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ORIGINAL ARTICLE

In vitro Studies on the Inhibition of α-Amylase and α-Glucosidase by Hydro-ethanolic Extract of *Pluchea lanceolata, Alhagi pseudalhagi, Caesalpinia bonduc*

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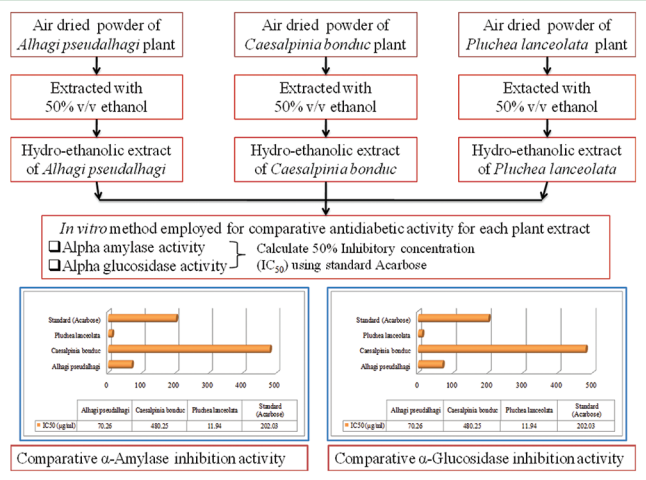
ABSTRACT

Background: *Pluchea lanceolata* (Rasna), *Alhagi pseudalhagi* (Jawasa), and *Caesalpinia bonduc* (Latakaranja) important medicinal plants widely used in India as folk medicine. These plants have been used to control diabetes in traditional medicinal systems. **Objective:** In the present study, 50% volume per volume ethanolic extracts of *P. lanceolata*, *A. pseudalhagi*, and *C. bonduc* subjected to *in vitro* analysis of antidiabetic effect by alpha-amylase and alpha-glucosidase inhibitory assay. **Materials and Methods:** Inhibitory activity of the hydro-ethanolic extract of the all three 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. **Results:** The percentage inhibition of *P. lanceolata* showed the highest alpha-amylase and alpha-glucosidase inhibitory activity. Half-maximal inhibitory concentration value *P. lanceolata* was found to be 4.83 μg/ml and 11.94 μg/ml for alpha-amylase and alpha-glucosidase inhibition. **Conclusion:** This study suggests that the hydro-ethanolic extract of all three plants have antidiabetic property, among these three plants *P. lanceolata* showed potent enzyme inhibition as compared to other plant extracts and standard acarbose.

Key words: Alpha-amylase, alpha-glucosidase, antidiabetic, hydro-ethanolic, *in vitro*

SUMMARY

- In the present study, 50% volume per volume ethanolic extracts of *Pluchea lanceolata*, *Alhagi pseudalhagi*, and *Caesalpinia bonduc* 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 three 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 *Pluchea lanceolata* showed the highest alpha-amylase and alpha-glucosidase inhibitory activity. Half-maximal inhibitory concentration value *Pluchea lanceolata* was found to be 4.83 μg/ml and 11.94 μg/ml for alpha-amylase and alpha-glucosidase inhibition
- Among these three plants, *Pluchea lanceolata* showed potent enzyme inhibition as compared to other plant extracts.



Abbreviations Used: PPA: Porcine pancreatic α-amylase; DNS: 3,5-dinitrosalicylic acid, PBS: Potassium phosphate buffer solution, pNPG: p-Nitrophenyl-α-D-glucopyranoside, IC_{50}: Half-maximal inhibitory concentration.

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INTRODUCTION

Diabetes mellitus is a chronic multifactorial disorder and one of the non-communicable life-threatening metabolic diseases involving huge health-care cost and high mortality rate. In 2015, it was found that it affecting 422 million adults globally. The majority of them were between 40 and 59 years and around 80% lived in middle- and low-income countries. It was found that more than 4.9 million deaths were caused alone with diabetes and the number of diabetes patients will increase up to 55% by 2035, reaching 592 million aging between 20 and 79 years.[1,2] These are non-infectious and non-transmissible. It is characterized by chronic hyperglycemia with disturbance of carbohydrate, fat, and protein metabolism due to the insufficient secretion of insulin by the pancreas and by the resistance to the action on insulin in various issues, i.e., muscle, liver, and adipose, which results in impaired uptake of glucose.[3,4] Postprandial hyperglycemia is one of the earliest observable abnormalities of glucose homeostasis, in which blood glucose level remains high after consuming meal and plays an important role in the development of type 2 diabetes and associated chronic complications, such as micro- and macro-vascular disorder.[5,6] Management of plasma glucose levels is essential for delaying or preventing type-2 diabetes. Insulin secretion

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through medication and/or dietary supervision, it is possible to reach this goal.[7] Decreasing the postprandial glucose level is one of the therapeutic approaches for treating type-2 diabetes; for example slowing the glucose absorption through inhibition of the carbohydrates-hydrolyzing enzymes present in the small intestinal brush border, α-glucosidase, and α-amylase. These are responsible for the breakdown of oligosaccharides and disaccharides into monosaccharides.[8-10] Fruits and vegetables that are consumed worldwide have excellent sources of bioactive compounds and having capacity reducing the risk of developing diabetes.[11,12]

Postprandial glucose levels can be regulated through α-glucosidase inhibition. Inhibition of these enzymes delay and in some cases halt carbohydrate digestion, thus prolonging overall carbohydrate digestion time, causing a reduction in the rate of glucose absorption and consequently reducing postprandial plasma glucose rise.[13]

Nowadays, α-glycosidase inhibitors such as acarbose, miglitol, and voglibose are oral blood glucose-lowering drugs commonly used. They decrease postprandial hyperglycemia without inducing insulin secretion; these compounds do not induce hypoglycemia and have good safety; although, the gastrointestinal adverse effect may limit long-term compliance to therapy.[14]

Several medicinal plants species have been used to control diabetes in the traditional medicinal systems of many cultures worldwide. The potential role of medicinal plants as inhibitors of α-amylase and α-glucosidase has been reviewed by several authors. A variety of plants has been reported to show an enzymatic inhibitory activity, and so many are relevant to the treatment of type-2 diabetes.[8,15-19]

The research for a new group of agents from natural resources, especially from traditional medicine becomes an attractive approach for the treatment of postprandial hyperglycemia. It is revealed that there is a direct relationship between phenolic compounds, flavonoids, and tannins and the ability to inhibit α-amylase and α-glycosidase activities. These phenolic compounds have a positive effect on diabetes, by inhibiting the two keys enzymes hydrolyzing carbohydrates in the digestive tract.[8,20-23]

The current study was undertaken to evaluate the hydro-ethanolic extract of whole plants of *Pluchea lanceolata, Alhagi pseudalhagi*, and *Caesalpinia bonduc* for α-amylase and α-glycosidase inhibiting *in vitro* activities.

In the present study, a survey was conducted in the remote villages of Chambal Valley (Etawah District-Uttar Pradesh) with the help of nongovernmental organization named Shri Jhabbulal Jan Jagrati Samiti, Etawah to interact with the people living in small groups. Out of various medicinal plants from Chambal Valley (Etawah District-Uttar Pradesh), three plants were selected on the basis of their ethnobotanical information used for the treatment of diabetes. Information was recorded, especially from native people and local traditional healers who were consulted for their experiences for these plants for curing certain diseases and disorders. Data were collected through questionnaires. The aqueous solution of the outer shell of the seeds of *C. bonduc* is conventionally used by the tribal people of Andaman and Nicober Islands for the relief of the symptoms of diabetes mellitus. The aqueous and 50% ethanolic extract of seeds produced antihyperglycemic and hypolipidaemic effect in normal and diabetic rats.[24] Hydroethanolic extract of the whole plant of *P. lanceolata* produced antidiabetic and wound healing activity in normal and diabetic rats.[25] Whole plant of *A. pseudalhagi* useful in the treatment of diabetes.[26] The hydro-ethanolic extract of *A. pseudalhagi* obtained by hot continuous extraction was subjected to phytochemical examination and pharmacological screening for antidiabetic activity in male Wistar rats after intraperitoneal administration using 18 h rat fasted model, oral glucose tolerance test, and streptozotocin-induced diabetic rat model.[27]

MATERIALS AND METHODS

Chemicals and reagents

Porcine pancreatic α-amylase (PPA), 3,5-dinitrosalicylic acid (DNS color reagent), Potassium phosphate buffer solution (PBS), p-Nitrop henyl-α-D-glucopyranoside (pNPG), α-glucosidase, and ascorbose were purchased from Sigma-Aldrich (St. Louis, USA). 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.

Collection and preparation of plant material

The plant material (whole plant) of *P. lanceolata* (Rasna), *A. pseudalhagi* (Jawasa), and *C. bonduc* (Kant karaj) were collected from the wild area of Chambal Valley, District Etawah, Uttar Pradesh, in the month of June and July during 2016. The plants were identified and authenticated at source by Pharmacognosy and Ethnopharmacology Division Council of Scientific and Industrial Research-National Botanical Research Institute (NBRI), Lucknow. A voucher specification (No.: NBRI-standard operating procedures-216) has been deposited in Institute repository. The plant materials were air dried and grounded into uniform powder with a grinder, passed through a sieve and stored in airtight glass container for further use.[28]

The air-dried powder of the plant was extracted by hot continuous extraction with 500 ml of 50% volume per volume (v/v) ethyl alcohol as menstruum using Soxhlet extractor.[29,30] The hydro-ethanolic extracts so obtained were filtered through muslin cloth, and filtrates were evaporated under reduced pressure by using rotary evaporator and vacuum dried. The residue were then stored in desiccators. The extracts derived are referred as hydro-ethanolic extract of *P. lanceolata* (Rasna), hydro-ethanolic extract of *A. pseudalhagi* (Jawasa), and hydro-ethanolic extract of *C. bonduc* (Latakaranja).

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 µl 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.[22,31]

α-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.[32]

- 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.

Calculation of half-maximal inhibitory concentration

The concentration of plant extracts required to scavenge 50% of the radicals (IC_{50}) was calculated by using the percentage scavenging activities at five different concentrations of the extracts.

Percentage inhibition (I%) was calculated by

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

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

RESULTS AND DISCUSSION

Anti-diabetic 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 anti-diabetic activity of hydro-ethanolic 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.[33]

α–Amylase inhibition activity

In this study, the *in vitro* α-amylase inhibitory activities of the hydro-ethanolic extract of the whole plant of *A. pseudalhagi, C. bonduc,* and *P. lanceolata* 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. *P. lanceolata* extract showed highest α-amylase inhibitory activity as compared to the standard drug (acarbose). IC_{50} value *P. lanceolata* was found to be 4.83 µg/ml. IC_{50} value of *C. bonduc* extract was found to be 9.81 µg/ml. It was also showed potent enzyme inhibition as compared to acarbose ($IC_{50} \approx 29.33$ µg/ml). *A. pseudalhagi* showed minimum α-amylase inhibitory activity and IC_{50} value was found to be 25.48 µg/ml. A comparison of α-amylase inhibitory activity between the standard drug and extract of plants has been depicted in Figure 1.

α-glucosidase inhibition activity

In this study, the *in vitro* α-glucosidase inhibitory activities of the hydro-alcoholic extract of the whole plant of *A. pseudalhagi*, *C. bonduc*, *P. lanceolata* was investigated. The results of experiment showed that there was a dose-dependent increase in percentage inhibitory activity against α-glucosidase enzyme [Table 2]. Hydro-alcohalic extracts of the whole plant of *A. pseudalhagi*, *C. bonduc*, *P. lanceolata* 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. *P. lanceolata* extract showed highest α-glucosidase inhibitory activity as compared to standard drug (acarbose). IC_{50} value *P. lanceolata* was found to be 11.94 µg/ml. IC_{50} value of *A. pseudalhagi* extract was found to be 70.26 µg/ml. It was also showed potent enzyme inhibition as compared to acarbose ($IC_{50} \approx 202.03$ µg/ml). *C. bonduc* showed minimum α-glucosidase inhibitory activity and IC_{50} value was found to be 480.25 µg/ml. A comparison of α-glucosidase inhibitory activity between the standard drug and extract of plants is depicted in Figure 2.

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.[34,35] 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.[32,36]

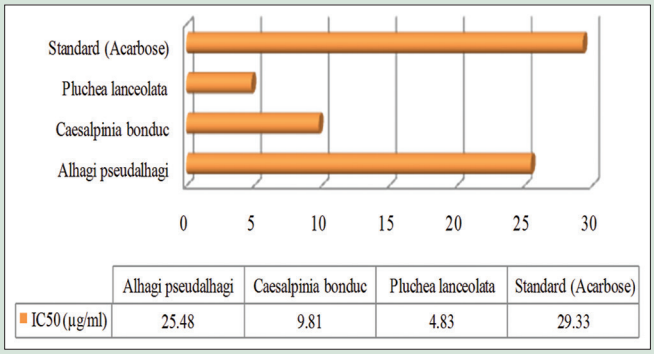

Figure 1: Half-maximal inhibitory concentration value (µg/ml) of test samples showed α-amylase inhibition potential. Note: Lower half-maximal inhibitory concentration value means higher efficacy

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

Concentration (µg/ml)	Percentage of inhibition			
	Alhagi pseudalhagi	*Caesalpinia bonduc*	*Pluchea lanceolata*	Standard (acarbose)
3.90	28.22	32.39	45.80	12.49
7.81	39.12	48.56	51.36	34.96
15.63	45.56	61.53	57.51	45.51
31.25	57.25	74.12	65.23	51.03
62.50	65.12	84.37	74.25	59.62
125.00	70.21	97.11	81.23	75.92
250.00	88.14	100	92.97	84.20
500.00	98.35	100	100.00	97.52

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

Concentration (µg/ml)	Percentage of inhibition			
	Alhagi pseudalhagi	***Caesalpinia bonduc***	***Pluchea lanceolata***	**Standard (acarbose)**
3.90	22.02	0.36	39.43	4.63
7.81	30.25	5.31	45.12	10.64
15.63	38.90	8.65	57.23	13.62
31.25	44.31	12.36	69.25	37.75
62.50	51.10	16.96	80.49	39.83
125.00	60.85	24.88	89.21	47.18
250.00	72.04	31.12	93.08	56.20
500.00	97.98	50.63	99.82	66.52

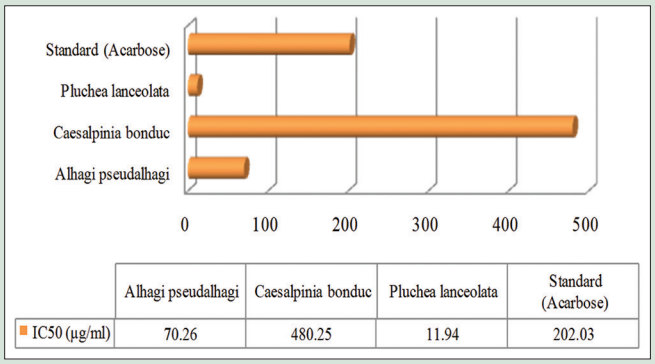

Figure 2: Half-maximal inhibitory concentration value (µg/ml) of test samples showed α-glucosidase inhibition potential. Note: Lower half-maximal inhibitory concentration value means higher efficacy

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 ethanolic extracts of all three plants *P. lanceolata*, *A. pseudalhagi* and *C. bonduc* 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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Nil.

Conflicts of interest

There are no conflicts of interest.

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