Pharmacogn Res. 2021; 13(3):140-148
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogres.com | www.phcog.net

Original Article

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

Basanta Kumar Sapkota[1,2], Karan Khadayat[1], Bikash Adhikari[1], Darbin Kumar Poudel[1], Purushottam Niraula[1], Prakriti Budhathoki[1], Babita Aryal[1], Kusum Basnet[1], Mandira Ghimire[1], Rishab Marahatha[1], Niranjan Parajuli[1,*]

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.

Basanta Kumar Sapkota[1,2], Karan Khadayat[1], Bikash Adhikari[1], Darbin Kumar Poudel[1], Purushottam Niraula[1], Prakriti Budhathoki[1], Babita Aryal[1], Kusum Basnet[1], Mandira Ghimire[1], Rishab Marahatha[1], Niranjan Parajuli[1,*]

[1]Biological Chemistry Lab, Central Department of Chemistry, Tribhuvan University, Kathmandu, NEPAL.
[2]Department of Chemistry, Butwal Multiple Campus, Tribhuvan University, Butwal, NEPAL.

Correspondence

Dr. Niranjan Parajuli

Professor of Chemistry, Central Department of Chemistry, Tribhuvan University, Kathmandu, NEPAL.

Phone no: +977-1-4332034

Email id: niranjan.parajuli@cdc.tu.edu.np

History
- Submission Date: 30-03-2021;
- Review completed: 29-04-2021;
- Accepted Date: 11-05-2021

DOI : 10.5530/pres.13.3.6

Article Available online
http://www.phcogres.com

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,

Cite this article: Sapkota BK, Khadayat K, Adhikari B, Poudel DK, Niraula P, Budhathoki P, Aryal B, Basnet K, Ghimire M, Marahatha R, Parajuli N. Phytochemical Analysis, Antidiabetic Potential and *in-silico* Evaluation of Some Medicinal Plants. Pharmacog Res. 2021;13(3):140-8.

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

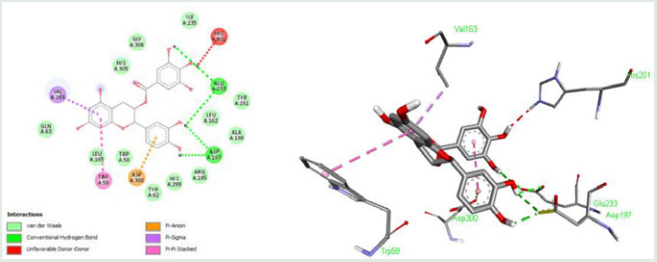


Figure 1: Molecular docking of (-)-3-O-galloylcatechin with α-amylase. (A - 2D view and B- 3D view).

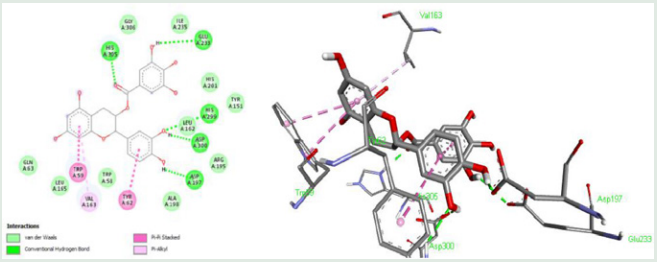


Figure 2: Molecular docking of (-)-3-O-galloylepicatechin with α-amylase. (A - 2D view B- 3D view).

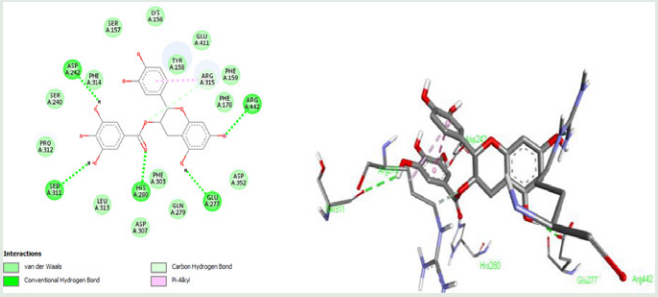


Figure 3: Molecular docking of (-)-3-O-galloylcatechin with isomaltase. (A - 2D view B- 3D view).

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).

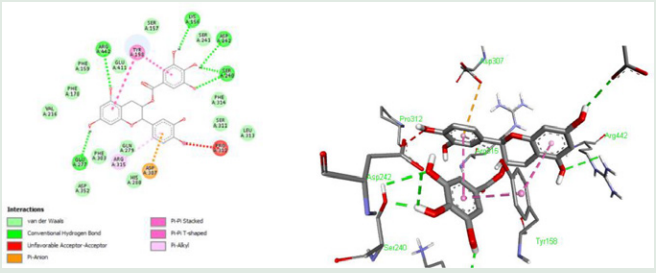


Figure 4: Molecular docking of (-)-3-O-galloylepicatechin with isomaltase. (A - 2D view B- 3D view).

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.

ACKNOWLEDGEMENT

Basanta Kumar Sapkota is grateful to University Grant Commission, Nepal for providing a Ph.D. research grant (Grant No. Ph.D. 76/77-S&T-12). This project is also supported by Higher Education Reform Project (Tribhuvan University), Kathmandu, Nepal to Niranjan Parajuli.

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.

REFERENCES

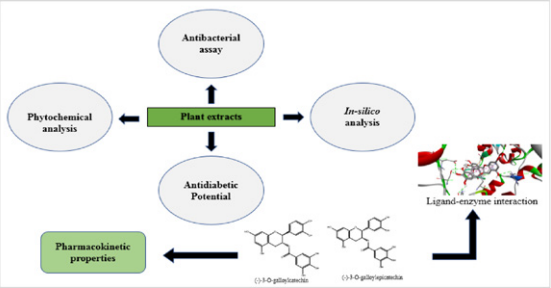
1. Jéquier E. Carbohydrates as a source of energy. Am J Clin Nutr. 1994;59(3 Suppl):682S-5S.
2. Björck I, Granfeldt Y, Liljeberg H, Tovar J, Asp NG. Food properties affecting the digestion and absorption of carbohydrates. The American Journal of Clinical Nutrition. 1994;59(3):699S-705S.
3. Ceriello A. Postprandial Hyperglycemia and Diabetes Complications: Is It Time to Treat?. Diabetes. 2005;54(1):1-7.
4. Van Dijk JW, Manders RJF, Hartgens F, Stehouwer CD, Praet SFE, Loon VLJC. Postprandial hyperglycemia is highly prevalent throughout the day in type 2 diabetes patients. Diabetes Research and Clinical Practice. 2011;93(1):31-7.
5. IDF. IDF Diabetes Atlas 9th Edition. Brussels, Belgium: International Diabetes Federation; 2019. Available from: https://www.diabetesatlas.org/upload/resources/2019/IDF_Atlas_9th_Edition_2019.html
6. Kazi AA, Blonde L. Classification of diabetes mellitus. Clinics in Laboratory Medicine. 200;21:1-13.
7. Deutschländer MS, Lall N, Venter VDM, Hussein AA. Hypoglycemic evaluation of a new triterpene and other compounds isolated from *Euclea undulata* Thunb. var. myrtina (Ebenaceae) root bark. Journal of Ethnopharmacology. 2011;133(3):1091–5. [cited 2019 May 27]Available from: https://linkinghub.elsevier.com/retrieve/pii/S0378874110008238
8. Zaharudin N, Staerk D, Dragsted LO. Inhibition of α-glucosidase activity by selected edible seaweeds and fucoxanthin. Food Chemistry. 2019;270:481-6. [cited 2020 Jul 8]Available from: https://linkinghub.elsevier.com/retrieve/pii/S0308814618312871
9. Tiwari SP, Srivastava R, Singh CS, Shukla K, Singh RK, Singh P, *et al*. Amylases: An overview with special reference to alpha amylase. J Global Biosci. 2015;4:1886–901.
10. Yan S, Wu G. Analysis on evolutionary relationship of amylases from archaea, bacteria and eukaryota. World Journal of Microbiology and Biotechnology. 2016;32(2):1–16.
11. Windish WW, Mhatre NS. Microbial Amylases. Advances in Applied Microbiology. 1965;7(C):273–304.
12. Stiefel DJ, Keller PJ. Preparation and some properties of human pancreatic amylase including a comparison with human parotid amylase. BBA: Enzymology. 1973;302(2):345–61.
13. Cheng AYY. Oral antihyperglycemic therapy for type 2 diabetes mellitus. Canadian Medical Association Journal. 2005;172(2):213-26. [cited 2019 Aug 6] Available from: http://www.cmaj.ca/cgi/doi/10.1503/cmaj.1031414
14. Kleinberger JW, Pollin TI. Personalized medicine in diabetes mellitus: Current opportunities and future prospects: Personalized medicine in diabetes mellitus. Ann NY Acad Sci]. 2015;1346(1):45–56. [cited 2020 Sep 7]Available from: http://doi.wiley.com/10.1111/nyas.12757
15. Modak M, Dixit P, Londhe J, Ghaskadbi S, Devasagayam TPA. Indian Herbs and Herbal Drugs Used for the Treatment of Diabetes. J Clin Biochem Nutr. 2007;40(3):163-73.
16. Shai LJ, Masoko P, Mokgotho MP, Magano SR, Mogale AM, Boaduo N, *et al*. Yeast alpha glucosidase inhibitory and antioxidant activities of six medicinal plants collected in Phalaborwa, South Africa. South African Journal of Botany. 2010;76(3):465–70.
17. Verma S, Gupta M, Popli H, Aggarwal G. Diabetes mellitus treatment using herbal drugs. International Journal of Phytomedicine. 2018;10(1):01.
18. Yuan H, Ma Q, Ye L, Piao G. The Traditional Medicine and Modern Medicine from Natural Products. Molecules. 2016;21(5). [cited 2021 Apr 16] Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6273146/
19. Grover JK, Yadav S, Vats V. Medicinal plants of India with anti-diabetic potential. Journal of Ethnopharmacology. 2002;81(1):81–100.
20. Mukherjee PK, Maiti K, Mukherjee K, Houghton PJ. Leads from Indian medicinal plants with hypoglycemic potentials. Journal of Ethnopharmacology. 2006;106(1):1-28.
21. Tadera K, Minami Y, Takamatsu K, Matsuoka T. Inhibition of α-glucosidase and α-amylase by flavonoids. Journal of Nutritional Science and Vitaminology. 2006;52(2):149–53.
22. Shrestha P, Jamarkattel-pandit N. Survey on Medicinal Plants used for Anti-diabetic Activity in Kaski. 2018;7(1).
23. Bhandari MR, Jong-Anurakkun N, Hong G, Kawabata J. α-Glucosidase and α-amylase inhibitory activities of Nepalese medicinal herb Pakhanbhed (*Bergenia ciliata*, Haw.). Food Chemistry. 2008;106(1):247–52.
24. Dhalwal K, Shinde VM, Biradar YS, Mahadik KR. Simultaneous quantification of bergenin, catechin and gallic acid from *Bergenia ciliata* and *Bergenia ligulata* by using thin-layer chromatography. Journal of Food Composition and Analysis. 2008;21(6):496–500.
25. Singh M, Pandey N, Agnihotri V, Singh KK, Pandey A. Antioxidant, antimicrobial activity and bioactive compounds of *Bergenia ciliata* Sternb.: A valuable medicinal herb of Sikkim Himalaya. Journal of Traditional and Complementary Medicine. 2017;7(2):152–7.
26. Koul B, Kumar A, Yadav D, Jin JO. Bergenia Genus: Traditional Uses, Phytochemistry and Pharmacology. Molecules. 2020;25(23):5555.
27. Fakhruddin S, Alanazi W, Jackson KE. Diabetes-Induced Reactive Oxygen Species: Mechanism of Their Generation and Role in Renal Injury. Journal of Diabetes Research. 2017;2017:e8379327.
28. Gupta S, Mediratta PK, Singh S, Sharma KK, Shukla R. Antidiabetic, antihypercholesterolaemic and antioxidant effect of *Ocimum sanctum* (Linn) seed oil. Indian Journal of Experimental Biology. 2006;44(4):300–4.
29. Sathisha AD, Lingaraju HB, Prasad KS. Evaluation of Antioxidant Activity of Medicinal Plant Extracts Produced for Commercial Purpose. E-Journal of Chemistry. 2011;8(2):882–6.
30. Joseph JA, Shukitt-Hale B, Denisova NA, Bielinski D, MartinA, McEwen JJ, *et al*. Reversals of age-related declines in neuronal signal transduction, cognitive and motor behavioral deficits with blueberry, spinach, or strawberry dietary supplementation. The Journal of Neuroscience: The Official Journal of the Society for Neuroscience. 1999;19(18):8114–21.
31. Bum EN, Dawack DL, Schmutz M, Rakotonirina A, Rakotonirina SV, Portet C, *et al*. Anticonvulsant activity of *Mimosa pudica* decoction. Fitoterapia. 2004;75(3-4):309-14.
32. Kokane DD, More RY, Kale MB, Nehete MN, Mehendale PC, Gadgoli CH. Evaluation of wound healing activity of root of *Mimosa pudica*. Journal of Ethnopharmacology. 2009;124(2):311–5.
33. Volkov AG, Foster JC, Baker KD, Markin VS. Mechanical and electrical anisotropy in *Mimosa pudica* pulvini. Plant Signaling and Behavior. 2010;5(10):1211-21.
34. Patro G, Bhattamisra KS, Mohanty KB. Effects of *Mimosa pudica* L. leaves extract on anxiety, depression and memory. Avicenna J Phytomed. 2016;6(6):696–710.
35. Molina M, Contreras CM, Tellez-Alcantara P. *Mimosa pudica* may possess anti-depressant actions in the rat. Phytomedicine. 1999;6(5):319–23.
36. Jose J, Dhanya AT, Haridas KR, Kumar STM, Jayaraman S, Variyar EJ, *et al*. Structural characterization of a novel derivative of myricetin from *Mimosa pudica* as an anti-proliferative agent for the treatment of cancer. Biomed Pharmacother. 2016;84:1067–77.
37. Ganguly M, Devi N, Mahanta R, Borthakur MK. Effect of *Mimosa pudica* root extract on vaginal estrous and serum hormones for screening of antifertility activity in albino mice. Contraception. 2007;76(6):482-5.
38. Arka G, Anindita K, Ankit S, Kumar SA, Kumar MS. Preliminary evaluation of hepatoprotective potential of the polyherbal formulation. J Intercult Ethnopharmacol. 2014;4(2):118–24.
39. Hassan AN, Karunakaran R, Abdulmumin S. A review on the pharmacological and traditional properties of *Mimosa pudica*. Int J Pharm Pharm Sci. 2019;12-6.
40. Gandhiraja N, Sriram S, Meenaa V, Srilakshmi J, Sasikumar C, Rajeswari R. Phytochemical Screening and Antimicrobial Activity of the Plant Extracts of *Mimosa pudica* L. Against Selected Microbes. Ethnobotanical Leaflets. 2009;2009(5). Available from: https://opensiuc.lib.siu.edu/ebl/vol2009/iss5/8
41. Akinsinde KA, Olukoya DK. Vibriocidal activities of some local herbs. J Diarrhoeal Dis Res. 1995;13(2):127–9.
42. Mahanta M, Mukherjee AK. Neutralisation of lethality, myotoxicity and toxic enzymes of Naja kaouthia venom by *Mimosa pudica* root extracts. J Ethnopharmacol. 2001;75(1):55–60.
43. Momin FN, Kalai BR, Godse VS, Patole NS, Shikalgar T, Naikwade NS. Gastroprotective Effect of Mimosa Pudica Leaves Extract on *in-vivo* Test Models in Rats. Journal of Biologically Active Products from Nature. 2011;1(3):160-7.
44. Ahmad H, Sehgal S, Mishra A, Gupta R. *Mimosa pudica* L. (Laajvanti): An overview. Pharmacogn Rev. 2012;6(12):115–24.
45. Sinha S, Murugesan T, Maiti K, Gayen JR, Pal B, Pal M, *et al*. Antibacterial activity of *Bergenia ciliata* rhizome. Fitoterapia. 2001;72(5):550–2.
46. Walter NS, Bagai U, Kalia S. Antimalarial activity of *Bergenia ciliata* (Haw.) Sternb. against *Plasmodium berghei*. Parasitol Res. 2013;112(9):3123-8.
47. Sinha S, Murugesan T, Maiti K, Gayen JR, Pal M, Saha BP. Evaluation of anti-inflammatory potential of *Bergenia ciliata* Sternb. rhizome extract in rats. Journal of Pharmacy and Pharmacology. 2001;53(2):193–6.
48. Kakub G, Gulfraz M. Cytoprotective effects of *Bergenia ciliata* Sternb, extract on gastric ulcer in rats. Phytotherapy Research. 2007;21(12):1217–20.
49. Saha S, Verma RJ. *In vitro* and *in silico* study of antioxidant effect of *Bergenia ciliata* and *Terminalia chebula* against sodium oxalate induced oxidative stress. Toxicol Environ Health Sci. 2015 1;7(1):50–7.
50. Islam M, Azhar I, Usmanghani K, Gill MA, Ahmad A, Null S. Bioactivity evaluation of *Bergenia ciliata*. Pak J Pharm Sci. 2002;15(1):15–33.
51. Behera PC, Tripathy DP, Parija SC. Shatavari: Potentials for galactogogue in dairy cows. Indian Journal of Traditional Knowledge. 2013;12(1):9-17.
52. Lee DY, Choo BK, Yoon T, Cheon MS, Lee HW, Lee AY, *et al*. Anti-inflammatory effects of *Asparagus cochinchinensis* extract in acute and chronic cutaneous inflammation. Journal of Ethnopharmacology. 2009;121(1):28–34.
53. Hannan JM, Ali L, Khaleque J, Akhter M, Flatt PR, Abdel-Wahab YHA. Antihyperglycaemic activity of *Asparagus racemosus* roots is partly mediated by inhibition of carbohydrate digestion and absorption and enhancement of cellular insulin action. The British Journal of Nutrition. 2012;107(9):1316–23.
54. Sabde S, Bodiwala HS, Karmase A, Deshpande PJ, Kaur A, Ahmed N, *et al*.

Anti-HIV activity of Indian medicinal plants. Journal of Natural Medicines. 2011;65(3–4):662–9.
55. Thakur M, Thompson D, Connellan P, Deseo MA, Morris C, Dixit VK. Improvement of penile erection, sperm count and seminal fructose levels *in vivo* and nitric oxide release *in vitro* by ayurvedic herbs. Andrologia. 2011;43(4):273–7.
56. Deshmukh PT, Fernandes J, Atul A, Toppo E. Wound healing activity of *Calotropis gigantea* root bark in rats. Journal of Ethnopharmacology. 2009;125(1):178–81.
57. Nguyen KDH, Dang PH, Nguyen HX, Nguyen MTT, Awale S, Nguyen NT. Phytochemical and cytotoxic studies on the leaves of *Calotropis gigantea*. Bioorganic and Medicinal Chemistry Letters. 2017;27(13):2902–6.
58. Habib MR, Karim MR. Chemical characterization and insecticidal activity of *Calotropis gigantea* L. flower extract against *Tribolium castaneum* (Herbst). Asian Pacific Journal of Tropical Disease. 2016;6(12):996–9.
59. Srivastava SR, Keshri G, Bhargavan B, Singh C, Singh MM. Pregnancy interceptive activity of the roots of *Calotropis gigantea* Linn. in rats. Contraception. 2007;75(4):318–22.
60. Rathod NR, Chitme HR, Irchhaiya R, Chandra R. Hypoglycemic effect of Calotropis gigantea Linn. leaves and flowers in streptozotocin-induced diabetic rats. Oman Medical Journal. 2011;26(2):104.
61. D'souza JJ, D'souza PP, Fazal F, Kumar A, Bhat HP, Baliga MS. Anti-diabetic effects of the Indian indigenous fruit Emblica officinalis Gaertn: Active constituents and modes of action. Food Funct. 2014;5(4):635.
62. Elobeid M, Ahmed E. Antidiabetic Efficacy of Aqueous Fruit Extract of Amla (*Emblica officinalis*, Gaertn) in Streptozotocin-Induced Diabetes Mellitus in Male Rats. Trop J Pharm Res. 2015;14(5):801.
63. Krishnaven M, Mirunalini S, Karthishwa K, Dhamodhara G. Antidiabetic and Antihyperlipidemic Properties of *Phyllanthus emblica* Linn. (Euphorbiaceae) on Streptozotocin Induced Diabetic Rats. Pakistan J of Nutrition. 2010;9(1):43-51.
64. Atanu FO, Ebiloma UG, Ajayi EI. A review of the pharmacological aspects of *Solanum nigrum* Linn. BMBR. 2011;6(1):1–8.
65. Ainsworth EA, Gillespie KM. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. Nature Protocols. 2007;2(4):875–7.
66. Lu X, Wang J, Al-Qadiri HM, Ross CF, Powers JR, Tang J, *et al*. Determination of total phenolic content and antioxidant capacity of onion (*Allium cepa*) and shallot (*Allium oschaninii*) using infrared spectroscopy. Food Chemistry. 2011;129(2):637–44.
67. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of Food and Drug Analysis. 2002;10(3).
68. Sabudak T, Demirkiran O, Ozturk M, Topcu G. Phenolic compounds from *Trifolium echinatum* Bieb. and investigation of their tyrosinase inhibitory and antioxidant activities. Phytochemistry. 2013;96:305–11.
69. Subedi A, Amatya MP, Shrestha TM, Mishra SK, Pokhrel BM. Antioxidant and antibacterial activity of methanolic extract of *Machilus odoratissima*. Kathmandu University Journal of Science, Engineering and Technology. 2012;8(1):73–80.
70. Fouotsa H, Lannang AM, Mbazoa CD, Rasheed S, Marasini BP, Ali Z, *et al*. Xanthones inhibitors of α-glucosidase and glycation from Garcinia nobilis. Phytochemistry Letters. 2012;5(2):236–9.
71. Khadayat K, Marasini BP, Gautam H, Ghaju S, Parajuli N. Evaluation of the alpha-amylase inhibitory activity of Nepalese medicinal plants used in the treatment of diabetes mellitus. Clinical Phytoscience. 2020;6(1):34. [cited 2020 Jun 26] Available from: https://doi.org/10.1186/s40816-020-00179-8
72. Balouiri M, Sadiki M, Ibnsouda SK. Methods for *in vitro* evaluating antimicrobial activity: A review. Journal of Pharmaceutical Analysis. 2016;6(2):71–9. [cited 2020 Aug 31]Available from: http://www.sciencedirect.com/science/article/pii/S2095177915300150
73. Gilles C, Astier JP, Marchis-Mouren G, Cambillau C, Payan F. Crystal Structure of Pig Pancreatic α-amylase Isoenzyme II, in Complex with the Carbohydrate Inhibitor Acarbose. European Journal of Biochemistry. 1996;238(2):561-9.
74. Sui X, Zhang Y, Zhou W. *In vitro* and *in silico* studies of the inhibition activity of anthocyanins against porcine pancreatic α-amylase. Journal of Functional Foods. 2016;21:50–7.
75. Yamamoto K, Miyake H, Kusunoki M, Osaki S. Crystal structures of isomaltase from Saccharomyces cerevisiae and in complex with its competitive inhibitor maltose. The FEBS Journal. 2010;277(20):4205–14.
76. Trott O, Olson AJ. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. Journal of Computational Chemistry. 2010;31(2):455–61.
77. Mohammadi-Khanaposhtani M, Rezaei S, Khalifeh R, Imanparast S, Faramarzi MA, Bahadorikhalili S, *et al*. Design, synthesis, docking study, α-glucosidase inhibition and cytotoxic activities of acridine linked to thioacetamides as novel agents in treatment of type 2 diabetes. Bioorganic Chemistry. 2018;80:288–95.
78. Sun H, Ding W, Song X, Wang D, Chen M, Wang K, *et al*. Synthesis of 6-hydroxyaurone analogues and evaluation of their α-glucosidase inhibitory and glucose consumption-promoting activity: Development of highly active 5,6-disubstituted derivatives. Bioorganic and Medicinal Chemistry Letters. 2017;27(15):3226–30.
79. Pires DEV, Blundell TL, Ascher DB. PkCSM: Predicting Small-Molecule Pharmacokinetic and Toxicity Properties Using Graph-Based Signatures. J Med Chem. 2015;58(9):4066–72.
80. Banerjee P, Eckert AO, Schrey AK, Preissner R. ProTox-II: A webserver for the prediction of toxicity of chemicals. Nucleic Acids Research. 2018;46(W1):W257–63.
81. Xie F, Zhang W, Gong S, Gu X, Lan X, Wu J, *et al*. Investigating lignin from Canna edulis ker residues induced activation of α-amylase: Kinetics, interaction and molecular docking. Food Chemistry. 2019;271:62–9.
82. Zhang X, Jia Y, Ma Y, Cheng G, Cai S. Phenolic Composition, Antioxidant Properties and Inhibition toward Digestive Enzymes with Molecular Docking Analysis of Different Fractions from *Prinsepia utilis* Royle Fruits. Molecules. 2018;23(12):3373.
83. Jugran AK, Rawat S, Devkota HP, Bhatt ID, Rawal RS. Diabetes and plant-derived natural products: From ethnopharmacological approaches to their potential for modern drug discovery and development. Phytotherapy Research. 2020. [cited 2020 Nov 8]n/a(n/a). Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/ptr.6821
84. Ríos JL, Francini F, Schinella GR. Natural Products for the Treatment of Type 2 Diabetes Mellitus. Planta Med. 2015;81(12–13):975–94.
85. Yeung AWK, Tzvetkov NT, Durazzo A, Lucarini M, Souto EB, Santini A, *et al*. Natural products in diabetes research: quantitative literature analysis. Natural Product Research. 2020;1–15. [cited 2020 Nov 8]Available from: https://www.tandfonline.com/doi/full/10.1080/14786419.2020.1821019
86. Li WL, Zheng HC, Bukuru J, Kimpe DN. Natural medicines used in the traditional Chinese medical system for therapy of diabetes mellitus. J Ethnopharmacol. 2004;92(1):1–21.
87. Sun C, Zhao C, Guven EC, Paoli P, Simal-Gandara J, Ramkumar KM, *et al*. Dietary polyphenols as antidiabetic agents: Advances and opportunities. Food Frontiers. 2020;1(1):18-44. [cited 2020 Nov 8] Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/fft2.15
88. Zhao C, Yang C, Wai STC, Zhang YP, Portillo M, Paoli P, *et al*. Regulation of glucose metabolism by bioactive phytochemicals for the management of type 2 diabetes mellitus. Critical Reviews in Food Science and Nutrition. 2019;59(6):830–47. [cited 2020 Nov 8]Available from: https://www.tandfonline.com/doi/full/10.1080/10408398.2018.1501658
89. Sharma KR, Kalauni SK, Awale S, Pokharel YR. *In vitro* Free Radical Scavenging Activity of Methanol Extracts of Some Selected Medicinal Plants of Nepal. Austin J Biotechnol Bioeng. 2015;2(1):1035.
90. Etxeberria U, Garza DLAL, Campión J, Martínez JA, Milagro FI. Antidiabetic effects of natural plant extracts via inhibition of carbohydrate hydrolysis enzymes with emphasis on pancreatic alpha amylase. Expert Opinion on Therapeutic Targets. 2012;16(3):269–97.
91. Tunna TS. Analyses and profiling of extract and fractions of neglected weed *Mimosa pudica* Linn. traditionally used in Southeast Asia to treat diabetes. South African Journal of Botany. 2015;9.
92. Klepacka J, Gujska E, Michalak J. Phenolic Compounds as Cultivar- and Variety-distinguishing Factors in Some Plant Products. Plant Foods Hum Nutr. 2011;66(1):64–9. [cited 2020 Jul 8]Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3079089/
93. Loo KY, Leong KH, Sivasothy Y, Ibrahim H, Awang K. Molecular Insight and Mode of Inhibition of α -Glucosidase and α -Amylase by Pahangensin A from *Alpinia pahangensis* Ridl. C and B. 2019;16(6):e1900032.
94. Dolgonosov AM. The universal relationship between the energy and length of a covalent bond derived from the theory of generalized charges. Russian Journal of Inorganic Chemistry. 2017;62(3):344-50.
95. Vadivelan R, Krishnan GR, Kannan R. Antidiabetic potential of *Asparagus racemosus* Willd leaf extracts through inhibition of α-amylase and α-glucosidase. Journal of Traditional and Complementary Medicine. 2019;9(1):1–4. [cited 2020 Mar 15] Available from: http://www.sciencedirect.com/science/article/pii/S2225411017301268
96. Sutar NG, Sutar UN, Behera BC. Antidiabetic activity of the leaves of *Mimosa pudica* Linn. in albino rats. Journal of Herbal Medicine and Toxicology. 2009;3(1):123–6.
97. Cowan MM. Plant Products as Antimicrobial Agents. Clin Microbiol Rev. 1999;12(4):564–82. [cited 2020 Nov 5] Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC88925/
98. Faron MLB, Perecin MB, Lago AAD, Bovi OA, Maia NB. Temperatura, nitrato de potássio e fotoperíodo na germinação de sementes de *Hypericum perforatum* L. e H. Brasiliense Choisy. Bragantia. 2004;63(2):193–9.
99. Doan HV, Riyajan S, Iyara R, Chudapongse N. Antidiabetic activity, glucose uptake stimulation and α-glucosidase inhibitory effect of *Chrysophyllum cainito* L. stem bark extract. BMC Complementary and Alternative Medicine. 2018;18(1):267. Available from: https://doi.org/10.1186/s12906-018-2328-0
100. Sarian MN, Ahmed QU, Mat So'ad SZ, Alhassan AM, Murugesu S, Perumal V, *et al*. Antioxidant and Antidiabetic Effects of Flavonoids: A Structure-Activity Relationship Based Study. Bio Med Research International. 2017;2017:1–14.
101. Camargo DAC, Favero BT, Morzelle MC, Franchin M, Alvarez-Parrilla E,

Rosa DLLA, *et al*. Is Chickpea a Potential Substitute for Soybean? Phenolic Bioactives and Potential Health Benefits. International Journal of Molecular Sciences 2019;20(11):2644. [cited 2020 Sep 26] Available from: https://www.mdpi.com/1422-0067/20/11/2644

102. Pinaffi ACDC, Sampaio GR, Soares MJ, Shahidi F, Camargo DAC, Torres EAFS. Insoluble-Bound Polyphenols Released from Guarana Powder: Inhibition of Alpha-Glucosidase and Proanthocyanidin Profile. Molecules. 2020;25(3):679. [cited 2020 Sep 26] Available from: https://www.mdpi.com/1420-3049/25/3/679.

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.

Cite this article: Sapkota BK, Khadayat K, Adhikari B, Poudel DK, Niraula P, Budhathoki P, Aryal B, Basnet K, Ghimire M, Marahatha R, Parajuli N. Phytochemical Analysis, Antidiabetic Potential and *in-silico* Evaluation of some Medicinal Plants. Pharmacog Res. 2021;13(3):140-8.