012	0.637*	1

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed).

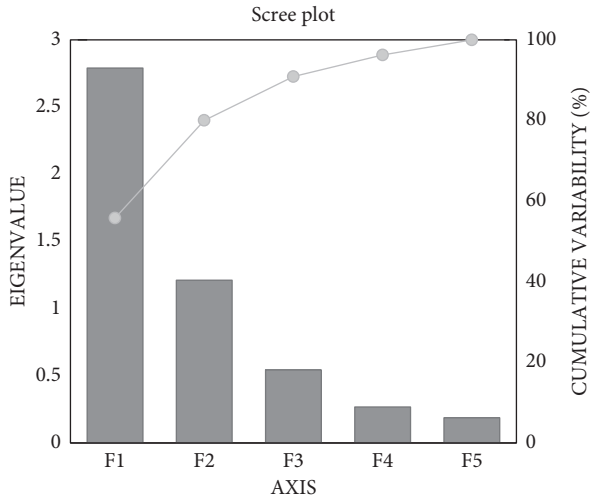

Figure 1: Scree plot showing eigenvalue and cumulative variability of studied parameters.

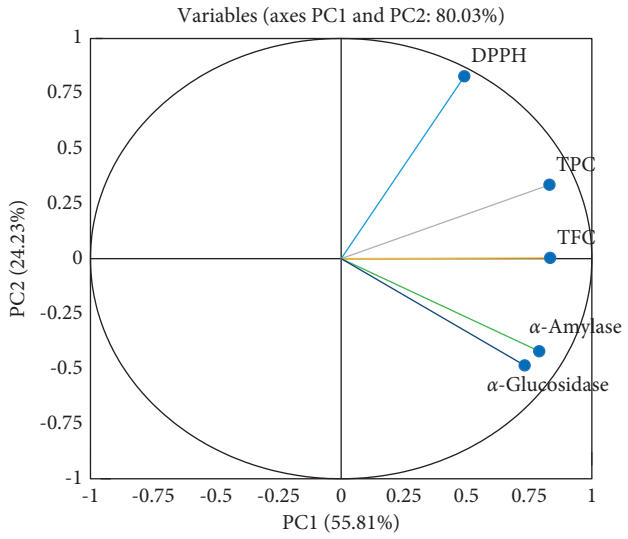

Figure 2: Loading plot of phytochemical content and pharmacological parameters.

and showed a positive correlation. In our study, we observed ethyl acetate, methanol, and aqueous extracts are mainly responsible for their activity against different variables.

Table 7: Principal component analysis for phytochemical content and pharmacological activities of different extracts of selected plants.

Principal component analysis (PCA)					
	F1	F2	F3	F4	F5
Eigenvalue	2.79	1.21	0.54	0.27	0.19
Variability (%)	55.81	24.23	10.87	5.36	3.75
Cumulative (%)	55.81	80.03	90.90	96.25	100.000
Factor loading	F1	F2	F3	F4	F5
TPC	0.831	0.335	−0.333	−0.043	0.290
TFC	0.834	0.003	0.451	−0.317	−0.015
DPPH	0.492	0.828	0.017	0.153	−0.220
α-Amylase	0.791	−0.421	0.245	0.366	0.058
α-Glucosidase	0.732	−0.485	−0.411	−0.089	−0.226

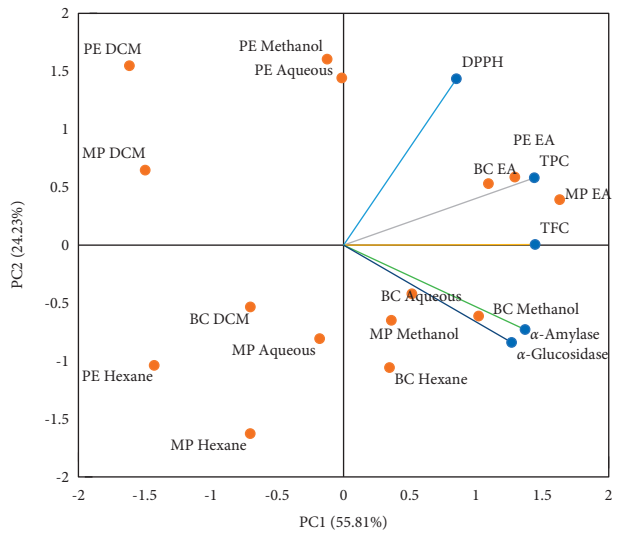

Figure 3: Factor score of 15 fractions of 3 medicinal plants in PC1 (x) and PC2 (y).

5. Conclusions

In conclusion, the study found that the ethyl acetate fraction has greater TPC and TFC content along with the potent antioxidant activity. The inhibitory activity of the ethyl acetate fraction against α-amylase was higher than that of the aqueous fraction against α-glucosidase. Thus, the ethyl

acetate fraction had significant activity as compared to other fractions. The chemical constituents (-)-3-O-galloylcatechin and (-)-3-O-galloylepicatechin from *B. ciliata*, stigmasterol, quercetin, and avicularin from *M. pudica* and chebulagic acid, chebulic acid, corilagin, gallic acid, and ellagic acid from *P. emblica* might be responsible for antioxidant and enzyme inhibitory activity from ethyl acetate fraction. As a whole, our findings support indigenous practices of using *B. ciliata, M. pudica,* and *P. emblica* as therapeutic herbs and provide the basis for their effective use as a significant inhibitor of α-amylase and α-glucosidase for the treatment of diabetes.

Data Availability

The datasets used in this study are available upon reasonable request to the corresponding author.

Conflicts of Interest

There are no conflicts of interest among authors.

Authors' Contributions

N. P. designed and supervised the research; B. K. S. performed research; B. K. S., B. B. T, and K.K. analyzed data; K. K., K. S., B. K. R., D. A., and B. K. S. wrote the manuscript.

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LC-HRMS-Based Profiling: Antibacterial and Lipase Inhibitory Activities of Some Medicinal Plants for the Remedy of Obesity

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Abstract: Globally, obesity is a serious health concern that causes numerous diseases, including type 2 diabetes, hypertension, cardiovascular diseases, etc. Medicinal plants have been used to aid in weight loss since ancient times. Thus, this research is focused on the exploration of pancreatic lipase inhibitory activity and secondary metabolite profiling of *Bergenia ciliata*, *Mimosa pudica*, and *Phyllanthus emblica*, selected based on an ethnobotanical survey. The lipase inhibition was investigated using 4-nitrophenyl butyrate (p-NPB) as a substrate. To uncover further therapeutic potentials of these medicinal plants, antimicrobial activity and minimum inhibitory concentration (MIC) of the extracts were also determined. The ethyl acetate plant extracts showed higher antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, and *Shigella sonnei*. The MIC of ethyl acetate extracts of medicinal plants considered in this study ranges from 1.56 to 6.25 mg/mL. The hexane fraction of *Mimosa pudica* and *Phyllanthus emblica* showed a higher lipase inhibitory activity as compared to others, with IC_{50} values of 0.49 ± 0.02 and 2.45 ± 0.003 mg/mL, respectively. In the case of *Bergenia ciliata*, the methanolic extract inhibited lipase more effectively than others, with an IC_{50} value of 1.55 ± 0.02 mg/mL (IC_{50} value of orlistat was 179.70 ± 3.60 µg/mL). A mass spectrometry analysis of various solvent/solvent partition fractions (extracts) revealed 29 major secondary metabolites. The research offers a multitude of evidence for using medicinal plants as antiobesity and antimicrobial agents.

Keywords: medicinal plants; lipase; antibacterial activity; mass spectrometry

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1. Introduction

A metabolic disorder is one of the known underlying reasons for the rise in obesity, and abdominal obesity is a direct or indirect consequence of a group of metabolic risk factors that also cause type II diabetes, cardiovascular disease, and non-alcoholic fatty liver disease [1]. The World Obesity Atlas 2022 predicted that approximately one billion people will be living with obesity globally by 2030 [2]. Prolonged maintenance of significant weight loss persists as a challenging problem in obesity treatment.

Pancreatic lipase inhibition is a considerable approach to treating metabolic syndrome since it is liable for 50–70% of all-out dietary fat hydrolysis. [3]. Alternative lipase inhibitors have piqued the interest of researchers because some lipase inhibitors have been suggested as effective weight-controlling medications. Additionally, the inordinate buildup of lipids in the pancreas is a leading cause of type II diabetes, which incites the dysfunction of insulin-producing pancreatic β-cells [4]. Intestinal lipase catalyzes the breakdown of triacylglycerols into fatty acids and glycerol in the intestinal lumen [5]. Slackening the lipolytic process can protect the pancreas by minimizing lipid absorption and eventually restoring regular insulin production [6]. Several FDA-approved antiobesity medications, such as orlistat, lorcaserin, topiramate extended-release, phentermine, naltrexone sustained-release, and liraglutide (injectable formulation), are available on the market [7]. Each drug fluctuates in its after-effect profiles and efficacy. Orlistat covalently

bonds to serine at the active site of lipase but is also associated with several gastrointestinal adverse effects [8].

Numerous studies on natural products for obesity management also yielded positive results in terms of long-term safety, mode of action, and metabolic activity [9]. The long history of using natural products for weight loss demonstrates a preference for investigation over synthetic drugs [10]. *Vitis vinifera*, *Rhus coriaria*, *Origanum dayi*, *Averrhoa carambola*, *Archidendron jiringa*, *Cynometra cauliflora*, and *Aleurites moluccana* imply their potential for obesity treatment by antilipase activity [11]. *Phyllanthus emblica* widely known as Indian gooseberry or amla, and belonging to the Euphorbiaceae family, is a significant herbal remedy utilized in both the Unani (Graceo—Arab) and Ayurveda traditions of medicine. The fruit, which has been used in traditional medicine and Ayurveda as a robust Rasayana to cure diarrhea, jaundice, and inflammation, is the most commonly utilized portion of the plant for medicinal purposes out of all its parts [12]. Similarly, the pharmacological profile of *Mimosa pudica* L. (Mimosaceae), commonly known as the touch-me-not, live-and-die, and shame plant, suggests that it is a good herbal candidate for further investigation. The plant has a long history of usage in traditional medicine, having been applied to wounds and used to treat piles, dysentery, sinuses, and urogenital diseases [13]. Additionally, *B. ciliata* has indeed been reported as a remedy for over 100 ailments, with the greatest potential in the solution of gastrointestinal problems. Hence, the investigation of the unexplored potential of medicinal plants could result in alternative lipase inhibitors with minimal side effects.

Bacterial infections are viewed as a global concern and are thus acknowledged as a threat to human life. Resistance to antibacterial and antifungal medications has intensified in recent years, amplifying serious concerns for global health. As a consequence, infectious diseases are now more challenging to treat in the healthcare system. This resistance is due to the misuse of antibiotics [14]. The prime factor for antibiotic resistance or drug failure is due to formation of biofilm by microorganisms, which can be overcome by using alizarin as a natural antibiofilm agent [15]). Additionally, different natural products, such as flavonoids, alkaloids, polyphenols, and many other phytochemicals, have been evidenced to display antimicrobial activity [16]. Plants with a diverse range of secondary metabolites, such as *P. emblica* [17] and *B. ciliata* [18], provide an appealing conclusion of potential phytochemicals to control microbial diseases. Similarly, a recent study also revealed that the modified polymeric form of Gum kayara polysaccharides exhibits compelling antibacterial activity against various bacteria [19].

Identification of secondary metabolites is an important prerequisite in validating and acquiring a decisive result in the analysis of plant bioactivities. Metabolic profiling allows for comprehensive analyses of a wide range of metabolites, which greatly increases the value of common findings of plant bioactivities [20]. The complementary analytical platform of liquid chromatography-mass spectrometry (LC-MS) is used to identify a wide range of primary and secondary metabolites [21]. Recent advancements in mass spectrometry with advanced data processing technology allow for the simultaneous measurement of hundreds of chemically different metabolites and investigate more thoroughly the regulation of metabolic networks to study their influence on complex traits as well [22].

The current study is focused on the identification of the lipase inhibitory activity of *B. ciliata*, *M. pudica*, and *P. emblica*, followed by their antimicrobial studies against four microbial strains**.** Moreover, this study is also aimed at secondary metabolite profiling, using LC-HRMS to precisely measure the mass of unknown molecules, parent ions, and fragment ions of the plant extracts. The overview of this study is represented in Figure 1.

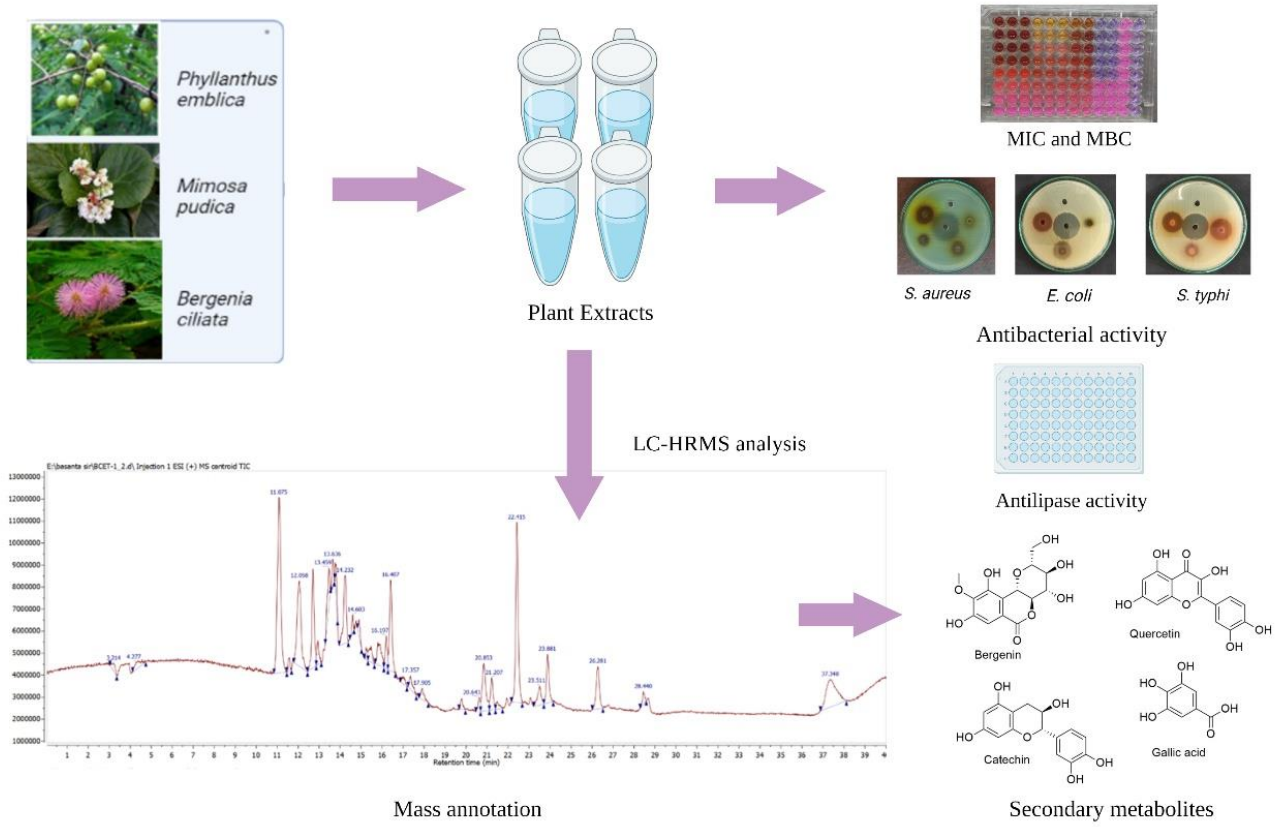

Figure 1. Overview of the study.

2. Materials and Methods

2.1. Chemicals and Reagents

Methanol, ethanol, ethyl acetate, dichloromethane, and hexane were purchased from Thermo Fisher Scientific (Powai, Mumbai, India). Resazurin was purchased from HiMedia (Thane West, Maharashtra, India). The lipase from the porcine pancreas (Type II), 4-nitrophenyl butyrate (p-NPB), orlistat, and neomycin were obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Plant Collection and Extract Preparation

M. pudica and *P. emblica* were collected from Shankar Nagar (27°39′48.3″ N; 83°28′52.3″ E), Rupandehi, Nepal, and *B. ciliata* were collected from Shantipur (28°11′24″ N; 82°13′48″ E), Gulmi, Nepal. Their taxonomy was authenticated and verified by the National Herbarium and Plant Laboratory (KATH), Godavari, Nepal. Plant materials harvested in the same climatic session were dried in the shade at room temperature. They were then pulverized by a grinder and drenched in methanol for 1 day. Subsequently, a cold percolation method was carried out and incubated for the next day, followed by filtration (Whatman filter paper 1). The procedure was done recurrently for 3 days in a row. The rotary evaporator was used under reduced pressure at 40 °C to evaporate the collected methanol from primary extracts, whereas the secondary extracts were prepared after the solvation of the primary extract in water followed by fractionation processes with different solvents, such as hexane, dichloromethane, and ethyl acetate-based on polarity.

The ethnobotanical uses and the pharmacological studies of the selected plant with the voucher specimen are listed in Table 1.

Table 1. List of selected medicinal plants for the study with their reported traditional uses.

Medicinal Plant	Family	Voucher Specimen	Indigenous Uses	Pharmacological Studies
Bergenia ciliata	Saxifragaceae	BS-02	Treatment of diarrhea, vomiting, fever, cough, diabetes, cancer, pulmonary disorders, and wound healing [23].	*B. ciliata* has antibacterial, anti-inflammatory, anticancer, antitussive, antidiabetic, antilithotriptic, antidiabetic, and antimicrobial properties [23].
Mimosa pudica	Fabaceae	BS-04	Treatment of urogenital disorders, piles, dysentery, sinusitis, and wounds [13].	Pharmacological activity as an antidiabetic, antitoxin, antihepatotoxic, antioxidant, and wound healer [13].
Phyllanthus emblica	Phyllanthaceae	BS-05	It is used to treat diarrhea, jaundice, and inflammation, and as a powerful Rasayana (life-extension technique) [24].	*P. emblica* has previously been reported to have antimicrobial, antioxidant, anti-inflammatory, analgesic, antipyretic, adaptogenic, hepatoprotective, antitumor, and antiulcerogenic potential [24]

2.3. Lipase Assay

The porcine pancreas lipase inhibition assay was performed by modifying the method previously reported [25]. The 20 µL of plant extracts, 40 µL of lipase, and 100 µL of 0.1 mM PBS were taken at pH 8.0 and incubated at 37 °C for 15 min. The initial absorbance was observed at 405 nM.

Henceforth, the 40 µL of 3 mM substrate and p-NPB prepared in ethanol was added to each well and incubated at 37 °C for 30 min. The final absorbance was noted at 405 nm (SynergyLX, BioTek, Winooski, VT, USA). Orlistat and 30% DMSO were used as positive and negative controls, respectively. The lipase inhibitory activity was calculated using the given formula:

$$\text{Inhibition } \% = \left(\frac{\text{ODcontrol} - \text{ODtest sample}}{\text{ODcontrol}}\right) \times 100$$

2.4. Antibacterial Assays

The antibacterial assay was performed using the agar well diffusion method [26]. The test microorganisms were inoculated in Mueller Hinton Broth and incubated at 37 °C until the turbidity matched 0.5 McFarland. Then, the lawn culture of test microorganisms was performed in Mueller Hinton Agar (MHA), with 1.5×10^8 CFU/mL microbial inoculum. Five wells were made on the lawn cultured MHA plate with the help of a sterile cork borer. A total of 1 mg/mL neomycin was used as a positive control, and 50% DMSO was used as a negative control. The plates were then incubated at 37 °C for 18–24 h, and the zone of inhibition was measured.

2.5. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The minimum inhibitory concentration was done according to the Clinical and Laboratory Standards Institute (CLSI) [27]. The sterile 96-plate with a flat bottom was used for 2-fold serial dilution of the extracts in MHB. Then, a bacterial concentration of 10^6 CFU/mL was used in each well except for the negative control. The plate was then covered with a lid and incubated at 37 °C for 18–24 h. After incubation, resazurin was added to each well at a 0.003% concentration and left for 3–4 h of incubation at the same temperature. The lowest concentration with a blue color was considered MIC and for the determination of MBC, the concentration with MIC and above were streaked in nutrient agar plates and incubated for 18–24 h at 37 °C. The resazurin is converted to pink by the reductase enzyme of bacteria, so it is considered bacterial growth, whereas the blue color showed no bacterial growth. The MIC was done in duplicate and triplicate.

2.6. Statistical Analysis

The Gen5 Microplate Data Collection and Analysis Software was used for the processing of results, followed by MS Excel. The data were expressed as mean ± standard error of the mean. The IC_{50} values were determined using GraphPad Prism version 8 (San Diego, CA, USA)

2.7. LC-HRMS Analysis

The LC-HRMS analyses of ethyl acetate and the hexane fraction were carried out using an Agilent 6520, Accurate-Mass Q-TOF Mass Spectrometer outfitted with a G1311A quaternary pump, a G1329A autosampler, and a G1315D diode array detector (DAD). The aforementioned parameters were set for the source and scan: gas temp: 30 °C, gas flow: 11.01/min, nebulizer: 40 psi, VCap: 3500, fragmentor: 175, skimmer 1: 65.0, and octopole RF Peak: 750. The components of the solvent elution included acetonitrile (ACN), a 5 mM acetate buffer, and water, which was carried out at a flow rate of 1.5 mL/min. The elution gradient was initiated with 5% acetonitrile for 0.1 min, followed by 30% acetonitrile for 10 min, 80% acetonitrile for 32 min, and eventually back to the initial conditions. Throughout the procedure, the column temperature was kept consistent at 30 °C. The column elute was channeled to Q-TOF HRMS fitted with an electrospray interface after passing through the flow cell of the diode array detector. Positive electrospray ionization (ESI-positive mode) was used to analyze the mass spectrum in the mass range of 100–2000 Daltons at a scan rate of 1.03 [28].

The collected data was analyzed using Gen5 Microplate Data Collection and Analysis Software, followed by MS Excel. Using GraphPad, the 50% inhibition of enzymatic hydrolysis of the substrate (IC_{50}) was determined. Each experiment was performed in triplicate, and the data were shown as mean ± standard deviation. Mestre Nova 12.0 was used to process and analyze data files from the LC-HRMS for compound annotation using PubChem, Dictionary of Natural Products 2, ChemSpider, and the METLIN database.

3. Results

3.1. Lipase Inhibition

At different concentrations, the ability of particular medicinal plants to inhibit lipase was tested. Lipase inhibition was tested at 5 mg/mL, and further dilution of different concentrations was performed based on the screening results. When compared to the IC_{50} value of orlistat, a positive control (179.70 ± 3.60 µg/mL), the results showed moderate to poor activity (IC_{50} values: 0.82 ± 0.05 to 5.37 ± 0.07 mg/mL). Among all fractions, crude, hexane, and EA showed higher activity than DCM and aqueous fractions. Table 2 shows the results for lipase inhibition.

Table 2. Lipase inhibition at different concentrations of medicinal plants and their IC_{50} values.

Standard/Plants	Fractions	Concentration	% Inhibition	IC_{50} Value
Orlistat (μg/mL)	-	500	65.66 ± 0.40	179.70 ± 3.60
		250	54.76 ± 1.38	
		125	44.61 ± 1.73	
		62.5	33.64 ± 3.81	
Bergenia ciliata (mg/mL)	Crude	2.5	79.05 ± 1.18	1.07 ± 0.03
		1.25	57.42 ± 1.21	
		0.625	28.60 ± 2.26	
	Hexane	5	62.49 ± 0.63	1.55 ± 0.02
		2.5	54.54 ± 1.41	
		1.25	48.00 ± 0.44	
	DCM	10	93.33 ± 3.88	3.11 ± 0.10
		5	57.39 ± 1.49	
		2.5	46.00 ± 1.54	
	EA	2.5	54.90 ± 0.39	2.01 ± 0.08
		1.25	38.74 ± 2.06	
		0.625	22.23 ± 3.90	
	Aqueous	5	59.37 ± 1.42	1.99 ± 0.17
		2.5	52.83 ± 1.56	
		1.25	45.26 ± 0.61	
Mimosa pudica (mg/mL)	Crude	2.5	79.35 ± 1.70	1.33 ± 0.05
		1.25	44.86 ± 2.81	
		0.625	19.94 ± 3.76	
	Hexane	1	73.68 ± 1.49	0.49 ± 0.02
		0.5	49.42 ± 0.75	
		0.25	26.18 ± 3.34	
	DCM	10	77.32 ± 1.06	5.37 ± 0.07
		5	45.02 ± 1.16	
		2.5	20.49 ± 0.96	
	EA	1.25	71.51 ± 4.71	0.82 ± 0.05
		0.625	34.17 ± 0.22	
		0.3125	18.78 ± 1.82	
	Aqueous	5	68.85 ± 1.73	1.84 ± 0.09
		2.5	55.72 ± 0.97	
		1.25	42.68 ± 1.74	
Phyllanthus emblica (mg/mL)	Crude	10	34.68 ± 0.14	-
		5	22.15 ± 1.48	
		2.5	17.66 ± 1.79	
	Hexane	5	73.02 ± 1.09	2.45 ± 0.03
		2.5	45.90 ± 0.97	
		1.25	34.82 ± 1.35	
	DCM	5	53.88 ± 0.85	4.19 ± 0.09
		2.5	37.56 ± 2.39	
		1.25	14.39 ± 3.67	
	EA	10	82.87 ± 1.22	3.64 ± 0.12
		5	60.86 ± 3.92	
		2.5	36.80 ± 1.49	
	Aqueous	10	32.10 ± 1.63	-
		5	19.67 ± 0.47	
		2.5	10.38 ± 1.15	

3.2. Analysis of Antimicrobial Activity

Different fractions of plant extracts were tested for antibacterial activity against ATCC strains of bacteria (Figure 2). The tested strains were: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25923, *Salmonella typhi* ATCC 14028, and *Shigella sonnei* ATCC 25931. The details of antibacterial activity with ZoI are displayed in Table 3.

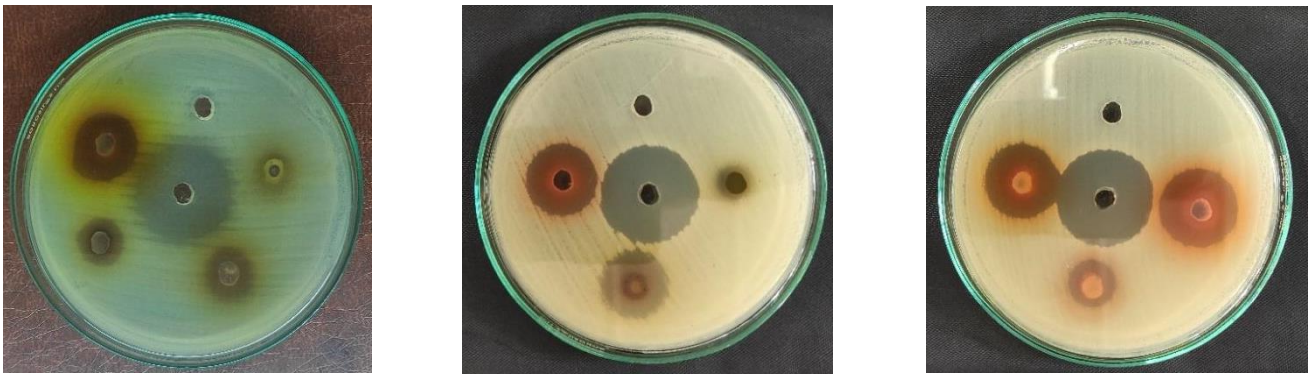

Figure 2. Antibacterial assays of plant extracts against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25923, *Salmonella typhi* ATCC 14028, and *Shigella sonnei* ATCC 25931.

Table 3. Zone of inhibition of different solvent fractions of plant extracts against *B. ciliata*, *M. pudica*, and *P. emblica*.

Microorganism	Zone of Inhibition (mm)																
	B. ciliata					*M. pudica*					*P. emblica*					Neomycin	50% DMSO
	C	H	D	E	A	C	H	D	E	A	C	H	D	E	A		
S. aureus	20	13	13	21	20	19	8	-	27	12	18	15	19	28	17	27	-
E. coli	18	13	9	21	18	8	-	-	12	-	-	9	-	11	-	17	-
S. typhi	13	10	9	14	11	12	-	-	17	12	11	7	14	14	8	23	-
S. sonnei	23	15	10	25	22	23	12	-	30	21	23	17	21	28	21	30	-

Note: C = crude, H = hexane, D = DCM, E = ethyl acetate, and A = aqueous.

3.3. Determination of MIC and MBC

The highest zone of inhibition against the tested microorganisms was seen in the ethyl acetate fraction from all plants which was then subjected to the determination of MIC/MBC (Figures 3 and S1). The MIC value ranges from 1562.5 to 6250 μg/mL, while the MBC ranges from 6250–12,500 μg/mL. Neomycin, the positive control, had demonstrated strong activity against the test microorganisms. Table 4 shows the details of MIC and MBC.

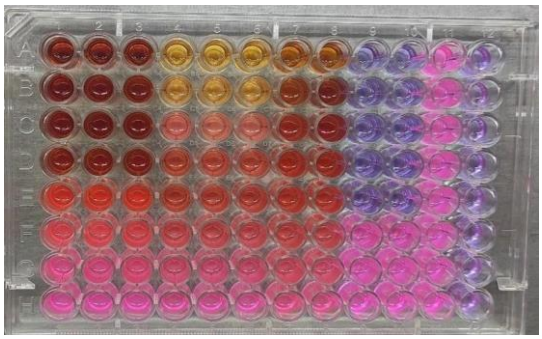

Figure 3. MIC of different extracts and antibiotics against *E. coli*: 1–3: *B. ciliata* (A–H: 12.5–0.098 mg/mL), 4–6: *P. emblica* (A–H: 12.5–0.098 mg/mL), 7–8: *M. pudica* (A–H: 12.5–0.098 mg/mL), 9–10: Antibiotic (Neomycin A–H: 250–1.95 μg/mL), 11: Positive control (A–H: Media + bacteria), 12: Negative control (A–H: Media only).

Table 4. Minimum inhibitory and minimum bactericidal concentration of plant extract ethyl acetate fraction.

Microorganism	Concentration (μg/mL)							
	B. ciliata		*M. pudica*		*P. emblica*		Neomycin	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
S. aureus	1562.5	12,500	3125	12,500	6250	12,500	1.56	12.5
E. coli	1562.5	6250	1562.5	12,500	6250	12,500	15.63	62.5
S. typhi	3125	6250	1562.5	12,500	3125	6250	1.56	12.5
S. sonnei	1562.5	12,500	3125	12,500	3125	12,500	1.56	6.25

3.4. LC-HRMS-Based Molecular Annotation

The raw LC-HRMS data were processed, and the fraction with the best total ion chromatogram (TIC) was considered for the study with the MestreNova 12.0 software (Mestrelab Research, Santiago de Compostela, Spain). Table 5 shows the details of the identified compounds, along with their theoretical and observed mass-to-charge ratio, double bond equivalence (DBE), molecular formula, and absolute errors in parts per million (ppm) and retention time (Rt) in the positive ion mode in ESI. The mass spectra of *B. ciliata*, *M. pudica*, and *P. emblica* are shown in Figures S2–S4.

Based on the observed mass spectra, the compounds were identified, and the results were evaluated by comparing them to literature data. Structures of secondary metabolites identified based on the mass spectra of *B. ciliata*, *M. pudica*, and *P. emblica* were drawn using Chemdraw (Figure 4). In the ethyl extract of *P. emblica*, we observed the presence of phenolic compounds methyl gallate (*m*/*z* = 185.05), gallic acid (*m*/*z* = 171.02), flavonoids quercetin (*m*/*z* = 303.05), an isoflavone irisflorentin (*m*/*z* = 387.1), galloyl-hexahydroxydiphenoyl (HHDP)-glucose—a hydrolysable tannin (*m*/*z* = 483.07), derivatives of hydroxycinnamic acid 2-O-Caffeoylhydroxycitric acid (*m*/*z* = 371.06), 3,4,8,9,10-pentahydroxydibenzo [b, d]pyran-6-one (*m*/*z* = 277.06), isoquercetin (*m*/*z* = 465.1), prodelphinidin B3 (*m*/*z* = 595.14), cassiaoccidentalin B (*m*/*z* = 576.15), trihydroxydimethoxyflavone (*m*/*z* = 331.08), aflotaxin B1 (*m*/*z* = 329.06), kaemferol (*m*/*z* = 287.05), emodin (*m*/*z* = 271.06), isorhamnetin (*m*/*z* = 317.06), and trigalloyllevoglucosan IX (*m*/*z* = 619.09). The phytochemicals annotated in the ethyl extract of *M. pudica* are catechin/epicatechin (*m*/*z* = 291.08), gallocatechin/epigallocatechin (*m*/*z* = 307.8), procyanidin B1/procyanidin B3 (*m*/*z* =578.15), chlorogenic acid (*m*/*z* = 355.10), vitexin (*m*/*z* = 433.11), and myricetin (*m*/*z* = 319.04), In addition, important phytochemicals observed in the fraction of *B. ciliata* are bergenin (*m*/*z* = 329.08), afzelechin/epiafzelechin (*m*/*z* = 275.08), orientin (*m*/*z* = 449.10), and diosmetin (*m*/*z* = 301.07).

Table 5. Secondary metabolites identified from *B. ciliata*, *M. pudica*, and *P. emblica* through mass spectrometry.

Annotated Compounds	Calculated Mass	Observed Mass (*m*/*z*)	Formula	DBE	Absolute Error (ppm)	Rt Minute	Fragment Peak	Source	Reference
Bergenin	328.08	329.08	$C_{14}H_{16}O_9$	7.0	2.84	11.20	314.78; 251.05; 237.07; 194.40	*B. ciliata*	[29]
Afzelechin	274.08	275.08	$C_{15}H_{14}O_5$	9.0	0.29	13.28	257.17, 233.08	*B. ciliata*	[30]
Epiafzelechin	274.08	275.08	$C_{15}H_{14}O_5$	9.0	0.29	13.28	257.17, 233.08	*B. ciliata*	[31–33]
Orientin	448.10	449.10	$C_{21}H_{20}O_{11}$	12.0	3.18	16.34	329.36; 299.30	*B. ciliata*	[34]

Catechin	290.07	291.08	$C_{15}H_{14}O_6$	9	1.25	12.22	313.07 [M + Na]$^+$, and 139.03	*M. pudica*	[35,36]
Epicatechin	290.07	291.08	$C_{15}H_{14}O_6$	9	1.25	12.22	313.07 [M + Na]$^+$, and 139.03	*M. pudica*	[35–37]
Trihydroxydi-methoxyfla-vone	330.07	331.08	$C_{17}H_{14}0_7$	11	0.90	15.83	301.08, and 315.09	*B. ciliata*	[38]
Gallocatechin	306.07	307.08	$C_{15}H_{14}O_7$	9	0.77	10.16	329.07 [M + Na]$^+$, 289.07, 139.03	*M. pudica*	[36]
Epigallocate-chin	306.07	307.08	$C_{15}H_{14}O_7$	9	2.26	7.15	329.07 [M + Na]$^+$, 289.07, 139.03	*M. pudica*	[36,37,39]
Procyanidin B1	578.15	579.15	$C_{30}H_{26}O_{12}$	18	0.01	11.82	427.10 [M + H -152] +, 289.07 (kaempfero l)	*M. pudica*	[36,40]
Procyanidin B3	578.15	579.15	$C_{30}H_{26}O_{12}$	18	0.01	11.82	427.10 [M + H -152] +, 289.07 (kaempfero l)	*M. pudica*	[36,41]
Chlorogenic acid	354.09	355.10	$C_{16}H_{18}O_9$	8.0	0.68	11.97	193.02	*M. pudica*	[42,43]
Vitexin	432.11	433.11	$C_{21}H_{20}O_{10}$	12.0	1.81	14.30	343.04; 313.07; 285.14	*M. pudica*	[44]
Myricetin	318.03	319.04	$C_{15}H_{10}O_8$	11	4.58	14.51	181.05; 153.01	*M. pudica*	[45,46]
Isoquercetin	464.09	465.1	$C_{21}H_{20}O_{12}$	12	3.59	14.72	303.05 (Querce-tin), 289.07 (Kaempfer ol)	*P. emblica*	[46]
Prodelphinidin B3	594.13	595.14	$C_{30}H_{26}O_{13}$	18	3.23	14.79	427.08, 169.07, 291.09, 305.07	*P. emblica*	[40,46,47]
Cassiaoccident-talin B	576.15	577.15	$C_{27}H_{28}O_{14}$	14.0	3.66	15.33	-	*P. emblica*	[48]
Aflotaxin B1	328.06	329.06	$C_{17}H_{12}O_7$	12.0	0.18	16.20	-	*P. emblica*	[49]
Kaempferol	286.04	287.05	$C_{15}H_{10}O_6$	11	0.90	18.33	259.13, 165.09, 153.12	*P. emblica*	[50]
Emodin	270.05	271.06	$C_{15}H_{10}O_5$	11	1.73	19.28	253.16, 243.17, 229.14, 225.13 and 197.08	*P. emblica*	[51]
Isorhamnetin	316.05	317.06	$C_{16}H_{12}O_7$	11	3.86	18.72	303.21, 274.20, 153.12	*P. emblica*	[52]
Methyl gallate	184.04	185.05	$C_8H_8O_5$	5.0	2.38	12.43	170.97; 127.03	*P. emblica*	[44]
Quercetin	302.04	303.05	$C_{15}H_{10}O_7$	11.0	4.82	15.28	273.12, 257.13	*P. emblica*	[53]

Irisflorentin	386.09	387.1	$C_{20}H_{18}O_8$	12	1.59	11.09	357.09 [M + H − CH_3 × 2] $^+$, 372.07 [M + H − CH_3] $^+$	*P. emblica*	[54–56]
Gallic acid	170.02	171.02	$C_7H_6O_5$	5.0	0.62	7.30	127.03 [M + H-CO_2]$^+$	*P. emblica*	[57]
HHDP-glglucose	482.07	483.07	$C_{20}H_{18}O_{14}$	12.0	2.12	12.07	251.21; 277.03; 303.20;	*P. emblica*	[58]
2-O-Caffeoylhydroxycitric acid	370.05	371.06	$C_{15}H_{14}O_{11}$	9.0	3.23	9.30	-	*P. emblica*	[59]
3,4,8,9,10-Pentahydroxydibenzo [b, d]pyran-6-one	276.04	277.06	$C_{13}H_8O_7$	10.0	1.03	13.54	-	*P. emblica*	[60]
Trigalloyllevoglucosan IX	618.09	619.09	$C_{20}H_{26}O_{22}$	8.0	4.33	13.76	-	*P. emblica*	[61]

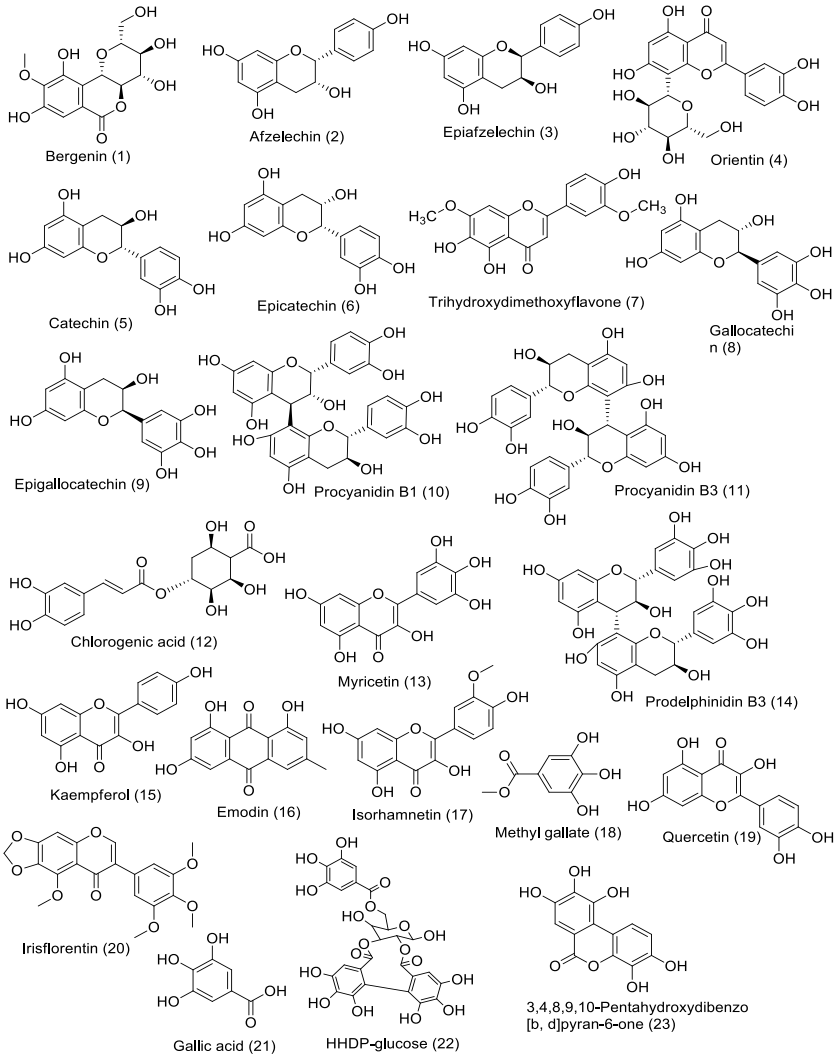

Figure 4. Chemdraw structures of secondary metabolites identified from *B. ciliata*, *M. pudica*, and *P. emblica* through mass spectrometry.

4. Discussion

Obesity is due to the unusual deposition of fat in the body and leads to different types of health problems, such as cardiovascular cancers, diabetes, hypertension, stroke, dyslipidemia, and osteoarthritis. Previous findings suggest that obese and diabetic patients are vulnerable to cardiovascular diseases [62]. These dietary fats are hydrolyzed by different types of lipases, such as tongue, gastric, and pancreatic lipases. Approximately 90% of dietary fats are composed of mixed triglycerides, and pancreatic lipase is responsible for the digestion of 50–70% of dietary fats into fatty acids and monoglycerides. Then, mixed micelles are formed with bile salts, cholesterol, and lysophosphatidic acid to produce triglycerides that are absorbed into enterocytes. The adipocytes present in the body are the main site for the storage of triglycerides and act as the major source of energy [63]. One of the strategies to combat obesity is to inhibit the lipase enzyme. Plant-based inhibitors are gaining popularity as safer, more affordable, and readily available alternatives to synthetic drugs due to their side effects, cost, and availability [64]. The plant contains different constituents, such as polyphenols, saponins, terpenes, flavonoids, and tannins, that are responsible for the inhibition of lipase enzymes [65]. The antilipase activity of our extracts also may be explained by the presence of these compounds, which were reported in our earlier study [66].

In contrast to DCM and aqueous fractions, hexane, methanol, and ethyl acetate fractions demonstrated higher inhibitory activity, according to our research. Methanolic extracts contain a mixture of compounds that might act synergistically to inhibit lipase enzymes. Drug combinations that work well together therapeutically are more prominent and highly effective. By preventing biological compensation, allowing lower dosages of each compound, or gaining access to context-specific multitarget mechanisms, synergistic combinations of two or more agents can overcome the toxicity and other side effects connected with high doses of single drugs [67]. At relatively low concentrations, the combination of kaempferol and orlistat demonstrated the synergistic inhibition of pancreatic lipase. When the combined concentrations of kaempferol and orlistat were less than 114.60 μM and 30.24 μM, the results showed the activity synergistically, however, kaempferol could partially replace orlistat to produce the same antiobesity results. [68]**.** The combination of the three drugs (ECG-EGCG-orlistat) exhibited potent synergy in inhibiting pancreatic lipase [69].

A previous study done on the hexane fraction of *M. flagellipes* and *P. mildbraedii* had shown significant activity against lipase enzyme. Besides that, hexane fraction significantly decreased total glycerides, total cholesterol, and low-density lipoprotein cholesterol as compared to hyperlipidemic control rats from both extracts. The GCMS analysis revealed two major compounds, 9-octadecenoic acid, and hexadecanoic acid, in *P. mildbraedii*, with hexadecanoic acid and 9,12-octadecadienoic acid in *M. flagellipes* [70]. So, the presence of these compounds in our study might be responsible for the inhibition of lipase enzyme from hexane fraction. The antiobesity activity of major constituent bergenin is due to increased norepinephrine-induced lipolysis in endogenous lipid droplets, slightly stimulated adrenocorticotropic hormone-induced lipolysis, and inhibited insulin-induced lipogenesis from glucose in fat cells obtained from rat epididymal adipose tissues [71]. The in vivo experiment in rats significantly reduced serum, cholesterol, triglycerides, and low-density lipoprotein-cholesterol levels after 21 days of oral administration [72].

The ethyl acetate fraction of *M. pudica* contains stigmasterol, quercetin, and avicularin. A previous study showed competitive inhibition by quercetin with an IC_{50} value of 53.05 μM, while non-competitive or mixed inhibition by avicularin with an IC_{50} value of 141.84 μM [73]. Stigmasterol showed weak porcine pancreatic lipase inhibition of 2.7 ± 0.4% at 100 μg/mL as compared to 34.5 ± 5.4% of orlistat at the same concentration [74]. Antidiabetic constituents, such as gallic acid, ellagic acid, chebulagic acid, and quercetin, along with other natural compounds, were reported earlier from *P. emblica* [75]. A previous study showed that chebulagic acid, ellagic acid, and gallic acid showed an IC_{50} value of 57.4 μg/mL, 90 μg/mL, and 5192 μg/mL, respectively, for pancreatic lipase inhibition

[76]. The ethanolic fruit extract of *P. emblica* showed antilipase activity due to decreased triglyceride accumulation and downregulating adiponectin, FABP4, PPARγ, and cEBPα, respectively [77]. There is a rise in the number of multidrug-resistant pathogens. So, to cope with this situation, antimicrobial drugs are in high demand globally, but their production is delayed. For this reason, scientists are now attracted to natural resources. Plants are an easily available option and have been in use since ancient times as an ethnobotanical remedy. A previous study showed that the highest zone of inhibition in EA extracts of *B. ciliata* with ZoI, was 21 mm for *S. aureus* and 11 mm for *E. coli*. Similarly, another study from ethanolic extracts showed ZoI (24.0 ± 0.10) mm against *S.aureus*, (23.7 ± 0.25) mm against *E. coli*, and (22.8 ± 0.15) mm against *S. typhi* at 50 mg/mL [78]. The MIC was reported as 2500 μg/mL [79]. We found the highest zone of inhibition in the EA fraction in all tested bacteria (Table 3), and the MIC value was 1250 μg/mL (Table 4). The antibacterial activity of methanolic extract of *M. pudica* was reported with a value of 15 mm for S. aureus, 20 mm for *K. pneumoniae*, 12 mm for *E. coli*, and 14.5 mm for *S. typhi*, using 5 mg/mL disc [80]. In our study, the following was observed: 19 mm for *S. aureus*, 8 mm for *E. coli*, 12 mm for *S. typhi*, and 23 mm for *S. sonnei* using 50 mg/mL methanolic extracts. In the previous study, the ZoI of *S. aureus* and *S. typhi* was reported as 9 and 8 mm, respectively, from *P. emblica* extract. The MIC and MBC were observed at 50 mcg/mL and 65 mcg/mL, respectively, for *S. aureus* (methanol extract), in contrast to our observed values of 6250 μg/mL and 12,500 μg/mL (ethyl acetate fraction). The MIC and MBC for *S. typhi* were reported as 35 and 45 mcg/mL as compared to 3125 and 6250 μg/mL in our study [81]. Our study revealed that the ethyl acetate fraction is the more potent fraction, followed by the methanolic (crude) extract for antimicrobial activity. The methanolic extracts contain several metabolites, such as phenols, flavonoids, tannins, terpenoids, alkaloids, saponins, glycosides, and steroids. The antimicrobial activity of these phenolic and flavonoid compounds is mediated by different mechanisms. These compounds may have a synergistic effect on antimicrobial activity, whereas the ethyl acetate fractions contain high phenolic and flavonoid content, as previously reported in our study [66]. The antimicrobial activity of these phenolic and flavonoid compounds is mediated by different mechanisms. These include inhibition of nucleic acid synthesis, inhibition of attachment and biofilm formation, inhibition of cytoplasmic membrane function, and alteration of membrane permeability, leading to cell destruction as well as attenuation of pathogenicity [82]. To the best of our knowledge, the lipase inhibitory activity of *B. ciliata, M. pudica,* and *P. embilica* was performed for the first time in Nepal. The study was limited to in vitro testing. However, additional research on the isolation of potent compounds, enzyme kinetics, in silico, and in vivo testing can be conducted.

The isolation and characterization of plant metabolites is still a significant challenge due to the lack of multivariate analyses, funding sources, and laboratory accessibility in our perspective. In this study, LC-HRMS was performed for the identification of bioactive metabolites in the fractions of *B. ciliata*, *M. pudica,* and *P. emblica*. Mestrenova 12.0 software, USA was used to annotate the metabolites based on *m*/*z*, retention time, and molecular formula, and other databases were used to search, assign formulas, and compound structures.

The compounds annotated from the ethyl extracts of *B. ciliata* with the base peak at *m*/*z* 329.08, molecular formula $C_{14}H_{16}O_9$, DBE 7, and fragment peaks at 314.78, 251.05, 237.07, and 194.40 are stated as bergenin [29]. The fragmentation pattern of bergenin is shown in Figure S5. Likewise, the base peak at *m*/*z* 275.08, molecular formula $C_{15}H_{14}O_5$, DBE 9, along with fragment peaks at 257.17 (loss of water) and 233.08 is considered afzelechin/epiafzelechin [32]. The compound with a base peak at *m*/*z* 449.10, molecular formula $C_{21}H_{20}O_{11}$, DBE 12, and the characteristic fragment ions at 431.12 $[M + H\text{-}H_20]^+$ corresponding to the losses of the molecule H_2O (18 Da) between the 2″-hydroxyl group of the sugar and the 5 or 7-hydroxyl group of the aglycone, 329.36 and 299.30 fragment peaks indicate the losses of C_4H_8O (120 Da) and 150 Da from the $[M + H]^+$ molecule is annotated as orientin [34]. In addition, the base peak at *m*/*z* 301.07, molecular formula

$C_{16}H_{12}O_6$, and DBE 11 with characteristic fragment ions 258.12, 153.31, and 149.09 are explicated as diosmetin. The fragmentation pattern of diosmetin is shown in Figure S6. Additionally, a base peak at *m/z* 331.08, molecular formula $C_{17}H_{14}0_7$, DBE 11, and fragment ions at 301.08 and 315.09 are considered trihydroxy methoxy flavone [38]. The fragmentation pattern of trihydroxy methoxyflavone is shown in Figure S7.

The compounds in the extract of *M. pudica* with the base peak at *m/z* 291.08, molecular formula $C_{15}H_{14}O_6$, DBE 9, and fragment peaks at 313.07 $[M + Na]^+$ and 139.03 are speculated to be catechin/epicatechin [35–37]. The fragmentation pattern of catechin and epicatechin is shown in Figure S8. Likewise, $[M + H]^+$ at *m/z* 307.08, molecular formula $C_{15}H_{14}O_7$, DBE 9 and along with fragment peak at 329.07 $[M + Na]^+$, 289.07 and 139.03 is considered as gallocatechin/ epigallocatechin [36–38]. The fragmentation pattern of gallocatechin/epigallocatechin is shown in Figure S9. Compounds with characteristic fragment ions 427.10 [M + H − 152], and 289.07 (kaempferol) and base peak $[M + H]^+$ at *m/z* 579.15, molecular formula $C_{30}H_{26}O_{12}$, and DBE 18 are annotated as procyanidin B1/procyanidin B3 [36,40]. The molecular ion at *m/z* 355.10, molecular formula $C_{16}H_{18}O_9$, DBE 8, and fragment ions at 193.02 is characterized as chlorogenic acid [41,42]. The fragmentation pattern of chlorogenic acid is shown in Figure S10. The base peak at *m/z* 433.11, molecular formula $C_{21}H_{20}O_{10}$, and DBE 12 with fragment peaks of 343.04; 313.07; and 285.14 manifested could be vitexin. The fragmentation peaks at *m/z* 343.04 and 313.07 were formed by the crisscross cleavage of the hexose unit and were formed due to the loss of $C_3H_6O_3$ (90 Da) and $C_4H_8O_4$ (120 Da) from $[M + H]^+$ ion, respectively. Due to the loss of CO (28 Da) from the *m/z* 313.07 ion, the product ions at *m/z* 285.14 were produced [44]. The base peak at *m/z* 319.04 with molecular formula $C_{15}H_{10}O_8$ and DBE 11 with fragments peaking at 181.05 and 153.01 is assigned as myricetin. The fragmentation pattern of myricetin is shown in Figure S11 [31,79]. Likewise, the compound in the extract of *P. emblica* base peak with *m/z* 465.1, molecular formula $C_{21}H_{20}O_{12}$, and the fragment ion at 303.05 (quercetin) and 289.07 (kaempferol) are interpreted as isoquercetin [60]. The fragmentation pattern of isoquercetin is shown in Figure S12. The mass spectrum with a base peak at *m/z* 594.13, molecular formula $C_{30}H_{26}O_{13}$, DBE 18, and fragment peaks at 427.08, 169.07, 291.09, and 305.07 is predicted to be prodelphinidin B3 [40,47]. The fragmentation pattern of prodelphinidin B3 is shown in Figure S13. Moreover, in our spectra base peak with *m/z* 577.15, molecular formula $C_{27}H_{28}O_{14}$, DBE 14 is annotated as cassiaoccidentalin [83]. The molecular ion peak at *m/z* 329.06, molecular formula $C_{17}H_{12}O_7$, and DBE 12, is annotated as aflatoxin B1 [49]. Likewise, the base peak with *m/z* 287.05, molecular formula $C_{15}H_{10}O_6$, DBE 11, and fragment peak at *m/z* 259.13, 165.09, and 153.12 are predicted to be kaempferol [50]. The fragmentation pattern of kaempferol is shown in Figure S14. Additionally, a base peak at *m/z* 271.06, molecular formula $C_{15}H_{10}O_5$, DBE 11, and fragment ions at 253.16, 243.17, 229.14, 225.13, and 197.08 is considered emodin [51]. The fragmentation pattern of emodin is shown in Figure S15. The molecular ion peak at *m/z* 317.06, molecular formula $C_{16}H_{12}O_7$, and DBE 11, and fragment ions at 303.21, 274.20, and 153.12 are annotated as isorhamnetin [52]. The fragmentation pattern of isorhamnetin is shown in Figure S16.

Additionally, the ethyl extract of *P. emblica* with $[M + H]^+$ at *m/z* 185.05, molecular formula $C_8H_8O_5$, DBE 5, and fragment ions at 170.97 and 127.03 is considered methyl gallate [61,84]. The fragmentation pattern of methyl gallate is shown in Figure S17. Base peak *m/z* 303.05, molecular formula $C_{15}H_{10}O_7$, DBE 11, and fragment ions at 273.12 and 257.13 due to the loss of $[Y\text{-}CHO]^+$ and $[CO + H_2O]^+$ is annotated as quercetin [53]. The base peak at *m/z* 387 molecular formula $C_{20}H_{18}O_8$, and DBE 12 with the fragment ions at 357.09 $[M + H - CH_3 \times 2]^+$, 372.07 $[M + H - CH_3]^+$ manifested it could be irisflorentin [54,55]. Moreover, another annotated compound is gallic acid with a base peak at *m/z* 171.02, molecular formula $C_7H_6O_5$ and DBE 5, with the fragment ions peak at 127.03 $[M + H\text{-}CO_2]^+$ [57]. Likewise, $[M + H]^+$ at 483.07, molecular formula $C_{20}H_{18}O_{14}$. DBE 12 along with a fragment peak at m/z 423.04 with glucose ring cleavage *m/z* 303.20 equivalent to an HHDP residue, and *m/z* 277.03 by the decarboxylation of the HDDP moiety is considered as HHDP-glucose [57]. Additionally, $[M + H]^+$ at *m/z* 371.06, molecular formula $C_{15}H_{14}O_{11}$, and DBE 9 are

considered as 2-O-Caffeoylhydroxycitric acid [59]. Base peak *m*/*z* 277.06, molecular formula $C_{13}H_8O_7$, and DBE 10 annotated as 3,4,8,9,10-pentahydroxydibenzo [b, d] pyran-6-one [84]. Likewise, the base peak at *m*/*z* 619.09, molecular formula $C_{20}H_{26}O_{22}$, and DBE 8 is annotated as trigalloyllevoglucosan IX [61]. However, to confirm their various pharmacological significance, additional research on the isolation and characterization of plant extract-derived compounds is required.

5. Conclusions

The current research is centered on the observable evidence of *B. ciliata*, *M. pudica*, and *P. emblica*'s lipase inhibitory action, preceded by their antimicrobial examinations. One of the strategies to combat obesity is to inhibit the lipase enzyme. The antilipase activities of different extracts were evaluated by pancreatic lipase inhibition assay. Among all fractions of the plants, crude, hexane, and EA showed higher activity for lipase inhibition than DCM and aqueous fractions. From the study, it was found that the highest zone of inhibition against the tested micro-organism was in EA fractions of all the plant extracts depicting higher antimicrobial activity than other fractions.

The antilipase activity of a plant is explained by the presence of these compounds, which were reported in our earlier study as well as analyzed through LC-HRMS. A further experiment on the isolation of potent inhibitory compounds and their mechanism of action in animal models is required to favor the drug discovery program.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/scipharm90030055/s1, Figure S1: MBC of antibiotic and *P. emblica* against *E. coli*; Figure S2: Mass spectrum of *Bergenia ciliata*; Figure S3: Mass spectrum of *Mimosa pudica*; Figure S4: Mass spectrum of *Phyllanthus emblica*; Figure S5: Fragmentation pattern of bergenin; Figure S6: Fragmentation pattern of diosmetin; Figure S7: Fragmentation pattern of trihydroxy-dimethoxyflavone; Figure S8: Fragmentation pattern of catechin/epicatechin; Figure S9: Fragmentation pattern of gallocatechin/epigallocatechin; Figure S10: Fragmentation pattern of chlorogenic acid; Figure S11: Fragmentation pattern of myricetin; Figure S12: Fragmentation pattern of isoquercetin; Figure S13: Fragmentation pattern of prodelphinidin B3; Figure S14: Fragmentation pattern of kaempferol; Figure S15: Fragmentation pattern of emodin; Figure S16: Fragmentation pattern of isorhamnetin; Figure S17: Fragmentation pattern of methyl gallate.

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Original Article

Phytochemical Analysis, Antidiabetic Potential and *in-silico* Evaluation of Some Medicinal Plants

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ABSTRACT

Background: The increasing frequency of diabetes patients and the reported side effects of commercially available anti-hyperglycemic drugs have gathered the attention of researchers towards the search for new therapeutic approaches. Inhibition of activities of carbohydrate hydrolyzing enzymes is one of the approaches to reduce postprandial hyperglycemia by delaying digestion and absorption of carbohydrates. **Objectives:** The objective of the study was to investigate phytochemicals, antioxidants, digestive enzymes inhibitory effect, and molecular docking of potent extract. **Materials and Methods:** In this study, we carry out the substrate-based α-glucosidase and α-amylase inhibitory activity of *Asparagus racemosus*, *Bergenia ciliata, Calotropis gigantea, Mimosa pudica, Phyllanthus emblica,* and *Solanum nigrum* along with the determination of total phenolic and flavonoids contents. Likewise, the antioxidant activity was evaluated by measuring the scavenging of DPPH radical. Additionally, antibacterial activity was also studied by Agar well diffusion method. Molecular docking of bioactive compounds from *B. ciliata* was performed via AutoDock vina. **Results:** *B. ciliata, M. pudica,* and *P. emblica* exhibit significant inhibitory activity against the α-glucosidase and α-amylase with IC_{50} (µg/ml) of (2.24 ± 0.01, 46.19 ± 1.06), (35.73 ± 0.65, 99.93 ± 0.9) and (8.12 ± 0.29, no significant activity) respectively indicating a good source for isolating a potential drug candidate for diabetes. These plant extracts also showed significant antioxidant activity with the IC_{50} ranges from 13.2 to 26.5 µg/mL along with the significant antibacterial activity towards *Staphylococcus aureus* and *Klebsiella pneumonia*. **Conclusion:** *Bergenia* extract appeared to be a potent α-glucosidase and α-amylase inhibitor. Further research should be carried out to characterize inhibitor compounds.

Key words: Diabetes, Medicinal plants, α-Amylase, α-Glucosidase, Molecular docking.

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INTRODUCTION

In our meals, we consumed carbohydrates as one of the important sources of energy,[1] for survival whose digestion starts from mouth to intestine. These carbohydrates are hydrolyzed into absorbable monomers via the action of enzymes (α-amylase and α-glucosidase) and hence leading to postprandial hyperglycemia,[2] which eventually leads to diabetes.[3,4] Diabetes is a chronic endocrine metabolic disorder that occurs when the glucose level is raised in the person's blood when the body cannot produce enough insulin or cannot effectively use it. In 2019, 463 million people have diabetes and it is projected to reach 578 million by 2030 and 700 million by 2045.[5] In 2019, it was reported that the prevalence rate of diabetes in Nepalese adults is 4% out of the total adult population with 696,900 sufferings.[5] People with diabetes are also at higher risk of heart, peripheral arterial and cerebrovascular disease, obesity, cataracts, erectile dysfunction, and nonalcoholic fatty liver disease.[6] Retinopathy, nephropathy, and neuropathy are the effects of long-term diabetes.

Various strategies, such as proper diets, regular exercises, and digestive enzyme inhibitors have been used to control blood glucose levels.[7,8] α-Amylase is a calcium metallo-endoenzymes[9,10] that hydrolyze the α-l, 4-glucosidic linkages of starch, amylose, amylopectin, and glycogen,[11] secreted by salivary gland and pancreas in human with similar amino acid composition, mode of action, and optimum pH.[12] α-Glucosidase are exo-enzyme hydrolyzing terminal glycosidic bonds and discharging α-glucose from the non-reducing end of the substrate. Clinically, some potential drugs such as insulin secretagogue sulfonylureas (gliclazide, glimepiride, glyburide), insulin secretagogue non-sulfonylureas (repaglinide, nateglinide), sulphonylureas, biguanides (metformin), thiazolidinediones (rosiglitazone, pioglitazone), intestinal lipase inhibitor (orlistat), and α-glucosidase inhibitors (acarbose, miglitol, voglibose) are commercially available.[13,14] but their high cost, low tolerability possessing severe side effects such as abdominal pain, bloating, flatulence,

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oily stools, diarrhea, development of hypoglycemia, weight gain, liver toxicity, and many more are provoking the researchers to exfoliate the digestive enzyme inhibitors from natural products with negligible side effects.[15,16]

As per world ethnobotanical, 800 restorative plants are utilized for the prevention of diabetes mellitus. Clinical studies demonstrated that only 450 therapeutic plants have diabetic properties from which 109 restorative plants have a total method of activity. Herbal drugs end up being a superior decision over manufactured medications on account of fewer side effects and unfriendly impacts.[17] The search for bioactive compounds from natural products for the development of conventional drugs is now reviving and becoming more commercialized in modern medicine throughout the world.[18] with the latest development of technology in separation methods, spectroscopic techniques, and advanced bioassays. Plants can provide a potential source of hypoglycemic drugs as they contain several phytochemicals.[19,20] incorporating flavonoids, glycosides, alkaloids, saponins (triterpenoid and steroidal glycosides), glycolipids, dietary fibers, polysaccharides, peptidoglycans, coumarins, xanthones, etc., which are thought to have an antidiabetic impact. Flavonoids such as luteolin, apigenin, quercetin dehydrate, kaempferol, fisetin, genisteinmyricetin and daidzein have been shown as inhibitors of α-amylase and α- glucosidase.[21] *Asparagus racemosus, Momordica charantia, Berberis aristata, Azadiracta indica, Holorhena pubences, Eugenia jambolana, Aegle marmelous,* and *Gymnema sylvestre* are the most widely used Nepalese flora for anti-diabetic purposes.[22] The potential antidiabetic activity of Nepalese herb *Bergenia ciliata*, Haw (Pakhanved), comprises two α-glucosidase and α-amylase inhibitors namely (-)-3-O-galloylepicatechin and (-)-3-O-galloylcatechin.[23] Besides, bergenin, catechin, and gallic acid were found predominately on rhizomes, petioles, and leaves of *B. ciliata,*[24,25] 150 bioactive compounds with their activities from *Bergenia* species have been reviewed elsewhere.[26]

Free radicals are constantly being produced in the body during metabolism as they are required to serve various essential functions essential for survival. Hyperglycemia also generates reactive oxygen species (ROS),[27] playing a dual role as both deleterious and beneficial to the living system. The beneficial effect of ROS occurs at low/moderate concentrations and involves physiological roles in cellular responses to anoxia, for example in defense against infectious agents, several cellular signaling systems, and induction of a mitogenic response.[28] Plant-sourced food antioxidants like Vitamin C, Vitamin E, carotenes, phenolic acids, phytate, and phytoestrogens have been recognized as having the potential to reduce disease risk.[29] Through several studies, it was found that plant-derived antioxidant nutraceuticals scavenge free radicals and modulate oxidative stress-related degenerative effects.[30] Extracts from various medicinal plants with biologically active principles are used in ayurvedic preparations are prepared in bulk for commercial purposes.[29]

Mimosa pudica is an annual or perennial herb grown mostly in moist ground or lawns of tropical areas.[31,32] famous as touch me not, live and die, shame and humble plants and shows thigmonastic and seismonastic movements.[33] *M. pudica* has been shown as an antidepressant,[34,35] anticancer,[36] antihelminthic, antifertility,[37] antihepatotoxic,[38] hypolipidemic,[39] antimicrobial,[40] antiviral,[41] antivenom,[42] antiulcer[43] and wound-healing activity.[44] *Bergenia* species has been shown with diverse biological activities such as antimicrobial,[25,45] antimalarial,[46] antipyretic,[26] anti-inflammatory,[47] anti-ulcer,[48] anticancer,[26] anti-urolithic,[26] antioxidant,[49] and antidiabetic.[50] Similarly, *A. racemosus* also showed galactogogue,[51] anti-inflammatory,[52] anti-diabetic,[53] anti-HIV,[54] and fertility activity.[55] Additionally, *C. gigantea* claimed to have different activities such as wound healing,[56] cytotoxic,[57] insecticidal,[58] pregnancy interceptive,[59] antidiabetic,[60] and so on. Nonetheless, other selected medicinal plants i.e. *P. emblica*[61-63] and *S. nigrum*[64] were also reported with diverse ethnopharmacological importance.

Plant-derived therapeutic agents are being used for various diseases and complications from the ancient period. The diversity of species in Nepalese flora offers wide chances for the search for medicinal substances. The assorted variety of species in Nepalese flora offers incredible open doors for the hunt of medicinal substances, the identification of natural inhibitors of digestive enzymes is most probable.

MATERIALS AND METHODS

Chemicals

α-Glucosidase from *Saccharomyces cerevisiae* (CAS: 9001-42-7), 4-Nitrophenyl-α-D-glucopyranoside (pNPG) (CAS: 3767-28-0), α-Amylase from porcine pancreases (CAS No: 9000-90-2), 2-chloro-4-nitrophenyl-α-D-maltotrioside (CNPG3) (CAS No:118291-90-0), Acarbose (CAS No: 56180-94-0) and Quercetin (CAS No: 117-39-5) were purchased from Sigma-Aldrich (Germany).

Collection of medicinal plants

Different parts of medicinal plants were collected from various regions of Nepal based on ethnobotanical use with the help of local healers. They were identified by National Herbarium and Plant Laboratories (Lalitpur, Nepal) and the voucher specimens (from BS-01 to BS-06) are compared and deposited. The name of collected plants is listed in Table 1. Plant parts were shade dried and ground into fine powder.

Preparation of crude extracts

The crude extracts were prepared by using the cold percolation method as the powder was soaked in methanol for 24hr and filtered. The process was repeated for 3 days and then methanol was evaporated using a rotary evaporator below 50°C. The working solution was prepared in 50% dimethyl sulfoxide (DMSO).

Determination of total phenolic contents (TPC)

The TPC was done as previously described Folin-Ciocalteau's method.[65,66] The reaction was done in 200µL final volume by adding 20µL of plant extract, 100µL Folin-Ciocalteau's reagent, and 80µL of sodium carbonate. It was left for 15 min at room temperature and then absorbance was taken at 765 nm using a spectrophotometer. The standard curve was generated using gallic acid of different concentrations and extract concentration was expressed as milligrams of gallic acid per gram dry weight basis of extract (mg GAE/g).

Table 1: Name of plants and parts used in this study.

Voucher Specimen	Scientific Name	Family	Local Name	Parts used
BS-01	*Asparagus racemosus* Willd.	Asparagaceae	Kurilo	Root
BS-02	*Bergenia ciliata* (Haw.) Sternb.	Saxifragaceae	Pakhanved	Stem
BS-03	*Calotropis gigantea* (L.)	Apocynaceae	Aakh	Leaves
BS-04	*Mimosa pudica*L.	Fabaceae	Lajjwati	Whole plant
BS-05	*Phyllanthus emblica* L.	Phyllanthaceae	Amla	Fruit
BS-06	*Solanum nigrum* L.	Solanaceae	Kaligedi	Whole plant

Determination of total flavonoid content (TFC)

The TFC was also done as previously described aluminum trichloride-based method.[67] The reaction was done in 200µL final volume by adding 20µL of plant extract with 110µL distilled water, 60µL ethanol, 5µL aluminum trichloride ($AlCl_3$, 10%), and 5µL of 1 M potassium acetate. Then, it was left for 30 min at room temperature and then absorbance was taken at 415 nm using a spectrophotometer. The standard curve was generated using quercetin of different concentrations and the concentration of the extract was expressed as milligrams of quercetin equivalent per gram dry weight basis of extract (mg QE/g).

In vitro free radical scavenging activity

The antioxidant activity of the extracts was determined by the colorimetric method.[68] with slight modifications. The reaction was done in 200µL by mixing DPPH (0.1 mM) and plant extract in 1:1 volume. Then it was incubated in dark for 15 min and absorbance was taken at 517 nm.[68,69] The % scavenging was calculated by the following formula:

$$\% \text{ Scavanging} = \frac{A_o - A_t}{A_o} \times 100$$

Where A_o = Absorbance of DPPH radical with 50% DMSO and A_t = Absorbance of DPPH radical with test or reference sample.

In vitro α-glucosidase inhibition assay

The α-glucosidase inhibitory activity of crude extracts was done according to Fouotsa *et al.* with slight modification.[70] Various concentrations of 20µL plant extracts were mixed with 20µL enzyme (0.2 Units) along with 120µL 50 mM phosphate buffer saline (pH 6.8) and incubated for 10 min at 37°C. Then, 0.7 mm pNPG as substrate was added and incubated again for 15 min at the same temperature. The absorbance was taken for p-nitrophenyl from the hydrolysis of pNPG at 405 nm in Synergy LX microplate reader with Gene 5 software. The assay was performed in triplicate. The % α-glucosidase inhibitory activity is calculated by the following formula:

$$\% \text{ Inhibition} = \frac{A_o - A_t}{A_o} \times 100$$

Where Ao is the absorbance of enzyme-substrate reaction with 30% DMSO and A_t is the absorbance of enzyme-substrate with plant extract.

In vitro α-amylase inhibition assay

The α-amylase inhibition was done in 200µL volume, the enzyme and substrate were prepared in 50 mM phosphate buffer pH 7.0 with 0.9 % NaCl. Initially, 20µL of various concentrations of plant extracts were mixed with 80µL of PPA (1.5 units/mL) and was incubated at 37°C for 10 min. Then 100µL substrate CNPG3 was added at 0.5 mM incubated again at the same temperature for 15 min. The absorbance was noted at 405 nm for the release of p-nitroaniline.[71] The assay was done in triplicate by using a microplate reader (SynergyLX, BioTek, Instruments, Inc., USA). The percentage of inhibition was calculated as:

$$\% \text{ Inhibition} = \frac{A_o - A_t}{A_o} \times 100$$

Where A_o is the absorbance of enzyme-substrate reaction with 30% DMSO and A_t is the absorbance of enzyme-substrate with plant extract.

Antibacterial assay

The agar well diffusion method was used for antibacterial activity.[72] The inoculum turbidity in Mueller-Hinton broth (MHB) was matched with 0.5 McFarland standard resulting in 1.5×10^8 CFU/mL. Then, lawn cultured was done in a Mueller- Hinton Agar (MHA) plate using a sterile cotton swab with matched inoculum turbidity. The well was prepared by using a sterile cork borer of 6 mm and 50µL of plant extract (50 mg/mL) along with positive control neomycin (1mg/mL) and negative control 50% DMSO was placed in a different well. It was then left for 15 min to allow diffusion and incubated at 37°C for 18-24hr. The zone of inhibition was measured in mm.

Molecular docking study

The PDB structure of PPA (PDB ID: 1OSE),[73,74] and isomaltase (PDB ID: 3A4A),[75] was taken from protein database (http://www.rcsb.org) and molecular docking was done using AutoDock 4.2.6 program.[76] The water molecules and ligands were removed from the protein structure before performing docking. The 3D structures of the most active compounds were taken from NCBI PubChem and were converted to a PDB file using PyMol Molecular Graphics System (San Carlos, CA, USA) and finally to pdbqt file using AutoDock 4.2.6. The cubic grid dimensions were set at 88 × 104 × 104 and was placed in coordinates x = 35.098, y = 31.028, z = 15.155 for PPA while for isomaltase cubic grid dimension were set at 50 × 50 × 50 and was placed in coordinates x = 22.6225, y = – 8.069, z = 24.158 as previously described with a spacing of 0.375 Å. The docking of the active compound was done with isomaltase instead of α-glucosidase because till now no report of the crystallographic structure of *S. cerevisiae* α-glucosidase is reported which was used in our *in vitro* assay. The reason to choose *S. cerevisiae* isomaltase for docking was due to its 71% identity and 84% similarity toward the *S. cerevisiae* α-glucosidase.[77,78] Finally, the best pose of ligand was used for analyzing the interactions of enzyme and inhibitor via Biovia Discovery Studio 4.0.

ADMET analysis

The parameters of absorption, distribution, metabolism, excretion, and toxicity were checked by using the pkCSM web server.[79] Furthermore, toxicity was also observed using the ProTox II web server.[80]

Data analysis

The results were processed by using Gen5 Microplate Data Collection and Analysis Software and then by MS Excel. The IC_{50} (Inhibition of enzymatic hydrolysis of the substrate pNPG and CNPG3 by 50%) value was calculated using the GraphPad Prism software version 8. Values were expressed as a mean ± standard error of the mean of triplicate.

RESULTS

In this present work, seven medicinal plants were assessed for TPC, TFC, DPPH, enzyme assay, antibacterial assay, docking, and ADMET analysis. Methanol was used as a choice of solvent for extraction. Previous studies also showed these plants contain pharmacologically active constituents for biological activity.

Total phenolic and flavonoid contents

The TPC and TFC were expressed as the mg GAE/gm and mg QE/gm using a calibration curve of gallic acid and quercetin, respectively (Table 2). The highest TPC and TFC was found to be 159.43 ± 1.29 mg GAE/g in *B. ciliata* and 404.17 ± 15.06 mg QE/gm in *M. pudica* respectively and the lowest phenol and flavonoid content was 18.30 ± 1.03 mg GAE/g and 19 ± 2.65 mg QE/gm was observed in *A. racemous*. The TPC and TFC of all plants are shown in Table 2.

Table 2: Results of TPC and TFC of medicinal plants.

Name of plants	TPC (mg GAE/gm)	TFC (mg QE/gm)
Asparagus racemous	18.30 ± 1.03	19 ± 2.65
Bergenia ciliata	159.43 ± 1.29	25.17 ± 3.63
Calotropis gigantean	22.95 ± 3.52	23 ± 1.44
Mimosa pudica	123.62 ± 8.91	404.17 ± 15.06
Phyllanthus emblica	135.52 ± 19.74	44 ± 3.14
Solanum nigrum	38.30 ± 2.84	51.83 ± 14.90

Table 3: Antioxidant screening of plant extract.

Name of plants	Free radical scavenging IC_{50} (µg/mL)
Asparagus racemous	3.10%
Bergenia ciliata	92.35%
Calotropis gigantean	10.50%
Mimosa pudica	90.051%
Phyllanthus emblica	80.29%
Solanum nigrum	6.60%

Table 4: IC_{50} values of potent plant extracts.

Plants	IC_{50} values (µg/mL)
Mimosa pudica	26.5 ± 1.1
Bergenia ciliata	23.7 ± 0.4
Phyllanthus emblica	13.2 ± 0.1
Quercetin (standard)	6.3 ± 1.0

α-Glucosidase and α-Amylase inhibitory activity

Table 5: Screening of plant extracts for enzyme inhibition.

Name of plants	α-Glucosidase	α-Amylase
Asparagus racemous	-	0.2%
Bergenia ciliata	98.31%	96.81%
Calotropis gigantean	0.35%	2.81%
Mimosa pudica	99.9%	90.71%
Phyllanthus emblica	96.29%	32.87%
Solanum nigrum	35.61%	4.73%

Free radical scavenging activity

The antioxidant of seven plant extracts was evaluated using a DPPH radical scavenging assay. Among seven plant extracts, only three of them showed more than 50% inhibition and were further examined for their IC_{50} value. The free radical scavenging activity of medicinal plants are given in Table 3 and 4.

α-Glucosidase and α-Amylase inhibitory activity

Screening of plant extracts was done at 500 µg/mL concentration for both α-glucosidase and α-amylase. Only those extracts which have shown more than 50% inhibitory activity against both enzymes were further examined for their IC_{50} value. Among seven plants, only three plants showed over 50% inhibition. The inhibitory activity of different plant extracts for both enzymes are shown in Table 5 and 6.

Antimicrobial assays

The antibacterial activity of crude plant extracts against *Staphylococcus aureus* ATCC 43300, *Escherichia coli* ATCC 2591, *Klebsiella pneumoniae* ATCC 700603, *Salmonella typhi* ATCC 14028 were performed. The antibacterial activity is measured in terms of zone of inhibition (ZoI) diameter in millimeters (mm) as shown in Table 7.

Table 6: α-Glucosidase and α-amylase inhibitory activities of different plant extracts.

Name of plants	α-Glucosidase (µg/mL)	α-Amylase (µg/mL)
Bergenia ciliata	2.24 ± 0.01	46.19 ± 1.07
Mimosa pudica	35.73 ± 0.65	99.93 ± 0.65
Phyllanthus emblica	8.11 ± 0.29	< 50%
Acarbose (Standard)	344.2 ± 1.0	6.02 ± 0.1

Table 7: Diameter of ZoI of different medicinal plants against tested micro-organisms.

Plant name	Bacterial strains (ZoI)			
	S. aureus ATCC 43300	***E. coli*** ATCC 25922	***K. pneumoniae*** ATCC 700603	S. typhi ATCC 14028
Berginia ciliata	14 mm	-	16 mm	-
Mimosa pudica	11 mm	-	10 mm	-
Phyllanthus emblica	12 mm	-	15 mm	-
Solanum nigrum	-	-	-	-
Asparagus racemous	-	-	-	-
Calotropis gigantea	-	-	-	-
Positive control (Neomycin 1 mg/mL)	20 mm	16 mm	19 mm	18 mm

Molecular docking study

From literature, it was known that *B. ciliata* contain two active compounds (-)-3-O-galloylepicatechin and (-)-3-O-galloylcatechin responsible for the inhibition of α-glucosidase and α-amylase.[23] In our study, potent activity was also shown by the same plant, so docking was performed with both enzymes.

Porcine pancreatic amylase (PPA)

The results showed that (-)-3-O-galloylcatechin interact with the active site of PPA with the best binding energy of −9.5 kcal/mol. It was surrounded by ILE 235, HIS 201, GLU 233, TYR151, LEU 162, ALA 198, ASP197, ARG 195, HIS 299, TYR 62, ASP300, TRP58, TRP59, LEU165, GLN63, VAL163, HIS 305, GLY 306 and form hydrogen bonds with certain amino acid residues of PPA (Glu 233 and Asp 197) (Figure 1).

Similarly, (-)-3-O-galloylepicatechin also binds into the active site with the best binding energy of −9.4 kcal/mol. The (-)-3-O-galloylepicatechin was surrounded by ILE 235, HIS 201, GLU 233, TYR151, LEU 162, ALA 198, ASP197, ARG 195, HIS 299, TYR 62, ASP300, TRP58, TRP59, LEU165, GLN63, VAL163, HIS 305, GLY 306 and form hydrogen bonds with certain amino acid residues of PPA (Glu 233, Asp 197, ASP 300, HIS 299 and HIS 305) (Figure 2). Hence, both compounds might inhibit the

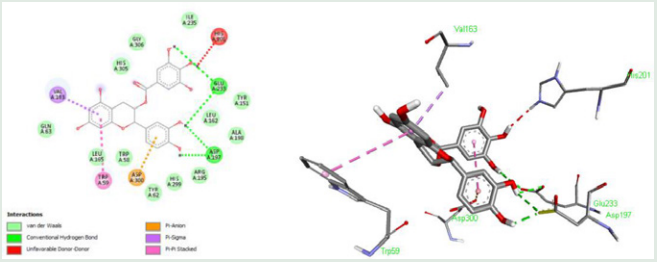

Figure 1: Molecular docking of (-)-3-O-galloylcatechin with α-amylase. (A - 2D view and B- 3D view).

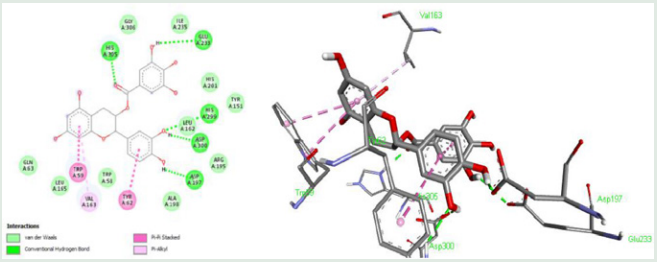

Figure 2: Molecular docking of (-)-3-O-galloylepicatechin with α-amylase. (A - 2D view B- 3D view).

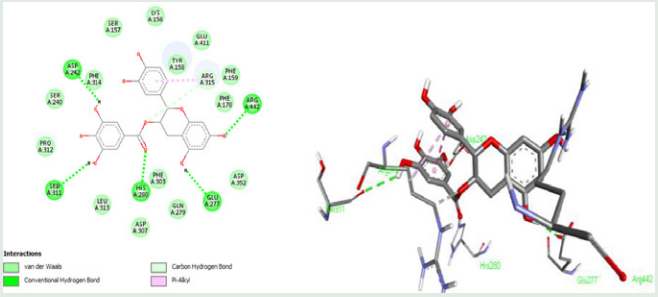

Figure 3: Molecular docking of (-)-3-O-galloylcatechin with isomaltase. (A - 2D view B- 3D view).

catalytic activity of PPA by binding to the enzyme's active site including Glu 233, Asp 300, and Asp 197 amino acids residue.[81,82]

Isomaltase

The results showed that (-)-3-O-galloylcatechin binds into the active site of isomaltase with the best binding energy of –9.5 kcal/mol. It interacted with amino acid residues LYS 156, TYR 158, GLU 411, ARG 315, PHE 159, PHE 178, ARG 442, ASP 352, GLU 277, GLN 279, PHE 303, HIS 280, ASP 307, LEU 313, SER 311, PRO 312, SER 240, ASP 242, SER 157. Among them, ARG 442, GLU 277, HIS 280, SER 311, and ASP 242 formed a hydrogen bond (Figure 3).

The results showed that (-)-3-O-galloylepicatechin binds into the active site with the best binding energy of –10.0 kcal/mol. It interacted with residues LYS 156, TYR 158, GLU 411, ARG 315, PHE 159, PHE 178, ARG 442, ASP 352, GLU 277, GLN 279, PHE 303, HIS 280, ASP 307, LEU 313, SER 311, PRO 312, SER 240, ASP 242, SER 157, PHE 314, SER 241 and VAL 216. Among them, LYS 156, ASP 242, SER 240, GLU 277, and ARG 442 formed a hydrogen bond. Both compounds interacted with the active site of isomaltase via two hydrogen bonds with residues Glu277 and Arg442 (Figure 4).

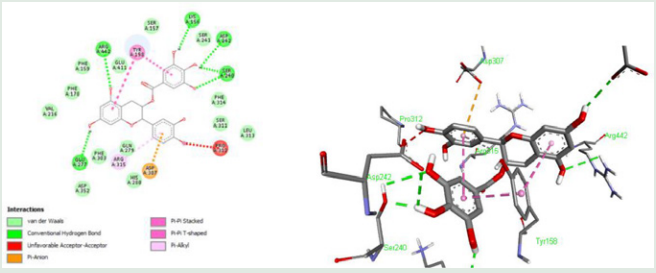

Figure 4: Molecular docking of (-)-3-O-galloylepicatechin with isomaltase. (A - 2D view B- 3D view).

ADMET properties

The ADMET properties and toxicity analysis of both active compounds were found the same as they are epimers and are presented in Table 8 and Table 9.

DISCUSSION

Natural products have immense potential in the management of diabetes.[83-85] Major digestive enzymes such as α-amylase and α-glucosidase are responsible for the digestion of starch into oligosaccharides, disaccharides, and ultimately into glucose. This results in high glucose levels in blood without being used for energy and results in type II diabetes. Bioactive compounds from natural products help in the management of diabetes via stimulation of the pancreas to secrete insulin and increase its sensitivity, protection, and promotion of β-cell proliferation, activation of insulin signaling, inhibition of digestive enzymes action, reduction of glucose absorption, inhibition of the formation of glycation end products, reduction on inflammation, depletion of oxidative stress, resisting lipid peroxidation and limiting the metabolic disorder of lipids and proteins.[86-88]

In literatures, *B. ciliata* showed TPC (145.85 ± 0.15 mg GAE/gm), TFC (15.71 ± 0.10 mg QE/gm) and significant antioxidant activity (IC_{50} = 11.21 ± 1.88 µg/mL).[89] The TPC, TFC and α-amylase inhibitory activity(IC_{50}) of *P. emblica* were shown as 154.15 ± 0.85 mg GAE/gm, 15.60 ± 0.20 mg QE/gm and 94.3 µg/mL respectively.[89,90] Similarly, the TPC and, TFC value of *M. pudica* was reported as 57.431 ± 1.096 mg GAE/gm and, 16.97 ± 1.472 mg QE/gm. The IC_{50} value for free radical scavenging activity (DPPH) was recorded as 7.18 ± 0.0005 µg/mL. The α-amylase and α-glucosidase inhibition by methanolic extract at the concentration of 1 mg/mL was 33.86 ± 5.599 % and 95.65 ± 0.911% respectively.[91] The TPC, TFC, and antioxidant values for *B. ciliata* and *P. emblica* were nearly similar to our study but there is variation in the case of *M. pudica* which might be due to climate, harvest time, storage conditions, variability, and genetic factors.[92]

From *B. ciliata*, two active compounds (-)-3-O-galloylepicatechin and (-)-3-O-galloylcatechin were isolated with α-amylase inhibitory activity of 739µM and 401µM, respectively.[23] The antidiabetic activities of these compounds were also further verified by our study through *in silico* molecular docking. Compounds namely (-)-3-O-galloylepicatechin and (-)-3-O-galloylcatechin were found to bind in the active site of the PPA with a binding energy value of -9.5 and -9.4 Kcal/mol respectively compared to standard drug acarbose -8.8 Kcal/mol. Furthermore,

Table 8: ADMET properties of active compounds.

Properties	(-)-3-O-galloylepicatechin/ (-)-3-O-galloylepicatechin
PSA	179.948
logP	2.5276
Absorption	
Water solubility(logmol/L)	-2.911
Caco-2 permeability (log papp 10^6 cm/s)	-1.264
Intestinal absorption (human) % absorbed	62.096
Skin permeability (log Kp)	-2.735
Distribution	
VDss (human log L/kg)	0.664
BBB permeability (logBB)	-1.847
CNS permeability (log PS)	-3.743
Metabolism	
CYP 1A2 inhibitor	No
CYP2C19 inhibitor	No
CYP2C9 inhibitor	No
CYP2C19 inhibitor	No
CYP2D6 inhibitor	No
CYP3A4 inhibitor	No
Excretion	
Total clearance (log ml/min/kg)	-0.169
Renal OCT2 substrate	No
Toxicity	
AMES toxicity	No
Hepatotoxicity	No
Skin sensitization	No
Oral Rat Acute Toxicity (LD_{50}) (mol/kg)	2.558
Oral Rat Chronic Toxicity (LOAEL) (log mg/kg bw/day)	2.777

Table 9: Toxicity profile of active compounds.

LD_{50} mg/kg	Toxicity class	Active target	Probability
1190	4	Immunotoxicity	0.96
		Aromatase	1
		Estrogen receptor alpha (ER)	0.99
		Estrogen receptor ligand-binding domain (ER-LBD)	0.99

compounds (-)-3-O-galloylepicatechin and (-)-3-O-galloylcatechin bind in the active site of isomaltase with a strong binding energy value of -9.5 and -10.0 Kcal/mol respectively compared to standard drug acarbose -8.4 Kcal/mol.[93] The lower the binding free energy of any protein-ligand complex, the higher is the stability.[94] Additionally, these compounds also have significant ADMET parameters (Table 8 and 9). Both the compounds have been found with better absorption values in the human intestine as well as the volume of distribution (VDss). Besides, it is mandatory to check the toxicity parameters like ames toxicity, oral toxicity, and hepatotoxicity of any metabolites before the selection of drugs.

Methanolic extract of *A. racemosus* has shown IC_{50} of 55.52 ± 1.21 mg/ml against α-amylase.[95] It has been reported that the IC_{50} value of methanolic extract of *P. emblica* for α-amylase is 94.3 µg/mL.[90] Ethanolic extract of *M. pudica* also showed a significant decrease in blood glucose level.[96] In our study, the highest α-glucosidase and α-amylase inhibition were exhibited by three methanolic extracts *B. ciliata, M. pudica,* and *P. emblica.* These plant extracts also have higher phenolic content than the remaining plants in our study.

Some of the plant extracts under study showed a significant antibacterial activity which is due to inhibition of nucleic acid synthesis, cytoplasmic membrane structure, energy metabolism, attachment and biofilm production, porin on the cell membrane, and modification of membrane permeability contributing to cell destruction and attenuation of pathogenicity of phenolic as well as flavonoids compounds within the extracts.[97,98]

Cells containing high glucose levels generate free radicals and ROS, which damage the cellular macromolecules (lipids, proteins, and nucleic acids), leading to the progression of diabetes and its complications.[99] These polyphenol compounds have a wide range of pharmaceutical importance. The structural features like a large number of hydroxyl groups and their configuration, the ketonic functional group at C_4, and a double bond at C_2–C_3 on flavonoids enhanced the antioxidant ability.[100] So, the use of natural antioxidants to manage diabetes has received attention. The plant extracts under study which have shown higher enzyme inhibition and higher phenolic content also show a significant antioxidant ability. Plant extracts with higher phenolic compounds are already proved to have a higher antidiabetic ability through the inhibition of α-amylase and α-glucosidase via the formation of hydrogen bonds and hydrophobic interactions between them and reduce the activity of enzymes.[101,102]

Therefore, further studies on *B. ciliata, M. pudica* and *P. emblica* is required for the isolation of active compounds in pure form, to carry out kinetics, *in vivo* assays, molecular docking, and toxicity to prepare high-value natural pharmaceutical products.

CONCLUSION

The medicinal plants historically used by local and indigenous people contain certain inhibitory compounds of digestive enzymes to prevent the hydrolysis of carbohydrates, which eventually reduces the blood glucose level. The current study suggests that *B. ciliata, M. pudica* and *P. emblica* could be a good source of medicine for the treatment of diabetes but still, active compounds from the plants are not well characterized to develop as future drug candidates.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

ABBREVIATIONS

ADMET: Absorption, Distribution, Metabolism, Excretion and Toxicity; **CNPG3:** 2-chloro-4-nitrophenyl-α-D-maltotrioside; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl; **PPA:** Porcine Pancreatic Amylase; **pNPG:** 4-Nitrophenyl-α-D-glucopyranoside; **ROS:** Reactive Oxygen Species; **ZoI:** Zone of Inhibition.

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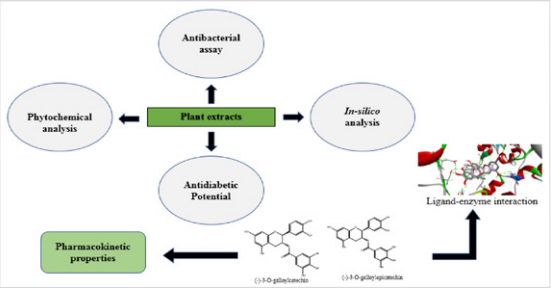
GRAPHICAL ABSTRACT



SUMMARY

The present study investigated the inhibition of major diabetic enzymes from medicinal plants. Among them, *Bergenia ciliata* showed the most potent activity against both enzymes. From literature, it was known that (-)-3-O-galloylepicatechin and (-)-3-O-galloylcatechin was a major component. Molecular docking revealed that these two compounds can interact at active sites of the enzyme with various configurations and binding affinities. Thus, our findings support the traditional use of *Bergenia ciliata* as an antidiabetic plant.

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CERTIFICATES RECEIVED

Tribhuvan University
Institute of Science and Technology
Central Department of Chemistry
Kirtipur, Kathmandu

CERTIFICATE OF APPRECIATION

We present this to Mr./Mrs./Miss. Basanta Sapkota
with appreciation and sincere thanks for attending the One-Day Seminar on "COVID-19 Research Project" held on 28th December 2021.

Prof. Dr. Niranjan Parajuli
President
Nepal Chemical Society

Dr. Pramod Poudel
Research Director, UGC
Special Guest

Prof. Dr. Dhyanendra Bahadur Rai
Principal, University Campus
Chief Guest

Prof. Dr. Ram Chandra Basnyat
Head CDC and Principal Investigator
Chairperson

CERTIFICATE
of Participation

It is certified that Dr/Mr/Ms. Basanta Kumar Sapkota *actively participated in a two-days-workshop on "Tools and Techniques and Advanced Data Analysis in Chemistry" that was jointly organized by* ***Nepal Chemical Society and Central Department of Chemistry, Tribhuvan University*** *from August 30-31, 2019 in Central Department of Chemistry, Kathmandu, Nepal.*

(Chief guest)
Prof. Dr. Ram Chandra Basnyat
HOD
Central Department of Chemistry, Tribhuvan University

Prof. Dr. Niranjan Parajuli
President
Nepal Chemical Society

Dr. Bhanu B Neupane
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in recognition as a PRESENTER in the Eighth Graduate Conference held in Mid-West University, Birendranagar, Surkhet, Nepal on April 4-5, 2022.

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Vice-Chancellor
Mid-West University

Sudeep Thakuri, PhD
Convener
Dean, Faculty of Science and Technology
Mid-West University

Nepal Academy of Science and Technology (NAST)

CERTIFICATE OF PARTICIPATION

Awarded to

Basanta Kumar Sapkota

for Presentation in Oral / ~~Poster~~ / ~~Participation~~ in the

9th National Conference on Science and Technology

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THIS IS PRESENTED TO

Mr. Basanta Kumar Sapkota

for their presentation at the second
Research Project Dissemination Seminar
held at the Central Department of Chemistry, Tribhuvan University
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Certificate

This is to certify that

Prof./Dr./Mr./Ms. Basanta Kumar Sapkota

From Butwal Multiple Campus, Butwal Participated as resource person/participant in 3 days workshop held from April 2-4, 2019 (Chaitra 19-21, 2075) at Central Department of Chemistry, TU, Kirtipur.

Prof. Dr. Amar Prasad Yadav
President
Nepal Chemical Society

Dr. Surya Kant Kalauni
General Secretary
Nepal Chemical Society

Prof. Dr. Ram Chandra Basnyat
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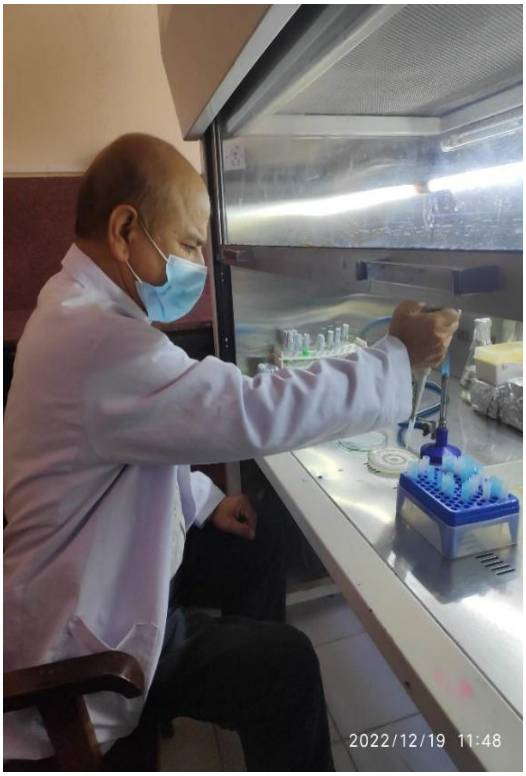
EXPERIMENTAL EQUIPMETS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