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 IC50 (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 IC50 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.

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.[5-7] α-Amylase is a calcium metallo-endoenzyme[8,9] that hydrolyze the α-1, 4-glucosidic linkages of starch, amylose, amylopectin, and glycogen,[10] secreted by salivary gland and pancreas in human with similar amino acid composition, mode of action, and optimum pH.[11] 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), sulphfonylureas, biguanides (metformin), thiazolidinediones (rosiglitazone, pioglitazone), intestinal lipase inhibitor (orlistat), and α-glucosidase inhibitors (acarbose, miglitol, voglibose) are commercially available.[11,12] but their high cost, low tolerability possessing severe side effects such as abdominal pain, bloating, flatulence,
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 steroid glycosides), glycolipids, dietary fibers, polysaccharides, teigidolignans, coumarins, xanthones, etc., which are thought to have an antidiabetic impact. Flavonoids such as luteolin, apigenin, quercetin dehydrate, kaempferol, fisetin, genisteinmyrinctic and daidzein have been shown as inhibitors of α-amylase and α-glucosidase.[21] Asparagus racemosus, Momordica charantia, Berberis aristata, Azadiracta indica, Holohenra pubences, Eugenia jambolana, Agle marmelois, 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 (Pakhwaned), 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, phyrate, 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 responses.[33] *M. pudica* has been shown as an antidepressant,[34,35] antitumor,[36] antihypertensive, antihypolipidemic,[37] antimicrobial,[38] antiviral,[39] antivenom,[40] antiallergic,[41] and wound-healing activity.[42] *Bergenia* species has been described with diverse biological activities such as antimicrobial,[25,43] antimalarial,[26] antipyretic,[27] anti-inflammatory,[28] anti-ulcer,[29] anticancer,[30] anti-urologic,[31] antioxidant,[32] and anti-diabetic.[33] Similarly, *A. racemosus* also showed galactogogue,[34] anti-inflammatory,[35] anti-diabetic,[36] anti-HIV,[37] and fertility activity.[38] Additionally, *C. gigantea* claimed to have different activities such as wound healing,[39] cytotoxic,[40] insecticidal,[41] pregnancy contraceptive,[42] and so on. Nonetheless, other selected medicinal plants i.e. *P. emblica* and *S. nigrum* were also reported with diverse ethnopharmacological importance.

**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), Acrabose (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.[63]

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 | Asparagaceae | Kurilo     | Root          |
| BS-02            | Bergenia ciliata (Haw.) | Saxifragaceae | Pakhanwed | Stem          |
| BS-03            | Calotropis gigantea (L.) | Apocynaceae | Aakh       | Leaves        |
| BS-04            | Mimosa pudica L. | Fabaceae | Lajwati    | 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 determined as previously described aluminum trichloride-based method.\(^{[67]}\) The reaction was performed in 200 \(\mu\)L final volume by adding 20 \(\mu\)L of plant extract with 110 \(\mu\)L distilled water, 60 \(\mu\)L ethanol, 5 \(\mu\)L aluminum chlorohydric \((\text{AlCl}_3, 10\%\), and 5 \(\mu\)L 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 200 \(\mu\)L 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:

\[
\% \text{Scavenging} = \left(1 - \frac{A_t}{A_o}\right) \times 100
\]

Where \(A_o\) = Absorbance of DPPH radical with 30% DMSO and \(A_t\) = Absorbance of DPPH radical with test or reference sample.

**In vitro \(\alpha\)-glucosidase inhibition assay**
The \(\alpha\)-glucosidase inhibitory activity of crude extracts was done according to Fouotsa et al. with slight modification.\(^{[70]}\) Various concentrations of 20 \(\mu\)L plant extracts were mixed with 20 \(\mu\)L enzyme (0.2 Units) along with \(20 \mu\)L 50 mM phosphate buffer saline (pH 6.8) and incubated for 10 min at 37°C. Then, 0.7 \(\mu\)L 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 % \(\alpha\)-glucosidase inhibitory activity is calculated by the following formula:

\[
\% \text{Inhibition} = \left(1 - \frac{A_o}{A_t}\right) \times 100
\]

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.

**In vitro \(\alpha\)-amylase inhibition assay**
The \(\alpha\)-amylase inhibition was done in 200 \(\mu\)L volume, the enzyme and substrate were prepared in 50 mM phosphate buffer pH 7.0 with 0.9 % NaCl. Initially, 20 \(\mu\)L of various concentrations of plant extracts were mixed with 80 \(\mu\)L of PPA (1.5 units/mL) and was incubated at 37°C for 10 min. Then 100 \(\mu\)L 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 (Synergy-LX, BioTek, Instruments, Inc., USA). The percentage of inhibition was calculated as:

\[
\% \text{Inhibition} = \left(1 - \frac{A_o}{A_t}\right) \times 100
\]

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 \(\times\) 10\(^6\) CFU/mL. Then, lawn culture 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 50 \(\mu\)L 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: 3AA4),\(^{[75]}\) 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 \(\times\) 104 \(\times\) 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 \(\times\) 50 \(\times\) 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 a-glucosidase because till now no report of the crystallographic structure of \(S.\) cerevisiae \(\alpha\)-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 \(\alpha\)-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 racemos  | 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 IC₅₀ (µg/mL) |
|-------------------|--------------------------------------|
| Asparagus racemos  | 3.10%                                |
| Bergenia ciliata  | 92.35%                               |
| Calotropis gigantean | 10.50%                         |
| Mimosa pudica     | 90.051%                              |
| Phyllanthus emblica | 80.29%                        |
| Solanum nigrum    | 6.60%                                |

Table 4: IC₅₀ values of potent plant extracts.

| Plants            | IC₅₀ 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 (µg/mL) | α-Amylase (µg/mL) |
|-----------------|----------------------|------------------|
| Asparagus racemos | -                    | 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 IC₅₀ 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 IC₅₀ 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 were performed with both enzymes.

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 Zol of different medicinal plants against tested micro-organisms.

| Plant name              | Bacterial strains (Zol) | ATCC 43300 | ATCC 25922 | ATCC 700603 | ATCC 14028 |
|-------------------------|-------------------------|------------|------------|-------------|------------|
| Bergenia ciliata        | S. aureus               | 14 mm      | -          | 16 mm       | -          |
|                         | E. coli                 | -          | 10 mm      | -           | -          |
| Mimosa pudica           | S. aureus               | 11 mm      | -          | 15 mm       | -          |
| Phyllanthus emblica     | S. aureus               | 12 mm      | -          | -           | -          |
|                        | ATCC 43300              | -          | -          | -           | -          |
|                        | ATCC 25922              | -          | -          | -           | -          |
|                        | ATCC 700603             | -          | -          | -           | -          |
|                        | ATCC 14028              | -          | -          | -           | -          |
|                        | 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.[28] 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-galloylcatechin also binds into the active site with the best binding energy of −9.4 kcal/mol. The (-)-3-O-galloylcatechin 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
catalytic activity of PPA by binding to the enzyme’s active site including Glu 233, Asp 300, and Asp 197 amino acids residue.\cite{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 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).

**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.\cite{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.\cite{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\textsubscript{50} = 11.21 ± 1.88 µg/mL).\cite{89} The TPC, TFC and α-amylase inhibitory activity(IC\textsubscript{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.\cite{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\textsubscript{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.\cite{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.\cite{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.\cite{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-galloylepticin/ (-)-3-O-galloylcalcechin |
|-----------------------------------|-----------------------------------------------|
| PSA                               | 179.948                                       |
| logP                              | 2.5276                                        |
| **Absorption**                    |                                               |
| Water solubility(logmol/L)        | -2.911                                        |
| Caco-2 permeability (log papp 10^8 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 (LD50) (mol/kg) | 2.558                                         |
| Oral Rat Chronic Toxicity (LOAEL) (log mg/kg bw/day) | 2.777                                         |

Table 9: Toxicity profile of active compounds.

| LD50 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 |

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.

**CONCLUSION**

The authors declare that they have no conflicts of interest.

**ABBREVIATIONS**

ADMET: Absorption, Distribution, Metabolism, Excretion and Toxicity; CNP3G: 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; Zol: Zone of Inhibition.
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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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