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Research Article

ANALYSIS OF PHYTO-CONSTITUENTS, ANTIOXIDANT, AND ALPHA AMYLASE INHIBITORY ACTIVITIES OF *PERSEA AMERICANA* MILL., *RHODODENDRON ARBORETUM* SM. *RUBUS ELLIPTICUS* SM. FROM ARGHAKHANCHI DISTRICT NEPAL

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ABSTRACT

Objective: To evaluate the phytochemical, antioxidant activities, and α-amylase inhibition assay for methanolic extract of three ethnomedicinal plants, namely *Persea americana* Mill., *Rubus ellipticus* Sm., and *Rhododendron arboretum* Sm. collected from Arghakhanchi District of Nepal using *in vitro* studies.

Methods: Methanolic plant extracts were prepared by cold percolation method. Analysis of phytochemical constituents was carried out using standard methods. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was used to evaluate *in vitro* antioxidants activities. Furthermore, inhibition effect of extracts on α- amylase enzyme was carried out by using starch as a substrate, pancreatic α-amylase as the enzyme, and acarbose as standard.

Results: Phytochemical screening of methanolic extract of all three selected plants displayed the presence of different chemical constituents such as alkaloids, polyphenols, flavonoids, terpenoids, saponins, glycosides, and tannins. The results of DPPH assay revealed that *R. ellipticus* and *R. arboreum* were most active with half maximal inhibitory concentration (IC_{50}) values 33.41 µg/ml and 47.28 µg/ml, respectively. *R. ellipticus was* found to be effective toward α-amylase inhibition with IC_{50} values 269.94 µg/ml.

Conclusion: The preliminary results of this study have put forward *R. ellipticus* into promising herbs with good antioxidant activities and α-amylase inhibition potential although further studies are needed to assess its mechanism of action.

Keywords: Amylase inhibitor, Antioxidant, Diabetes, Medicinal plants, Phytochemical.

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INTRODUCTION

Diabetes is a thoughtful disease increasing at frightening rates around the world. Type II diabetes is a widespread metabolic disorder of fat and carbohydrate metabolism, which is totally connected with food that we ate [1]. Postprandial hyperglycemia (PPHG) is key factors for the development of diabetic complications such as cardiovascular disease, neuropathy, nephropathy, and retinopathy [2,3]. The literature survey revealed that one of the best accepted therapeutic approaches for treating Type II diabetes mellitus is to lower PPHG by preventing the absorption of glucose through the inhibition of the α-glucosidase, and α-amylase enzymes. These two carbohydrate hydrolyzing enzymes in small intestine break oligosaccharides and disaccharides into monosaccharides, making them available for intestinal absorption to increase blood glucose level immediately after meal [4,5]. Unluckily, the inhibitors of the alpha-amylase enzyme designed as drugs so far, that is, acarbose and sulfonylureas are underwent with several side effects, as well as with financial cost [6,7].

It is well known fact that pancreatic cell damage initiated by reactive oxygen species (ROS) is root foundation of Type II diabetes. ROS also causes damage to cell components such as proteins, lipids, and nucleic acids, which are responsible for causing oxidative stress in many diabetic patients [8]. It is identified that phytochemicals such as polyphenols and flavonoids act as chemopreventive agents against these ROS and also inhibit α-glucosidase, and α-amylase enzyme, helping to lower down the PPHG with no known side effects so far. These phytochemicals are commonly found in nuts, vegetables, fruits, tea, and medicinal plants [9]. Recently, many papers had been published exposing the chemistry relating the antidiabetic activity of plants [10-12].

Nepal is enrich with variable climate conditions, geographical variation and immense variety of plants with potential antidiabetic activities, but no efforts have been made to seek more safe and efficient antioxidant, α-amylase inhibitors from its natural sources so far. Many plants have been commonly used for diabetic treatment all around the world without their scientific evaluations. Therefore, it is urgent to identify and explore the antioxidant amylase inhibitors from natural resources of Nepal. Thus, the present investigation was undertaken to make a comparative study of the ability to inhibit α-amylase activity, phytochemical constituents, and assessed their antioxidant properties of the selected plants, that is, *Persea americana,* Family: Lauraceae., *Rhododendron arboreum* (Family: Ericaceae), and *Rubus ellipticus,* belonging to family Rosaceae which are used traditionally for the treatment a number of illnesses including diabetic.

METHODS

Chemicals and reagent

The chemicals used in this study were methanol (Merck, Germany), porcine pancreatic α-amylase, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and ascorbic acid (Sigma-Aldrich, USA). All additional chemicals used in this research work were of the commercially available analytical grade.

Plant collection and extracts preparation

Leaves of *P. americana, R. ellipticus, and Rhododendron arboreum* were collected from Arghakhanchi District. All collected plants were identified by expert from the Central Department of Botany, Tribhuvan University. Kirtipur, Kathmandu. All collected plant materials were washed with tap water, dried in an oven and powdered. These powders were packed in separate labeled plastic bags and put away from sunlight in cool and dry place until used for the further experiment. The dried powdered were extracted with methanol by cold percolation method. The solvent

was replaced every 24 h until extraction was completed. All extracts of each plant were combined and filtered through a porous plug of absorbable cotton. The rotary evaporator was used to concentrate filter at 60 °C temperature. Finally, the concentrated extracts were fridge dried to yield a dry powder.

Phytochemical screening

Phytochemical screening is the method of finding the chief group of chemical constituents present in the plant extracts. The phytochemicals were analyzed by following the standard procedures put forward by Ciulei [13]. The diverse phytochemicals in the extracts were identified by the color reaction with different reagents.

Free radical scavenging ability assay

We followed the adopted methods in our laboratory by Subba *et al*. 2014 to evaluate the antioxidant activity of test extracts [14,15].

Briefly, 1 ml the each extracts solution comprising appropriate concentration was mixed with 1 ml, 0.4 mM DPPH in methanol. The absorbance of the reaction mixture was measured at 516 nm in a spectrophotometer immediately after incubation for 30 min in the dark. The DPPH free radical scavenging ability was then calculated using following formula thus:

$$\% \text{ of free radical scavenging activity} = \frac{A_0 - A_T}{A_0} \times 100$$

Where A_0 is the absorbance without samples extract and A_T is the absorbance of samples extract.

Alpha-amylase inhibition assay

Alpha-amylase inhibition assay was done using a standard protocol with minor variations as explain in brief [16]. The undigested starch due to enzyme inhibition was detected at 630 nm (blue, starch-iodine complex). The starch solution was ready by dissolving 200 mg starch in 25 ml of NaOH (0.4 M) through heating at 100 °C for 5 min, and it was left at room temperature to cool down. The pH of the cool solution was adjusted to 7.0, and the finally volume was made up 100 ml by adding distilled water. Acarbose was used as a standard inhibitor for alpha-amylase enzyme. 400 µl of starch solution was preincubated at 37 °C for 5 min with 200 µl of acarbose or plant extract at varying concentrations (40, 80, 160, 320, 640, and 1000 µg/ml), followed by 200 µl of 50 µg/ml α-amylase (20 mM phosphate buffer with 6.7 mM NaCl, pH 6.9), and incubated at 37 °C for 15 min. The reactions were terminated by adding 800 µl of HCl (0.1 M). Then, 1000 µl of iodine reagent (2.5 mM) was added, and absorbance was measured at 630 nm. Each assay was carried out in triplicates. Percentage of inhibition was calculated using the formula:

$$\% \text{ Inhibition} = (1 - [\text{Abs2} - \text{Abs1} / \text{Abs4} - \text{Abs3}]) \times 100$$

Where, Abs1 is the absorbance of the reaction mixture containing plant sample, starch, and α-amylase, Abs2 is the absorbance of the reaction mixture of sample and starch, Abs3 is the absorbance of the reaction mixture of starch and α-amylase, Abs4 is the absorbance of reaction mixture containing starch only.

RESULTS AND DISCUSSION

Plant collections

The tested plants on this research work were selected and collected on the basis of their used traditionally for the treatment a number of illnesses including diabetic (Table 1).

Phytochemical screening

The result obtained from phytochemical screening for each plant is tabulated as follows in Table 2. Preliminary phytochemical analysis of *P. americana, R. ellipticus,* and *R. arboreum* showed positive results for alkaloids, terpenoids, quinones, and flavonoids. These phytochemicals are reported for their various biological activities.

DPPH as an antioxidant

The methanol extractives of *P. americana, R. ellipticus,* and *R. arboreum* were evaluated for free radical scavenging activity. The graph of concentration against the corresponding percentage radical scavenging activity of different samples was plotted (Fig. 1) and concentration providing 50% inhibition was determined. Ascorbic acid was used as the standard in this experiment.

An half maximal inhibitory concentration (IC_{50}) value of *P. americana, R. ellipticus,* and *Rhododendron arboretum* was found 121.39±0.1333 µg/ml, 31±0.2641 µg/ml, and 38±0.15 µg/ml, respectively. IC_{50} value of the standard, that is, ascorbic acid was found to be 22.17 µg/ml. Among the test plants, *R. ellipticus* has lower IC_{50} values followed by other *R. arboretum,* and *P. americana,* respectively. The high antioxidant activity of the plant *R. ellipticus* is may be due to the phytochemicals such as quinones, flavonoids, terpenoids, and glycosides. These are the major phytochemicals which are known for their antioxidant behavior [17]. Flavonoids isolated from the leaves of *R. arboreum* were reported to have potent antioxidant property [18]. The result is well supported by previously reported the literature.

***In vitro* α-amylase inhibition study**

The mode of α-amylase inhibition by the tested plant's leaf extract was determined by the lowest IC_{50} according to the method given by Bernfeld [16]. Alpha-amylase inhibitory properties are presented in Fig. 2.

In the present study, among the medicinal plants tested, *R. ellipticus* leaves and *R. arboreum* leaves showed moderate inhibition of α-amylase activity and weak inhibition activity by *P. americana* on comparison with standard acarbose Table 3.

The aqueous leaves extracts of *P. americana* possess hypoglycemic activity [19]. Similarly, ether, ethanolic and aqueous extracts from *R. ellipticus* fruits (200 mg/kg) are reported for its antidiabetic activity on alloxan-induced diabetes and glucose tolerance test in rats [20]. Bhandary and Kuwabata reported the α-glucosidase inhibitory activity of *R. arboreum* and isolated active compounds from it [21]. According to the previous studies, the phytochemicals such as alkaloids, terpenoids, and polyphenols are the major antioxidants. Similarly, the presence of flavonoids and tannins in the extracts is accountable for the antidiabetic activity of the plants [22].

Hence, here the observed α-amylase inhibitory activity of test plant extracts might be due to the presence of analogous phytoconstituents which were marked during phytochemical screening, and also due to the witnessed antioxidant potential of the tested plant's extracts. Hence, this study here suggests *R. ellipticus* leaves as good antioxidant as well as good antidiabetic, and *R. arboreum* leaves as a good antioxidant but weak antidiabetic. Both plants have lower α-amylase inhibition IC_{50} value in comparison with acarbose so might be handier in controlling Type II diabetics than acarbose because its strong inhibitory activity toward α-amylase is credited for its side effects, that is, due to fermentation of excess undigested carbohydrates in the colon [23].

CONCLUSION

This study provides some scientific support for their traditional use for diabetes management and other ailments. The author also anticipated that antioxidant properties and inhibition of α-amylase by *R. ellipticus, R. arboretum,* and *P. americana* leaves extract as a probable mechanism for the antidiabetic action. Thus, it can be firm that use of these plant extracts will be greatly subsidized for effective management of Type II diabetes, but further experiments are necessary to find out whether the extract possesses antidiabetic activity under *in vivo* conditions. Here, it is concluded that further bioassay-guided fractionation approaches will be required on *R. ellipticus* and *R. arboretum* to identify the compounds responsible for their promising *in vitro* antidiabetic activity.

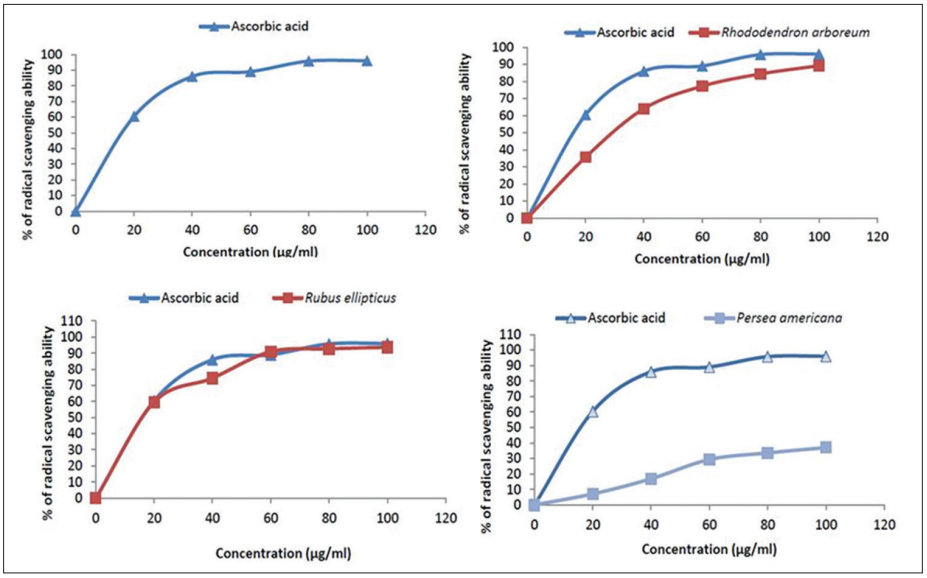

Fig. 1: Results of 2,2-diphenyl-1-picrylhydrazyl scavenging activities of the methanolic extracts of the plants

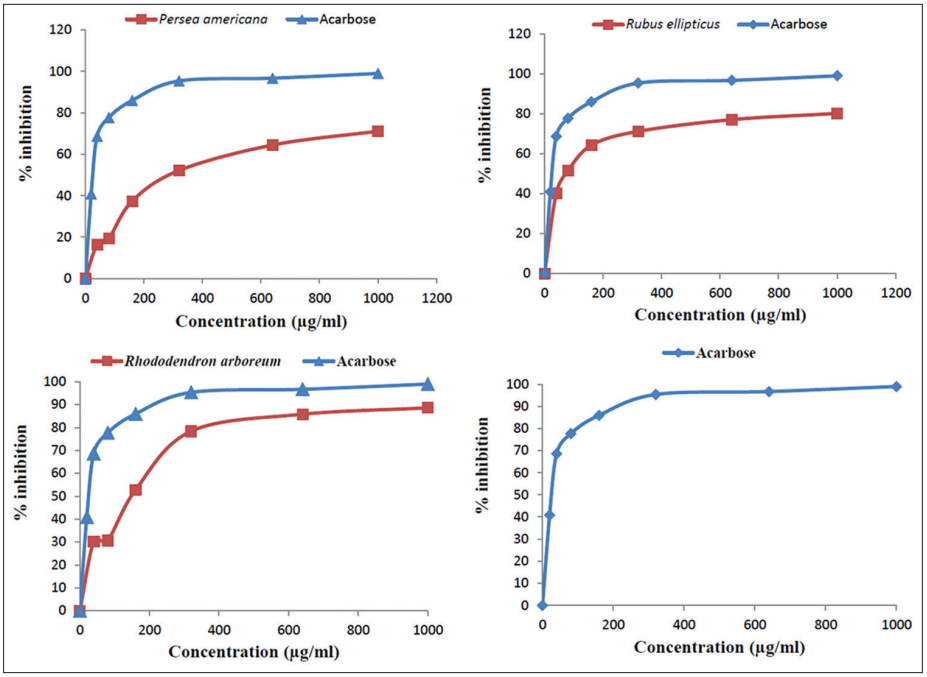

Fig. 2: Inhibition of a-amylase activities by plant extracts

Table 1: Names of the plants, respective family, parts used, and their uses

Scientific names	Common nepali names	Family	Parts used	Medicinal uses
Persea americana	Ghew Phal	Lauraceae	Leaves	Leprosy, fever, asthama, jaundice, and cancer
Rubus ellipticus	Ainselu	Rosaceae	Leaves	Bronchitis, ulcers, diarrhea
Rhododendron arboreum	Gurans	Apocynaceae	Leaves	Constipation, ringworm infection, and anti-inflammatory

Table 2: Results of phytochemical screening of different plant extracts

Phytochemicals										
Plants	**Alkaloids**	**Flavonoids**	**Glycosides**	**Polyphenols**	**Terpenoids**	**Steroids**	**Carbohydrates**	**Saponins**	**Tannins**	**Quinones**
Persea americana	+	-	+	+	+	+	-	+	+	+
Rubus ellipticus	+	+	+	-	+	-	+	-	-	+
Rhododendron arboreum	+	+	-	-	-	+	-	+	+	-

Where, (+)=Present and (-)=Absent

Table 3: IC_{50} value of extracts on inhibition of α-amylase

Sample name	IC_{50} (µg/ml) (mean)
Acarbose	144.56±0.26
Persea americana	479.16±0.10
Rubus ellipticus	269.94±0.11
Rhododendron arboreum	298.52±0.21

Values are expressed as mean+SD; Values are from triplicate readings. IC_{50}: Half maximal inhibitory concentration

STATISTICS

All the analysis was carried out in triplicate, and the results are expressed as mean ± SD.

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AUTHORS' CONTRIBUTIONS

B. Subba analyzed the data, and wrote the manuscript, whereas Sanjay Gaire carried out the laboratory work in the guidance of Dr. Khagraj Sharma. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

All authors have none to declare.

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