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Research Article

Determination of α-Amylase Inhibitory potential of leaf extracts of *Rhododendron arboreum* Sm. and *Rhododendron campanulatum* D. Don

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Abstract

The biological activity of plant-derived substances/metabolites may be considered as a source of new anti-enzyme drugs. Therefore, traditional Indian plants i.e. *Rhododendron arboreum* and *R. campanulatum* which are commonly used as remedies to control different diseases were screened to discover possible plant-derived α-amylase inhibitors. In the present investigation, inhibitory effects of leaf extracts of *Rhododendron arboreum* and *R. campanulatum* were determined against porcine α-amylase at a concentration range of 0.2-1.0 mg/mL. *R. arboreum* displayed 51.10, 44.00 and 35.40% inhibition at a concentration of 1 mg/mL for methanol, acetone and aqueous leaf extracts respectively. On the other hand, *R. campanulatum* showed 21.15, 18.25 and 15.85% α-amylase inhibition for methanol, acetone and aqueous extracts respectively at 1 mg/mL. The inhibitory activity increased altogether with increasing concentration of each plant extract. The results further indicated that methanol extracts of medicinal plants exhibited maximum inhibitory effects than other solvent (acetone, methanol) extracts.

Keywords: *Rhododendron arboreum*, *Rhododendron campanulatum*, porcine α-Amylase, Leaf extracts, Inhibition

INTRODUCTION

α-Amylases (EC 3.2.1.1) are widely distributed among various organisms and usually show diverse substrate specificities. Inhibition of mammalian α-amylase is a proven therapeutic approach in diabetes and other related disorders/ailments[1,2]. Inhibition of α-amylase delays the digestion process by hampering breakdown of starch thereby can be used as an effective strategy for regulating hyper-glycemic condition[3].

Inhibitors of α-amylase like acarbose, miglitol and voglibose usually delay carbohydrate digestion process and thus prolong overall carbohydrate digestion time causing reduction in the rate of glucose absorption and consequently blunting the post-prandial plasma glucose rise. However, these drugs are known to be associated with various gastrointestinal side effects such as abdominal pain, flatulence, diarrhoea etc.[4,5]. Therefore, it is the need of the hour to identify and explore α-amylase inhibitors from natural or plant-based sources having fewer or no side effects.

Rhododendron arboreum Sm. is an evergreen much branched tree whose young leaves are usually known to be poisonous (associated with intoxication in large quantities) as well as medicinal and applied on the forehead to alleviate headache[6]. The leaf extract of *R. arboreum* is reported to exert high antioxidant content with hepatoprotective, immunomodulatory and antimicrobial activities[7,8].

Rhododendron campanulatum D. Don, a shrub or a small tree, is a very important member of genus *Rhododendron*, which is known for its traditional medicinal significance for the different kind of ailments like body ache, sore throat, digestion, skin irritations, cold and fever, etc.[9] The leaves are generally used in treating chronic rheumatism, syphilis and sciatica. The mixture of powdered dried leaves and tobacco leaves can be used as snuff to cure hemicrania and colds.

In view of above mentioned useful properties of *R. arboreum* and *R. campanulatum*, I planned to determine *their* α-amylase inhibitory potential.

MATERIALS AND METHODS

Collection of plant material

Leaves of *Rhododendron arboreum* and *R. campanulatum* were collected from Churdhar area of District Sirmaur (Himachal Pradesh) during flowering season of the year. The collected plant material was finally brought to the laboratory for further analysis.

Processing of plant material

The Leaves were washed thoroughly under tap water and then treated with 2% Mercuric chloride ($HgCl_2$). Eventually the leaves were cut into smaller pieces for quick drying. The plant material obtained after drying was crushed into fine powder with the help of pestle mortar and stored in air tight containers at room temperature for further examinations.

α- Amylase inhibition assay

α- Amylase inhibition activity of different leaf extracts of *Rhododendron arboreum* and *R. campanulatum* was determined by some modifications in the method reported by Giancarlo *et al.*[10]. The starch solution (1% w/v) was prepared by boiling and stirring 1 g of potato starch in 100 mL of sodium phosphate buffer for about thirty minutes. The porcine pancreatic α- amylase enzyme (EC 3.2.1.1; purchased from Sigma Aldrich-3176) was obtained by mixing 0.01 g of α-amylase in 10 mL of sodium phosphate buffer (pH=6.9) containing 0.0006 mM sodium chloride (NaCl). The leaf extracts were then dissolved in DMSO to give concentrations ranging from 0.2 to 1.0 mg/mL (0.2, 0.4, 0.6, 0.8 and 1.0 mg/mL). The colour reagent was used (a solution containing 0.1 g of 3,5-dinitrosalicylic acid and 2.99 g sodium potassium tartrate in 0.16 g sodium hydroxide and 10 mL phosphate buffer). 50 µL of each plant extract plus 150 µL of starch solution along with 10 µL of enzyme were mixed in a 96 well-plate and then incubated at 37°C for 30 minutes. After this, 20 µL of sodium hydroxide (NaOH) and 20 µL of colour reagent were added and the closed plate was placed into a 100°C water bath. After 20 minutes, the reaction mixture was removed from the water bath, allowed for cooling and α-amylase activity was finally determined by measuring the absorbance of the mixture at 540 nm using a UV-VIS spectrophotometer. Blank samples, where the enzyme was replaced with the buffer solution were used to correct/set the absorption of the mixture. Also, a control reaction was used, where the plant extract was replaced with 50 µL of DMSO and the maximum enzyme activity was obtained. Acarbose solution (a positive (+) control) was used as in the concentration range of 0.2-1.0 mg/mL. The above experiment was performed in triplicate and the mean absorbance was used to calculate percentage of α- amylase inhibition. The inhibition percentage was assessed by using following formula:

$$\% \text{ α- Amylase inhibition} = \left(\frac{\Delta A_{control} - \Delta A_{sample}}{\Delta A_{control}}\right) \times 100$$

Where, $\Delta A_{control} = A_{test} - A_{Blank}$

$\Delta A_{sample} = A_{test} - A_{Blank}$

The concentration of the plant extract (enzyme inhibitor) was determined from corresponding dose-response curves of inhibition percentage versus inhibitor concentration and compared to acarbose, a known inhibitor of α- amylase activity and a logarithmic regression curve was established to calculate the IC_{50} value (the concentration of the given sample required to inhibit the activity of α- amylase enzyme by 50 percent) for each sample. Data were expressed as mean ± standard deviation (S.D.).

RESULTS & DISCUSSIONS

Inhibitory effects of different leaf extracts of *Rhododendron arboreum* and *R. campanulatum* were examined against porcine α-amylase at a concentration range of 0.2-1.0 mg/mL. *Rhododendron arboreum* showed 51.10, 44.00 and 35.40% inhibition at a concentration of 1 mg/mL for methanol, acetone and aqueous leaf extracts respectively while *R. campanulatum* exhibited 21.15, 18.25 and 15.85% α-amylase inhibition for methanol, acetone and aqueous extracts respectively at 1 mg/mL. This is the first report on α-amylase inhibitory activity of *R. arboreum* and *R.campanulatum* as there is no specific report available in literature regarding α-amylase inhibitory activity of these plants. Enzyme inhibitory activity can be attributed to the presence of different phytochemicals or chemical constituents present in these plants. Studies on green leaves of *Rhododendron arboreum* revealed the presence of phytochemicals like glucoside, ericolin (arbutin), ursolic acid, α-amyrin, epifriedelinol, triterpenoid campanulin, quercetin & hyperoside, lupeol and epifriedelinol. Quercetin-3-rhamnoside, a crystalline chemical compound has been extracted from the flowers of this plant[11]. The bark of this plant is found to be the richest source of single triterpenoid substance taraxerol and ursolic acid acetate[12].

Table 1: α-Amylase inhibitory activity (%) of *R. arboreum* leaf extracts at different concentrations

Concentration (mg/mL)	Methanol extract	Acetone extract	Aqueous extract	Acarbose
0.2	19.52±1.20	14.00±0.37	10.33±0.25	29.50±0.70
0.4	27.72±0.50	24.05±0.80	18.55±2.22	40.85±2.15
0.6	36.45±0.70	32.10±0.40	24.30±0.30	56.45±1.25
0.8	44.65±1.15	38.65±0.25	29.00±2.10	66.22±0.52
1.0	51.10±0.38	44.00±0.08	35.40±0.54	78.56±0.45
IC_{50} (mg/mL)	0.95	1.12	1.47	0.53

Values are given as mean ± S.E.

Table 2: α-Amylase inhibitory activity (%) of *R. campanulatum* leaf extracts at different concentrations

Concentration (mg/mL)	Methanol extract	Acetone extract	Aqueous extract	Acarbose
0.2	4.35±0.50	5.23±0.66	2.75±0.66	29.50±0.70
0.4	8.47±2.25	8.45±1.20	4.00±2.05	40.85±2.15
0.6	13.60±2.40	12.10±2.05	7.52±0.30	56.45±1.25
0.8	17.60±0.18	15.42±1.10	10.30±0.22	66.22±0.52
1.0	21.15±0.36	18.25±2.59	15.85±0.45	78.56±0.45
IC_{50} (mg/mL)	2.33	2.90	3.17	0.53

Values are given as mean ± S.E.

The leaves of *R. campanulatum* have shown the presence of active components such as epicatechin, syringic acid, quercetin, chlorogenic acid, gallic acid, proto-catechic acid and oleanane triterpenoid[13]. Besides, leaves are also reported to contain ericolin, ursolic acid, α-amyrin, friedelin, epifriedelinol, campanulin and a bitter yellowish brown resin. They also contain some pigments such as myricetin and quercetin. The leaves also contain a toxic substance which closely resembles andro-medotoxin in its chemical and pharmacological properties[14].

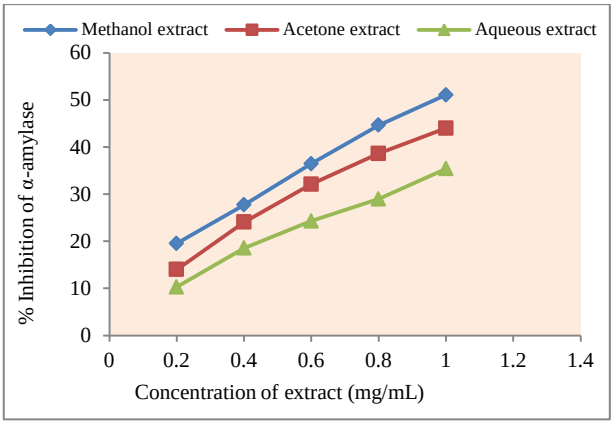

Figure 1: Inhibition profile of leaf extracts of *Rhododendron arboreum* against porcine α-amylase at a concentration range of 0.2-1.0 mg/mL

CONCLUSIONS

α-Amylase inhibitory effects of leaf extracts of *Rhododendron arboreum* and *R. campanulatum* were determined at a concentration range of 0.2-1.0 mg/mL. *R. arboreum* displayed 51.10, 44.00 and 35.40% inhibition at a concentration of 1 mg/mL for methanol, acetone and aqueous leaf extracts respectively whereas *R. campanulatum* showed 21.15, 18.25 and 15.85% α-amylase inhibition for methanol, acetone and aqueous extracts respectively at 1 mg/mL. The inhibitory potential increased with increasing concentration of leaf extract. The results further indicated that methanol leaf extracts of medicinal plants exhibited maximum inhibitory effects than other solvent extracts. This tends to show that the active metabolites/phytoconstituents of the different plant parts are better extracted with methanol than in other solvents. Therefore, the present study approves the medicinal value of these plants *viz*. *Rhododendron arboreum* and *R. campanulatum* and scientifically validates them for use as a component of medicinal preparations.

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CONFLICT OF INTERESTS

Author hereby declares no conflict of interest.

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