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In-vitro studies on the inhibition of α-amylase and α-Glucosidase by methanolic extract of *Lawsonia inermis* and *Malvastrum coromandelianum*

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Abstract
Lawsonia inermis and Malvastrum coromandelianum important medicinal plants widely used in India as folk medicine. These plants have been used to control diabetes in traditional medicinal systems. In the present study, 50% volume per volume ethanolic extracts of *Lawsonia inermis and Malvastrum coromandelianum* subjected to *in vitro* analysis of antidiabetic effect by alpha-amylase and alpha-glucosidase inhibitory assay. Inhibitory activity of the hydro-ethanolic extract of the all two plants individually against alpha-amylase enzyme and alpha-glucosidase enzyme were examined in different concentrations (3.90‑500 µg/mL), where acarbose used as a positive control. The percentage inhibition of Lawsonia inermis showed the highest alpha-amylase and alpha‑glucosidase inhibitory activity. Half-maximal inhibitory concentration value Malvastrum coromandelianum was found for two plants have antidiabetic property, from these two plants *Malvastrum coromandelianum* showed potent enzyme inhibition as compared to other plant extracts and standard acarbose.

Keywords: alpha-amylase, alpha-glucosidase, antidiabetic, hydro-ethanolic, *In vitro*

Introduction
Diabetes has caused a major burden to the health sector in the developing countries and has shown an increasing trend among the urban population [1]. It is estimated that most patients are with type II diabetes which could be easily treated with dietary changes, exercise, and medication [2, 3]. Sri Lanka carries a long history ayurvedic medicine where it uses the plant for treating many diseases. Therefore it is important to screen medicinal plants scientifically so they could be used safely and effectively in the traditional medical system and also be used for further investigations [3, 4]. It is estimated that the global prevalence of diabetes is increasing each year causing a major burden to the health sector, especially in the developing countries [5, 6]. It is estimated that the prevalence of diabetes is high among the urban population where 90% is accounted to be type II diabetic. Patients with type II diabetes can be easily treated with dietary changes, exercise, and medication. The complications arising with diabetes are closely related to the production of free radicals enhancing the oxidative stress. Hence the use of antioxidants has been effective in reducing the severity of diabetic complications [7, 8].

Materials and Methods
Chemicals and Reagents
Porcine pancreatic α-amylase (PPA), 3, 5-dinitrosalicylic acid (DNS color reagent), Potassium phosphate buffer solution (PBS), p-Nitrophenyl-α-D-glucopyranoside (pNPG), α-glucosidase, and ascorbose were purchased from Sigma Aldrich. Soluble starch potato, sodium potassium tartrate, sodium chloride, disodium hydrogen phosphate, and sodium hydroxide were from Merck Chemical Supplies (India). All the chemicals, including the solvents used in this study, were of analytical grade.

Plant Materials
The fresh matured leaves of the Lawsonia inermis and Malvastrum coromandelianum were collected randomly, from Sangli region, Maharashtra, India. Department of Botony, Yashwantrao Chavan College of Science, Karad has identified the plant and authenticated it.

Preparation of Plant Extract
Shade drying was done for almost a month to prevent sunlight chemical degradation. The dried material was grinded and transformed in coarse powder with the aid of a grinder. The extraction of Lawsonia inermis and Malvastrum coromandelianum with solvent methanol was carried out by microwave extraction, and excess solvent present was evaporated.

In vitro methods employed in antidiabetic studies
α-amylase inhibition activity
PPA (enzyme commission 3.2.1.1) solution was dissolved in 20 mM phosphate buffer (pH 6.9 with 6.7 mM sodium chloride) to give a concentration of 1 U/ml. Starch solution (1%, w/v) was obtained by stirring 0.1 g of potato starch in 100 ml of 20 mM of phosphate buffer (pH 6.9 with 6.7 mM sodium chloride) as a substrate. A total of 100 µl of plant extract solution and 100 µL of the enzyme were preincubated at 37°C for 30 min. After preincubation 100 µl of a 1% starch solution was added. The reaction mixtures were then incubated at 37°C for 20 min. The reaction was stopped with 200 µL of DNS color reagent and placed in boiling water for 5 min and cooled to room temperature. Add 200 μ l of reaction mixture into the 96‑well microplate after diluted with 1.5 ml of distilled water. The α‑amylase activity was

determined by measuring the absorbance of the mixture at 540 nm. Acarbose was used as positive control. Percentage inhibition was calculated by comparing against control optical density with the test group [9].

A-Glucosidase inhibitory activity

The α-glucosidase inhibitory activity was performed with a set of microwell. The enzyme solution containing 20 µl α-glucosidase (0.1 unit/ml) enzyme solutions were added in 96 microwell plate except blank well. A volume of 120 µl 0.1 M PBS solutions were added into the well-containing enzyme and 140 µl 0.1 M PBS in blank well and 160 µl PBS in extract blank well. Ten microliters of test samples (Acarbose or test samples) were added into the enzyme solution in microplate wells and then incubated for 15 min at 37° C. Twenty microliters of pNPG solutions were added to the microwell plate and incubated the plate for 15 min at 37°C. The reaction was terminated by adding 80 µl of 0.2 M sodium carbonate solution.

- Test solution contains: 20 µl enzyme + 120 µl PBS + 10 µl of test samples + 20 µl pNPG + 80 µl stop reagent.
- Control solution: All reaction mixture without test samples (20 µl enzyme + 130 µl PBS + 20 µl pNPG + 80 µl stop reagent).
- Blank solution: All reaction mixture except α-glucosidase enzyme (140 µl PBS + 10 µl of test samples + 20 µl pNPG + 80 µl stop reagent)
- Extract blank solution: 10 µl extract + 160 µl PBS + 80 µl stop reagent.

The absorbance of the wells was measured with a microplate reader. at 405 nm, while the reaction system without plant extracts was used as control. The system without α-glucosidase was used as blank, and acarbose was used as positive control. Each experiment was conducted in triplicate. The percentage enzyme inhibition and half-maximal inhibitory concentration (IC_{50}) was calculated [10].

Calculation of half-maximal inhibitory concentration

The concentration of plant extracts required to scavenge 50% of the radicals (IC50) was calculated by using the percentage scavenging activities at five different concentrations of the extracts. Percentage inhibition (I%) was calculated by [11-13].

$$I\% = (A_c - A_s)/Ac \times 100$$

Where,
A_c is the absorbance of the control and
A_s is the absorbance of the sample.

Results and Discussion

Antidiabetic plants have a major role in inhibiting the glucose level thus providing protection to human against hyperglycemia. Realizing the facts his research was carried out to evaluate the antidiabetic activity of methanolic extract of the selected plants. The *in vitro* antidiabetic activity of these plants extract was detected by measurement of glucose uptake in L6 cell lines.

A-Amylase inhibition activity

In this study, the *in vitro* α-amylase inhibitory activities of the hydro-ethanolic extract of the *Lawsonia inermis and Malvastrum coromandelianum* was investigated. The results of the experiment showed that there was a dose-dependent increase in percentage inhibitory activity against α-amylase enzyme [Table 1]. The IC_{50} values were determined using potato starch (1%, w/v) in 20 mM phosphate buffer (pH 6.9 containing 6.7 mM sodium chloride) is used as substrate *(in vitro)* and tested sample concentration ranged from 3.90 to 500 µg/ml. *B. prionitis* extract showed highest α-amylase inhibitory activity as compared to the standard drug (acarbose).

Table 1: α -Amylase inhibition data at different concentration of test samples

Concentration (µg/ml)	Percentage of inhibition		
	Lawsonia inermis	*Malvastrum coromandelianum*	Standard (acarbose)
3.90	49.12	31.20	18.14
7.81	55.40	45.80	29.54
15.63	62.23	54.38	38.91
31.25	69.10	59.19	41.67
62.50	70.18	62.18	52.92
125.00	88.32	75.97	61.56
250.00	95.02	82.86	85.76
500.00	99.15	88.01	98.47

A-Glucosidase inhibition activity

In this study, the *in vitro* α-glucosidase inhibitory activities of the hydro-alcoholic extract of the *Malvastrum coromandelianum* was investigated. The results of experiment showed that there was a dose-dependent increase in percentage inhibitory activity against α-glucosidase enzyme [Table 2]. Methanolic extracts of the *Malvastrum coromandelianum* showed α-glucosidase inhibitory potential. The half-maximal inhibitory concentration values were determined using paranitrophenyl - α - D - glucopyranoside as substrate *(in vitro)* and tested sample concentration ranged from 9.30 to 500 µg/ml. *B. prionitis* extract showed highest α-glucosidase inhibitory activity as compared to standard drug (acarbose). Inhibition of α-amylase and α-glucosidase enzymes involved in the digestion of carbohydrates, which can significantly decrease the postprandial increase of blood glucose after a mixed carbohydrate diet and therefore can be play an important role in the management of postprandial blood glucose level in type 2 diabetic patients and borderline patients. According to numerous *in vitro* studies, inhibition of α-amylase and α-glucosidase is believed to be one of the most effective approaches for diabetes care.

Table 2: α -Glucosidase inhibition data at different concentration of test samples

Concentration (µg/ml)	Percentage of inhibition		
	Lawsonia inermis	*Malvastrum coromandelianum*	Standard (acarbose)
3.90	32.54	10.12	7.72
7.81	44.12	30.62	28.18
15.63	49.67	39.45	34.45
31.25	59.12	47.66	46.33
62.50	68.56	53.02	52.07
125.00	73.13	61.56	59.12
250.00	89.71	72.56	60.45
500.00	96.10	79.62	78.10

Conclusion

Conventionally, many herbal formulations are using as single herb or in combinations of several different herbs. It believed that poly herbs show synergistic effect. The herbal formulation includes either plant raw material or plant extracts. Here, all selected plants are collected from the Chambal Valley of India to investigate the antidiabetic properties. This study provides the evidence that 50% v/v methanolic extracts of all two plants *Lawsonia inermis and Malvastrum coromandelianum* are having potent enzyme inhibitory actions which are responsible for hyperglycemia. However, more efforts are needed for the isolation and characterization of bioactive compounds and further evaluation of biological properties.

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