Research Article

In Vitro and In Silico Analysis of Bergenia ciliata and Mimosa pudica for Inhibition of α-Amylase

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The discovery of antidiabetic natural products is a flourishing field of opportunity in the sector of drug discovery. Various medicinal plants with diverse chemical constituents have been extensively studied for drug development. Bergenia ciliata and Mimosa pudica have been traditionally used for the treatment of diabetes and consist of valuable phytochemicals. In this study, we have analyzed total phenolic and flavonoid content along with the antioxidant and α-amylase inhibitory activity. The crude extract of B. ciliata contains higher levels of TPC whereas higher TFC was observed in M. pudica. The strong antioxidant activity was shown by B. ciliata with an IC50 value of 125.86 ± 4.16 μg/mL. The ethyl acetate extract of B. ciliata and M. pudica showed higher α-amylase inhibitory activity with an IC50 value of 13.97 ± 0.10 and 11.97 ± 0.36 μg/mL, respectively. The biological potential of the reported phytochemicals was also assessed by using bioinformatic tools. Furthermore, the active phytochemicals from these plants were docked with human pancreatic α-amylase to study their inhibitory activities to this enzyme. The docking analysis revealed that catechin has lower binding energy (−8.6 kcal/mol) as compared to the commercial drug acarbose (−7.3 kcal/mol) indicating higher affinity towards the enzyme. This study additionally sheds more light on medicinal plants’ antidiabetic activity. So, this study will aid in the investigation of the biological properties of these plants as well as the identification of potential compounds with antidiabetic properties.

1. Introduction

Diabetic mellitus (DM) is a metabolic disease characterized by high blood sugar levels in the body that result from a relative or absolute decrease in insulin secretion or its function. Currently, 537 million adults (20–79 years) are living with diabetes worldwide and this number is predicted to rise to 643 million by 2030 and 783 million by 2045 [1]. The increasing trend of diabetes mellitus and its complications has become a serious global medical concern. A major therapeutic approach for treating diabetes is by decelerating the absorption of glucose through the inhibition of α-amylase and α-glucosidase enzymes resulting in the decrease of hyperglycemia [2]. Enzyme inhibitors delay the rate of glucose absorption by preventing carbohydrate digestion, and ultimately check the surge of glucose rise. Hyperglycemia triggers the generation of free radicals, leading to oxidative stress when excessive free radicals react with proteins and nucleic acids. The inhibition of these enzymes by chemical constituents of plants has acquired good attention for controlling post-prandial blood glucose levels [3, 4].

Plants have been used as sources of medicine for millions of years. Still, today, in many countries, plants and their parts are used as a basic source of treatment for many ailments, and it has also been recognized by WHO (World Health Organization) as an important component of primary health care [5]. Secondary metabolites of plants like flavonoids and phenolics are known for their therapeutic qualities having antioxidant properties and demonstrating the highest inhibitory activities, also capable of scavenging free superoxide radicals [6]. Plant secondary metabolites have been linked to
diabetes management via a different mechanism. Polyphenols such as catechin, epicatechin, and epigallocatechin inhibit sodium-glucose cotransporter and S-Glut-1 mediated intestinal glucose transport, as well as α-glucosidase and α-amylase [7]. Furthermore, resveratrol inhibited K⁺ adenosine triphosphate and K⁺⁺⁺ channels, which delayed insulin resistance. It also increases insulin secretion, which leads to a decrease in glucose levels [8]. Quercetin also reduces lipid peroxidation and oxidative stress, which aids in the management of diabetes and its complications [9]. Furthermore, secondary metabolites from plants have been shown to inhibit digestive enzyme activity by forming hydrogen and hydrophobic bonds with digestive enzymes [10]. Traditional medicines have long been using plants and their extracts as antidiabetic agents [11]. Thus, more studies on such constituents from medicinal plants are important to find an effective and safe therapeutic agent for the treatment of diabetes.

*B. ciliata*, known as “Pashanbheda” in Nepalese communities, has been traditionally used for the treatment of diabetes, either singly or in conjunction with other types of treatment [12]. A variety of secondary metabolites found in *B. ciliata*, such as catechin, gallicin, gallic acid, β-sitosterol, bergenin, and tannic acid, are involved in biological activities including antibacterial, anti-inflammatory, antitussive, antiallergic, and anti-diabetic [13–18]. Additionally, *M. pudica* locally called “lajawati” consists of highly valued phytochemicals like mimosine, stigmasterol, β-sitosterol, betulinic acid, p-coumaric acid, mimopudine, 2-Hydroxymethylchroman-4-one, quercetin, and avicularin associated with numerous pharmacological properties, like antibacterial, antiviral, wound healing, anticancer, and antidiabetes effect [19–21]. Traditionally, *B. ciliata* and *M. pudica* have been used as anti-diabetic plants. Many phytochemicals or phytochemicals derived from chemically modified plants have been used to ensure a safer pharmaceutical drug. Many plant extracts have also been shown to have antidiabetic properties in-vitro, in-vivo, and in clinical trials [22]. LC-HRMS/MS, FTIR, HPLC, and other characterization tools have been used for the chemical profiling of plant extracts [23, 24]. The pharmacokinetics, toxicity, and drug-likeness properties of these chemical compounds can be assessed by using bioinformatic tools.

The bioinformatics tools offer the quickest way to identify a potential compound with therapeutic activity since the drug discovery process is indeed time-consuming. The study aims to identify the interaction of the active constituents found in these selected compounds with the catalytic domain of α-amylase in order to evaluate their antidiabetic activity. Therefore, the objective of our study is to conduct an in-vitro and in-silico analysis of the α-amylase inhibitory activity of *B. ciliata* and *M. pudica* plant extract and phytochemicals.

2. Materials and Methods

2.1. Chemical Reagents. 2-Chloro-4-nitrophenyl-α-D-maltotrioside (CNPG3), porcine pancreatic α-amylase (PPA), acarbose, and DPPH were purchased from Sigma–Aldrich, Germany. All other reagents were of analytical grade and purchased from Qualigens.

2.2. Collection of Plant Identification and Processing. *B. ciliata* was collected from the Bajhang district, (Coordinate: 29.7767° N, 81.2519° E) and the *M. pudica* was collected from the Dhading district, (Coordinate: 27.9711° N, 84.8985° E), Nepal, and were identified in the National Herbarium and Plant Laboratories, (Godawari, Nepal). The stems and leaves of *M. pudica* and the entire plant of *B. ciliata* were used for the experiment. The ethnobotanical use and chemical constituents of *B. ciliata*, and *M. pudica* are shown in Table 1 as well as Figures 1 and 2. The collected materials were shade dried and ground into a fine powder.

2.3. Preparation of Crude Extracts. Crude extracts of plants were prepared by the cold-percolation method; the powder was soaked in methanol for 24 hours and filtered. The same process was repeated for three successive days, and then, concentrated in a vacuum in a rotary evaporator at 40°C. The fractionation was done based on polarity using different solvents like hexane, dichloromethane (DCM), and ethyl acetate (EtOAc). Each extract was dissolved in 30 mL of distilled water, and an equal volume of hexane was added to it. Three successive fractionations were carried out using Hexane followed by DCM, and ethyl acetate. The organic solvent was concentrated using a rotary evaporator [25].

2.4. Determination of Total Phenol Content. The TPC was determined using Folin–Ciocalteau’s method [26, 27]. First, 20 μL of plant extract and 100 μL Folin–Ciocalteau’s reagent were added. Then, 80 μL of sodium carbonate was added to it and incubated at room temperature for 15 min. The absorbance was recorded at 765 nm using a microplate reader (Synergy LX, BioTek Instruments, Inc, USA). Different concentrations of gallic acid were used to generate a standard curve and expressed as milligrams of gallic acid per gram dry weight basis of extract (mg GAE/g).

2.5. Determination of Total Flavonoid Content. The aluminum trichloride method was used to determine TFC [27–29]. Initially, 20 μL of plant extract and 110 μL of distilled water were added. Then, 5 μL of 10% aluminum trichloride as well as 1 M potassium acetate were added to it, and finally, 60 μL ethanol was added. It was then incubated at room temperature for 30 min and absorbance was recorded at 415 nm using a microplate reader (Synergy LX, BioTek Instruments, Inc, USA). Different concentrations of gallic acid were used to generate a standard curve and expressed as milligrams of quercetin per gram dry weight basis of extract (mg GAE/g).

2.6. Determination of Antioxidant Activity. The antioxidant was done using a DPPH reagent [30, 31]. The reaction was carried out by adding 100 μL of plant extracts mixed with 100 μL of DPPH solution of 0.1 mM. After 30 min incubation in the dark at room temperature, the absorbance was taken at 517 nm. The percentage scavenging was calculated by the given formula:
\[
%\text{Scavenging} = \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100
\]

Where \( A \) = Absorbance of sample and control.

2.7. α-Amylase Inhibition Assay. The α-amylase inhibition assay was done using the previously described method. [32] In short, 20 μL of plant extract and 80 μL of PPA with a final concentration of 1.5 U/mL were incubated at 37°C for

| S.N. | Medicinal plant | Family | Ethnobotanical uses                                                                 | Chemical constituents                                                                 |
|------|-----------------|--------|-------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------|
| 1    | *B. ciliata*    | Saxifragaceae | It is used for antidiabetic coughs and colds [18], antibacterial activity [16], anti-inflammatory [15], antitussive [14], and antiulcer [17] | Bergenin, gallic acid, gallicin, tannic acid, catechin, (-)-3-0-galloylepicatechin, (-)-3-0-galloycatechin, stigmatosterol, β-sitosterol, galloylated leucoanthocyanidin-4-glucoside, allantoin, and rhabdiol [13] |
| 2    | *M. pudica*     | Fabaceae | It is traditionally used as an antidiabetic, antibacterial, wound healing, antivenom, anticancer, fever, and dyspepsia [20] | Mimosine, stigmatosterol, β-sitosterol, betulinic acid, p-coumaric acid, mimopudine, 2-hydroxymethyl-chroman-4-one [21] quercetin, and avicularin [19] |
10 min. Then, 100 μL of 0.8 mM CNPG3 as a substrate was added and incubated at the same temperature for 15 min. Both the enzyme and the substrate were prepared in 50 mM phosphate buffer supplemented with 0.9% NaCl. The absorbance was taken at 405 nm using a microplate reader (Synergy LX, BioTek Instruments, Inc, USA). The percentage inhibition was calculated using the given formula:

\[
\%\text{Inhibition} = \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100, \tag{2}
\]

where \( A \) = Absorbance of sample and control.

2.8. Pharmacokinetic Analysis. The pharmacokinetic analysis was carried out to determine the absorption, distribution, metabolism, excretion, and toxicity of the compounds using pkCSM and ProTox II webserver [33, 34]. Besides that, the drug-like properties of selected compounds were identified using bioinformatics tools: SwissADME and Lipinski’s rule of five [35].

2.9. Molecular Docking. Molecular docking was performed to determine the binding capacity of phytochemicals in the enzyme pocket using AutoDock Vina [36]. The optimization of protein was carried out by removing water, adding polar hydrogen, and Kollman charges. After converting protein and ligand to pdbqt format, a grid box of size 40 × 40 × 40 with attributes \( X = 7.130 \), \( Y = 47.451 \), and \( Z = 19.247 \) was created for the docking process. The exhaustiveness value was kept at eight. The docking process is validated by repeatedly docking the same ligand to get similar binding energy and superimposing the best results. The RMSD was less than 2, indicating that the docking process had been validated. After the docking process, the outputs were visualized in the Biovia discovery study for analysis of protein-ligand interactions.

2.10. Statistical Analysis. The Gen5 Microplate Data Collection and Analysis Software was used for result processing, followed by Microsoft Excel. The data were expressed as mean ± standard error of the mean. The IC\textsubscript{50} values were determined using GraphPad Prism Version 8 Software.

3. Result

3.1. Analysis of TPC, TFC, and Antioxidant. The total phenolic content (190.81 ± 0.63 and 44.88 ± 0.65) mg GAE/g and flavonoid content (9.22 ± 0.24 and 22.42 ± 0.46) mg GAE/g of B. ciliata and M. pudica were found, respectively, at 500 μg/mL. Similarly, the antioxidant activity of both plants at the same concentration was found to be (88.68 ± 1.81 and 53.41 ± 0.99), respectively. The IC\textsubscript{50} value of each plant as compared to the standard (quercetin) is shown in Table 2.

3.2. α-Amylase Inhibition. The screening of α-amylase inhibition was done at 500 μg/mL and only those fractions that showed more than 50% inhibition were further analyzed to determine the IC\textsubscript{50} value by diluting the extracts between 500 and 15.625 μg/mL. The dilution of acarbose was done between 50 and 1.56 μg/mL. The results revealed that the ethyl acetate fraction showed potent activity as compared to other fractions. The detail of α-amylase inhibition is given in Table 3.

3.3. Pharmacokinetic and ADMET Analysis. Different parameters such as absorption, distribution, metabolism, excretion, and toxicity were analyzed that play an important role during the drug development and approval stage. The result revealed that tannic acid showed 0% human intestinal absorption and CYP3A4 substrate inhibition, gallicin showed AMES toxicity, while betulinic acid and mimopudine showed hepatotoxicity. Quercetin falls under toxicity class 3, gallic acid, β-sitosterol, gallicin, galloylpeicatechin, galloylcatetchin, stigmasterol, mimosine, and mimopudine fall under toxicity class 4, while the remaining compounds fall under classes 5 and 6. The details of the ADMET and toxicity profile are shown in Table 4. The physical and chemical qualities for oral delivery of each compound were evaluated through Lipinski’s rule to determine possible drug candidates. The details of the study are given in Table 5.

3.4. Molecular Docking Analysis. The molecular docking was done based on ADMET and Lipinski’s rule of five analyses. The compounds that are isolated from selected medicinal
Plants were analyzed for pharmacokinetics analysis and the compounds that are nontoxic to humans were further analyzed via autodock vina to find the interaction between selected compounds and \( \alpha \)-amylase. The compounds like bergenin, catechin, allantoin, p-coumaric acid, and 2-hydroxymethyl-chroman-4-one were selected for further analysis. The details are shown in Figure 3 and Table 6.

4. Discussion

Diabetes is characterized by high concentrations of blood sugar levels, and the treatment goal is to maintain normal glucose levels or reduce fluctuations in blood sugar levels. Natural products have immense potential in the management of diabetes, with bioactive compounds stimulating the pancreas to secrete insulin and inhibiting digestive enzymes. \( \alpha \)-amylase catalyzes the hydrolysis of \( \alpha \)-1, 4-glucosidic linkages of starch, glycogen, and various oligosaccharides, and inhibition of \( \alpha \)-amylase is seen as one of the important strategies for managing glucose concentration by lowering the blood glucose level.

Phenolic compounds are reported to be the major phytochemicals in plants responsible for antioxidant activity due to their ability to scavenge free radicals. The TPC value of 190.81 ± 0.63 mg GAE/g and 44.88 ± 0.65 mg GAE/g was observed from the extract of *B. ciliata* and *M. pudica* respectively. In comparison to our study, a previous study reported a TPC value of 473.4 ± 15.1 mg GAE/g from *B. ciliata* and 57.431 ± 1.096 mg GAE/g from *M. pudica* respectively. Similarly, in our study, the TFC of *B. ciliata* and *M. pudica* was found to be 9.22 ± 0.24 and 22.42 ± 0.46 mg QE/g respectively as compared to 89.9 ± 0.1 mg QE/g and 16.97 ± 1.472 mg QE/g of previous findings. The antioxidant activity was found to be 53.5 μg/mL from methanolic leaf extract of *B. ciliata*, while 7.18 ± 0.0005 μg/mL from *M. pudica* methanolic, while in our study their antioxidant activity was found to be 125.86 ± 4.16 and 528.43 ± 6.53 μg/mL, respectively [37, 38]. The difference in polyphenol content might be due to different factors such as the degree of ripeness at the time of harvest, environmental factors, processing, and storage [39].

### Table 2: Total phenolic content, flavonoid content, and antioxidant activity of selected plants.

| Plants     | TPC (mg GAE/g) | TFC (mg QE/g) | DPPH (% inhibition) | IC\textsubscript{50} value (μg/mL) |
|------------|----------------|--------------|---------------------|-----------------------------------|
| *B. ciliata* | 190.81 ± 0.63  | 9.22 ± 0.24  | 88.68 ± 1.81        | 125.86 ± 4.16                    |
| *M. pudica*  | 44.88 ± 0.65   | 22.42 ± 0.46 | 53.41 ± 0.99        | 528.43 ± 6.53                    |
| Quercetin   | —              | —            | —                   | 13.41 ± 1.94                     |

### Table 3: Methanolic extract and its fraction IC\textsubscript{50} value (μg/mL) against \( \alpha \)-amylase.

| Plants     | Methanol | Hexane | DCM | Ethyl acetate |
|------------|----------|--------|-----|--------------|
| *B. ciliata* | 158.8 ± 1.30 | 32.18 ± 0.78 | 38.54 ± 0.61 | 13.97 ± 0.10 |
| *M. pudica*  | 49.85 ± 0.49# | 39.52 ± 1.91# | 40.85 ± 0.30# | 11.79 ± 0.36 |
| Acarbose    | 6.34 ± 0.07   |        |     |              |

#% Inhibition at 500 μg/mL.

### Table 4: Pharmacokinetic analysis of compounds isolated from *B. ciliata* and *M. pudica*.

| Compounds                  | Human intestinal absorption (HIA)% | BBB permeability (logBB) | CYP3A4 substrate inhibition | AMES toxicity | Hepato toxicity | Toxicity class | LD\textsubscript{50} mg/kg |
|----------------------------|-----------------------------------|--------------------------|-----------------------------|---------------|-----------------|---------------|-----------------|
| Gallic acid                | 43.374                            | −1.102                   | No                          | No            | No              | 4             | 2000            |
| \( \beta \)-sitosterol     | 94.464                            | 0.781                    | No                          | No            | No              | 4             | 1185            |
| Bergenin                   | 63.774                            | −1.091                   | No                          | No            | No              | 6             | 10000           |
| Gallicin                   | 76.635                            | −1.046                   | No                          | Yes           | No              | 4             | 1700            |
| Catechin                   | 68.829                            | −1.054                   | No                          | No            | No              | 6             | 10000           |
| Tannic acid                | 0                                 | −8.62                    | Yes                         | No            | No              | 5             | 2260            |
| Galloylereicatechin        | 62.096                            | −1.847                   | No                          | No            | No              | 4             | 10000           |
| Galloylereicatechin        | 62.096                            | −1.847                   | No                          | No            | No              | 4             | 10000           |
| Stigmastanol               | 94.97                             | 0.771                    | No                          | No            | No              | 4             | 890             |
| Mimosine                   | 45.69                             | −0.742                   | No                          | No            | No              | 4             | 2000            |
| Betulinic acid             | 99.763                            | −0.322                   | No                          | No            | Yes             | 5             | 2610            |
| Mimopudine                 | 50.165                            | −1.656                   | No                          | Yes           | No              | 4             | 780             |
| Quercetin                  | 77.207                            | −1.098                   | No                          | No            | No              | 3             | 159             |
| Avicularin                 | 57.226                            | −1.571                   | No                          | No            | No              | 5             | 5000            |
| Allantoin                  | 51.948                            | −0.566                   | No                          | No            | No              | 5             | 2600            |
| p-Coumaric acid            | 93.494                            | −0.225                   | No                          | No            | No              | 5             | 2850            |
| 2-hydroxymethyl-chroman-4-one | 89.486                         | −0.149                   | No                          | No            | No              | 5             | 2647            |
Our study revealed that the EtOAc fraction had high \( \alpha \)-amylase inhibitory activity. A previous study on \textit{B. ciliata} showed an 84.3 \( \pm \) 13.2\% in EtOAc fraction as compared to 65.3 \( \pm \) 2.7\% in water fraction. These compounds are also reported as anti-diabetic. Therefore, the presence of these compounds may be responsible for higher \( \alpha \)-amylase inhibitory activity by EtOAc fraction \textit{M. pudica} in our study.

Studies have shown that due to their insufficient efficacy and safety concerns, the majority of drugs fail to demonstrate their therapeutic efficacy. Therefore, the analysis of ADMET and drug-likeness properties of compounds is crucial to the process of developing new drugs. The pharmacokinetic results revealed that, with the possible exception of tannic acid, all remaining compounds showed more than 30\% absorption, which is considered good absorption [40]. Stigmasterol and \( \beta \)-sitosterol both showed a logBB value > 0.3 indicating high BBB permeability, while the remaining compounds have low brain permeability [34]. Compounds like bergenin, catechin, tannic acid, betulinic acid, avicularin, allantoin, p-coumaric acid, and p-hydroxymethyl-chroman-4-one lie under class v, may be harmful if swallowed, and classes v, nontoxic. Tannic acid showed 3A4A substrate inhibition, so it is most likely quickly metabolized in the liver.

The compounds such as bergenin, catechin, allantoin, p-coumaric acid, and p-hydroxymethyl-chroman-4-one showed suitable pharmacokinetic properties and zero violation of Lipinski’s rule of five. So, these compounds were further docked with human pancreatic \( \alpha \)-amylase to analyze their interaction with the amino acid residues of the catalytic domain. Table 6 shows the docking score, an interacted amino acid, distance, and bond responsible for the stability of the protein-ligand complex. The active site of \( \alpha \)-amylase contains different amino acid residues such as ARG61, ASP165, ASP197, LYS200, GLU233, ASP236, and ASP300. Besides that, different aromatic and nonpolar residues like TRP58, TRP59, TYR62, HIS101, PRO163, ILE235, TYR258, HIS299, HIS305, and ALA307 are also present [41, 42]. Our study revealed that catechin showed the lowest binding energy of \(-8.6\) kcal/mol among other
Figure 3: Continued.
phytochemicals, in comparison to the commercial drug acarbose. The phytochemicals interacted with the active amino acid residues i.e., TRP59, TYR62, PRO163, HIS299, ASP165, ASP197, GLU233, and ASP300 via electrostatic, hydrophobic, van der Waals, Pi-Pi Stacked, Pi-Anion, and hydrogen bond. The presence of such associated hydrogen and hydrophobic bonds indicates a positive response to the inhibition of the α-amylase enzyme. As a result, phytochemicals found in various plant extracts may be responsible for these extracts’ antidiabetic properties. In the future, these compounds can be studied for enzyme kinetics and in-vivo experiments.

5. Conclusions

In conclusion, the result of our study reveals the inhibition of α-amylase by B. ciliata and M. pudica extracts. The compounds reported from these two plants were subjected to
studies on pharmacokinetic properties, where five compounds were found to be nontoxic as well as have drug-like properties. These compounds, bergenin, catechin, allantoin, p-coumaric acid, and 2-hydroxymethyl-chroman-4-one were further analyzed through molecular docking. From docking analysis, it was found that the compound catechin has a higher binding affinity towards the α-amylase and interacted with key active amino acid residues GLU233 and ASP300 through hydrogen and hydrophobic bonds. So, it could serve as a potential drug candidate for the treatment of type 2 DM. Moreover, this study suggests further in-vivo studies to elucidate the potential of these compounds to treat diabetes efficiently and more safely.

Data Availability
The datasets used to support the findings of this study are included in the article.

Conflicts of Interest
The authors declare that they have no conflicts of interest.

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