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Research Article

IN VITRO HYPOGLYCAEMIC EVALUATION OF SEVEN CULINARY PLANTS OF NORTH EAST INDIA AGAINST TYPE 2 DIABETES

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ABSTRACT

Objective: The goal of the present study is to provide an *in vitro* evidence for potential inhibition of the α-amylase and α-glucosidase enzyme by seven culinary plants of North east India followed by their phytochemical screening.

Methods: The different parts (leaves, seeds, bark, and fruit) of the selected plants were chosen for the study. Collected plant parts were a shade dried, powdered, and successively extracted using petroleum ether, acetone, and methanol. The obtained extracts were quantitatively assayed for *in vitro* α-amylase and α-glucosidase enzyme inhibitory activity. The collected plant materials were screened qualitatively for detection of several bioactive compounds.

Results: In our study, we found that among all the screened culinary plants the highest α-amylase inhibitory activity was demonstrated by *Dillenia indica* Linn. fruit methanol extract with a minimum IC_{50} value of 02.45 ± 0.305 mg/ml, and the highest α-glucosidase inhibitory activity was demonstrated by *D. indica* leaves methanol extract with a very minimal IC_{50} value of 01.78 ± 0.331 mg/ml compared to the standard drug acarbose IC_{50} - 05.43 ± 0.280 mg/ml for α-amylase and IC_{50} - 03.06 ± 0.072 mg/ml for α-glucosidase, respectively. Phytochemical screening reveals the presence of several bioactive groups such as carbohydrate, protein, saponin, tannin, flavonoid, alkaloid, and terpenoids in all studied plants.

Conclusion: The study concludes that selected culinary plants of North East India are capable of inhibiting carbohydrate metabolizing enzyme α-amylase and α-glucosidase, and the presence of bioactive compounds in these plants add on the potentiality of these plants to reduce the post-prandial hyperglycemia.

Keywords: Diabetes mellitus, Post-prandial hyperglycemia, α-amylase, α-glucosidase, Culinary plants.

INTRODUCTION

Diabetes mellitus is a metabolic disorder resulting from a defect in insulin secretion or insulin action. Insulin deficiency, in turn, leads to chronic hyperglycemia with disturbance of carbohydrates, fats, and protein metabolism [1]. Post-prandial hyperglycemia is a prominent and early defect in diabetes, which can, in turn, lead to various secondary complications including risk factor for cardiovascular diseases [2]. One therapeutic approach for treating type 2 diabetes mellitus is to decrease the post-prandial glucose level. This could be done by retarding the absorption of glucose through the inhibition of the carbohydrates-hydrolyzing enzymes, α-glucosidase, and α-amylase [3]. Pancreatic α-amylase and intestinal α-glucosidase enzymes (including lactose, maltose, and sucrose) complete the breakdown to monosaccharide units. It is only the monosaccharide units that are absorbed into the body. Thus, the inhibitions of this enzyme can delay the digestion of carbohydrate and reduce the rate of glucose absorption. Consequently, the post-prandial rise in blood glucose is decreased [4]. Oral hypoglycemic agents/drugs may be effective for glycemic control, but they come with their attendant side effects such as liver disorders, flatulence, abdominal pain, renal tumors, hepatic injury, acute hepatitis, abdominal fullness, and diarrhea. Therefore, there is an increasing need for the development of natural and safe products without the side effects [5]. Ethnobotanical studies of traditional herbal remedies used for diabetes have identified more than 1200 species of plants with hypoglycemic activity [6-9]. A wide and diverse range of plants has been reported in the literature to prevent and treat diabetes.

In the present study, we have selected seven culinary plants of North East India - *Leucas aspera* Linn*. Enhydra fluctuans* DC*., Dillenia indica* Linn*., Citrus aurantifolia* Linn., *Aegle marmelos* Correa., *Paederia foetida* Linn., and *Carica papaya* Linn. Though the various medicinal properties and biochemical constituents of to these selected culinary plants are documented earlier by several researchers, we carried out our research work to shed light on the role of these culinary plants to combat the post-prandial hyperglycemia by a comparative study of its different extract (from non-polar to polar) to inhibit the carbohydrate metabolizing enzyme α-amylase and α-glucosidase.

METHODS

Plant material

Different parts (leaves, seeds, bark, and fruit) of the selected culinary plants were chosen for the study. These plants are commonly used by the different tribes of North East India to prepare several traditional culinary cuisines. The plant materials were collected from different villages of the Rural Kamrup district of Assam, during a survey conducted based on antidiabetic ethnomedicinal plants [10,11]. The plant material collected was authenticated by Dr. G.C Sarma, Curator, Department of Botany, Gauhati University, Guwahati. The plants were identified as *L. aspera* Linn. (Acc. No. 18085), *E. fluctuans* DC., (Acc. No. 18082), *D. indica* Linn., (Acc. No. 18086) *C. aurantifolia* Linn., (Acc. No. 18083), *A. marmelos* Correa., (Acc. No. 18089), *P. foetida* Linn., (Acc. No.18087), and *C. papaya* Linn., (Acc. No. 18088). A sample specimen of each collected plants was deposited as herbarium copy to the department of Botany, Gauhati University.

Preparation of plant extract

All collected plant parts were a shade dried, grounded into a fine powder, and stored in room temperature. The dried powder of each plant (40 g) was extracted successively using petroleum ether, acetone, and methanol in a soxhlet apparatus. The resulting extracts were filtered and concentrated using a rotary evaporator, under reduced pressure. The semisolid plant extracts were then stored at 4°C until enzyme inhibition assay.

α-amylase inhibition assay

The α-amylase inhibition was determined using the modified version of the method according to Bernfield [12]. Briefly, 100 µl of test extract was allowed to react with 200 µl of porcine pancreatic α-amylase enzyme (Sigma Aldrich-3176) and 100 µl of 2 mM of sodium phosphate buffer (pH 6.9). After 20 min of incubation at 37°C, 100 µl of 1% potato starch solution was then added. The same was performed for the control where 200 µl of the enzyme was replaced by the buffer. After incubation for 15 min, 500 µl of 3, 5 dintrosalicyclic acid reagent was added to both control and test. They were kept in boiling water bath for 10 min. The absorbance was recorded in 540 nm using a UV-VIS spectrophotometer and the percentage of inhibition of α-amylase enzyme was calculated using the formula:

Inhibition percentage (%) – 100 (control – test/control)

α-glucosidase inhibition assay

The α-glucosidase inhibitory assay was conducted according to Artani [13] with little modification. 100 µl of plant extract was added to a test tube containing 100 µl of 20 mM *p*-Nitrophenyl α-D glucopyranoside (pNPG) (pNPG, Himedia RM 10294 – 1G) and 2.2 ml of 100 mM phosphate buffer at pH 7.0, and then incubated for 10 mint at 37°C. The reaction was initiated by addition of 100 µl of α-glucosidase from *Saccharomyces cereviseae* (Sigma, G5003) solution (1 mg/0.1 ml) followed by 15 min incubation at 37°C. The reaction was stopped by addition of 2.5 ml of 200 mM Na_2CO_3. The absorbance of p Nitrophenol released from pNPG at 400 nm was measured in a spectrophotometer. Percentage of inhibition of the α-glucosidase activity was calculated by the equation:

Inhibition percentage (%) – 100 (control – test/control)

Suitable reagent blank and inhibitor controls were simultaneously carried out and subtracted.

Dose-dependent, α-amylase and α-glucosidase inhibitory activity was measured using 2, 4, 6, 8, and 10 mg of different extract. The IC_{50} value denotes the concentration of sample required to inhibit 50% of enzyme activity.

Phytochemical screening

Phytochemical screening was performed using standard procedures to identify the chemical constituents of the powdered aqueous extract of selected culinary plants using standard test/methods [14-16].

Test for carbohydrate

Benedict test was performed. 2 ml of Benedict's reagent was taken and 2-3 drops of the extract added to it. The solution was then boiled for 5 min in a water bath and allowed to cool. Formation of green color confirms the presence of carbohydrates in the sample.

Test for protein

Biuret test was performed. To 2 ml of the test extract solution, 2 ml of 10% NaOH and 2 drops of 0.1% $CuSO_4$ was added. Violet or pink color formation confirms the presence of protein in the sample.

Test for alkaloid

2 ml of HCl was added to the crude extract and heated gently. Mayer's and Wagner's reagent were added, and turbidity of the resultant precipitate was taken as evidence for the presence of alkaloids.

Test for saponin

5 ml of distilled water was added to the crude extract and shaken vigorously. The formation of stable foam indicated the presence of saponins.

Test for flavonoid

The alkaline reagent test was performed, where the crude extract was mixed with 2 ml of 2% solution of NaOH. An intense yellow which turns colorless on adding a few drops of diluted acid indicated the presence of the flavonoid.

Test for phenols

2 ml of 2% solution of $FeCl_3$ was mixed with crude extract. Blue-green or black indicated the presence of phenols.

Test for terpenoid

The crude extract was dissolved in 2 ml chloroform and evaporated to dryness. To this, 2 ml of conc. H_2SO_4 was added and heated for about 2 min. A reddish brown precipitate confirms the presence of terpenoid.

Statistical analysis

All values were expressed mean±standard error mean of three experiments in each group. The IC_{50} values were established by logarithmic regression curves with normalized data (using software Graph pad prism 6.05 for windows).

RESULTS AND DISCUSSION

In the present study, five different concentration viz., 2, 4, 6, 8, and 10 mg/ml each of methanol, acetone, and petroleum ether extracts of selected culinary plant parts were investigated for their potentiality to inhibit pancreatic α-amylase and yeast α-glucosidase.

α-amylase inhibitory activities varied widely among the tested plants. From (Table 1) among all the selected plant parts, the highest α-amylase inhibition was exhibited by the methanol and acetone extracts of all selected culinary plants. The highest α-amylase inhibition was demonstrated by fruit methanol extract of *D. indica* (IC_{50} - 02.45±0.305 mg/ml). Potent α-amylase inhibition was exhibited by the leaf methanol extract of *E. fluctuans* (IC_{50} - 03.42±0.243 mg/ml), followed by leaf methanol extract of *L. aspera* (IC_{50} - 03.65±0.461 mg/ml), and seed methanol extract of *C. papaya* (IC_{50} - 03.67±0.434 mg/ml) compared to standard drug acarbose. Similarly, acetone extract of *D. indica* fruit showed high α-amylase inhibition (IC_{50} - 02.58±0.047 mg/ml), followed by the acetone bark extract of *A. marmelos* (IC_{50} - 02.94±0.060 mg/ml), acetone seed extract of *C. papaya* (IC_{50} - 03.90±0.037 mg/ml), and acetone bark extract of *C. aurantifolia* (IC_{50} - 04.65±0.231 mg/ml) also exhibited much higher α-amylase inhibition than the standard drug acarbose (IC_{50} - 05.43±0.280 mg/ml).

When accessed, the highest α-glucosidase inhibition activity was demonstrated by *D. indica* leaf methanol extract (IC_{50} - 01.78±0.331 mg/ml), followed by *D. indica* fruit methanol extract (IC_{50} - 01.95±0.131 mg/ml) compared to standard acarbose (IC_{50} - 03.06±0.072 mg/ml). Similarly, the bark acetone extract of *A. marmelos* showed potent (IC_{50} - 02.14±0.222 mg/ml) α-glucosidase inhibition compared to the standard drug acarbose followed by acetone extract of *D. indica* fruit and leaves (IC_{50} - 02.16±0.025 mg/ml and 02.98±0.0252 mg/ml), respectively.

L. aspera though exhibited remarkable α-amylase inhibition but was unable to inhibit α-glucosidase. The petroleum ether extract, on the other hand, demonstrated very poor α-amylase and α-glucosidase inhibition in all studied plant samples except *C. papaya* seeds extract (IC_{50} - 03.43±0.274 mg/ml).

The phytochemical study (Table 2) reveals the presence of bioactive compounds such as carbohydrate, protein, tannin, flavonoid, alkaloid, and terpenoids. *D. indica* (leaves and fruits) and *A. marmelos* bark showed the presence of all phytochemicals. All studied plant extract

Table 1: IC_{50} calculation for α-amylase and α-glucosidase inhibition of selected culinary plant extracts

Serial number	Plants/parts	Solvent used for extraction	IC_{50} α-amylase inhibition (mg/ml)	IC_{50} α-glucosidase inhibition (mg/ml)
1	*Leucas aspera* (leaves)	Methanol	03.65±0.461	
		Acetone	05.90±0.085	No inhibition
		Petroleum ether	No inhibition	
2	*Enhydra fluctuans* (leaves)	Methanol	03.42±0.243	06.55±0.087
		Acetone	05.39±0.051	06.00±0.047
		Petroleum ether	No inhibition	09.19±0.024
3	*Dillenia indica* (leaves)	Methanol	05.52±0.194	01.78±0.331
		Acetone	16.08±0.023	02.98±0.252
		Petroleum ether	18.09±0.204	No inhibition
4	*Dillenia indica* (fruits)	Methanol	02.45±0.305	01.95±0.131
		Acetone	02.58±0.047	02.16±0.025
		Petroleum ether	12.60±0.131	No inhibition
5	*Carica papaya* (seeds)	Methanol	03.67±0.434	03.58±0.052
		Acetone	03.90±0.037	03.43±0.038
		Petroleum ether	No inhibition	03.43±0.274
6	*Citrus aurantifolia* (leaves)	Methanol	13.04±0.043	10.02±0.353
		Acetone	07.56±0.115	13.01±0.438
		Petroleum ether	07.43±0.035	09.30±0.354
7	*Citrus aurantifolia* (bark)	Methanol	No inhibition	No inhibition
		Acetone	04.65±0.231	03.28±0.041
		Petroleum ether	No inhibition	08.34±0.062
8	*Aegle marmelos* (bark)	Methanol	13.06±0.132	05.98±0.050
		Acetone	02.94±0.060	02.14±0.222
		Petroleum ether	07.32±0.621	No inhibition
9	*Paederia foetida* (leaves)	Methanol	No inhibition	07.48±0.091
		Acetone	10.05±0.063	11.50±0.132
		Petroleum ether	No inhibition	No inhibition
10	Acarbose		05.43±0.280	03.06±0.072

Each value is mean±standard error mean. (N=3)

Table 2: Phytochemical screening of the selected culinary plants

Plant/parts	Carbohydrate	Protein	Tannin	Flavonoid	Saponin	Alkaloid	Terpenoid
Leucas aspera (leaves)	-	+	++	+++	+	+	+++
Enhydra fluctuans (leaves)	++	-	++	+++	+	++	+ +
Dillenia indica (leaves)	++	++	+++	+++	+	+	+++
Dillenia indica (fruits)	++	+	+++	++	++	+++	++
Carica papaya (seeds)	-	-	+++	++	+	+++	+
Citrus aurantifolia (leaves)	-	-	++	+++	+	+++	+ +
Citrus aurantifolia (bark)	-	-	+	+++	+	-	-
Aegle marmelos (bark)	++	+	+	+++	+	++	+++
Paederia foetida (leaves)	+	-	+	++	-	+	+++

-: Absent, +: Less, ++: Moderate, +++: High

showed the presence of tannin and flavonoids. *P. foetida* leaves showed the absence of protein and saponin and *C. aurantifolia* bark showed absence for alkaloid and terpenoid. Carbohydrate and protein were detected absent in *C. papaya*, *C. aurantifolia* bark, and *C. aurantifolia* leaves.

The intestinal digestive enzymes α-amylase and α-glucosidase play a vital role in the carbohydrate digestion. One antidiabetic therapeutic approach to reduce the post-prandial glucose level in blood by the inhibition of the α-amylase enzyme. These can be an important strategy in the management of blood glucose [17]. These α-amylase inhibitors are also called as starch blockers since it prevents or slows the absorption of starch into the body mainly by blocking the hydrolysis of 1, 4-glycosidic linkages of starch, and other oligosaccharides into maltose, maltose, and other simple sugars [18,19]. In our study, we found that the *D. indica* fruit methanol extract (IC_{50} - 02.45±0.305 mg/ml) showed highest α-amylase and *D. indica* leaf methanol extract showed highest α-glucosidase (IC_{50} - 01.78±0.331 mg/ml) inhibition.

Medicinal plants have a wide range of phytochemical constituents ranging from both non-polar to polar. Thus, the collected plant materials were extracted to ensure complete extraction of all non-polar and polar phytochemical components and thereby inclusion of all constituents in the screening studies [20]. Certain bioactive compounds mainly

flavonoids and polyphenols are among the natural active antidiabatic agents that are capable of inhibiting the carbohydrate metabolizing enzyme α-amylase and α-glucosidase. Several phytochemicals including alkaloids, flavonoids, glycosides, polysaccharides, steroids, carbohydrates, glycopeptides, terpenoids, amino acids, saponins, dietary fibers, and inorganic ions affect various metabolic cascades, which directly or indirectly affect the level of glucose in the human body [21,22]. In our study, we found the presence of a bioactive phytochemical group like tannin and flavonoids in all extracts.

Studies have shown that plant-based α-amylase and α-glucosidase inhibitors offer a prospective therapeutic approach for the management of post-prandial hyperglycemia [23]. Compounds such as luteolin, myricetin, and quercetin flavonoids are being reported as potent pancreatic α-amylase inhibitor with IC_{50} values less than 0.5 mg/ml [24]. Several α-glucosidase inhibitors have been isolated from medicinal plants to develop as an alternative drug with increased potency and lesser adverse effects than the existing drugs [25]. At a concentration of 10 mg/ml, the highest α-amylase inhibition of *D. indica* fruit methanol extract (IC_{50} - 02.45±0.305 mg/ml) and highest α-glucosidase inhibition was demonstrated by *D. indicia* leaf methanol extract (IC_{50} – 01.78±0.331 mg/ml) is probably due to the fact that at high extract concentrations, there is a conformational change derived from the binding of compounds to enzyme rendering to their inactivity [26]. Our study suggests that culinary plants being not only a major source of nutrition, but they also possess immense medicinal potentiality to minimize the post-prandial hyperglycemia by inhibiting carbohydrate metabolizing enzyme α-amylase and α-glucosidase.

CONCLUSION

From the above result, it can be concluded that these culinary plants containing several bioactive molecules had a beneficial effect to reduce the rate of carbohydrate absorption and digestion, thereby decreasing post-prandial hyperglycemia. In future, specific α-amylase and α-glucosidase inhibitors are needed to be isolated from the crude extract, characterized and needed to be therapeutically explored *in vivo* using suitable animal model.

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