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RESEARCH ARTICLE

IN-VITRO STUDY ON α-AMYLASE INHIBITORY ACTIVITY AND PHYTOCHEMICAL SCREENING, TEST FOR INORGANIC ELEMENTS, PROXIMATE ANALYSIS, QUALITATIVE AND QUANTITATIVE CHARACTERIZATION OF PHYTOCONSTITUENTS OF *MAURITIUS PAPEDA(CITRUS HYSTRIX)* LEAVES HAVING ANTI-DIABETIC PROPERTIES

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ABSTRACT

The present study was to evaluate preliminary photochemical analysis, inorganic elements and proximate analysis by leaves extract of *Mauritius papeda* by using solvent like methanol and water. Phytochemical analysis showed the presence carbohydrate, reducing sugars, phenolics, flavonoids, glycosides, saponnins and steroids. The moisture content of the crude sample was found to be 10.01 ± 1.40 while the water extractive index was 1.26 ± 0.0710**.** Quantitative analysis of phenols, flavonoids and Triterpenoids was further performed. Additionally inorganic elements like iron, chloride and sulphate were identified by total ash analysis. The same extract was used for quantitative determination of total flavonoid content (85.88 mg/g), total phenolic content (69.78 mg/g) and total triterpenoids content (09.22 mg/g).The methanolic extract had the highest antioxidant property compared to other fractions. α-amylase inhibitors are used in the treatment of type II diabetes mellitus. This study aims to identify the α-amylase inhibitors in *Mauritius papeda* (*Citrus hystrix*). *Mauritius papeda* leaves were subjected to sequential solvent extraction tested for α-amylase inhibition. The results indicates methanolic extracts exhibited highest α-amylase inhibitory activity with IC50 of 39.11±1.33 µg/ml. Pancreatic α-amylase inhibitors offer an effective strategy to lower the levels of post prandial hyperglycemia via control of starch breakdown. This finding could be used to support the use of these plants for treatment of diabetes and also it is an interesting phyto-chemicals to be developed as a new drug.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia with disturbances of carbohydrate, lipid and protein metabolism resulting from defect in insulin secretion, insulin action or both. According to the recent data the prevalence of diabetes is on the rise from 143 million persons to 300 million persons by 2025 (Mitra *et al*., 2007). Among various therapeutic approaches to cure diabetes, lowering postprandial hyperglycemia is one such approach. Postprandial hyperglycemia is mainly due to high calorie and nutrition depleted foods leading to the formation of high glycation end products. This plays a role in diabetic complications, cardiovascular disease (CVD) and aging (Ashok *et al*., 2011). One of the effective method to control diabetes is to inhibit the activity of alpha amylase enzyme which is responsible for the breakdown of starch to more simple sugars (dextrin, maltotriose, maltose and glucose) (Alexander, 1992).

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This is contributed by alpha amylase inhibitors, which delays the glucose absorption rate thereby maintaining the serum blood glucose in hyperglycemic individuals (Dineshkumar *et al*., 2010). Some inhibitors in clinical use such as acarbose, miglitol, and voglibose produce serious side effects such as bloating, and abdominal discomfort. Oxidative stress (formation of free radicals) can be generated due to hyperglycemic status through both enzymatic and non-enzymatic processes. These free radicals would damage cellular proteins as well as mitochondrial DNA (Brajendra, 2006). Most of the ROS produced are scavenged by endogenous defense system under normoglycemic status. But in diabetes due to hyperglycemic condition the system depend on some exogenous antioxidants from natural resources. Medicinal plants are being used right from ancient times for they are an exemplary source of drug due to its high efficacy, reduced cost and side effect (Gauresh *et al*., 2012). Phytochemical constituents like saponin, phenols, flavonoids etc studied in various plants such as *P.vulgari, Euphorbia hirta, Cassia glauca*s howed potential alpha amylase inhibitors such as sapogenin, Cg-1(Sunil, 2010). The role of medicinal

plants in disease prevention is attributed to its antioxidant properties due to their bioactive constituents (RathiSre, 2012). Plants are the groundwork of subsistence on world and are central to people's livelihoods. The Plants have been used in conservative medicine for several centuries' knowledge of medicinal plants based on different medicinal systems such as Ayurveda, Unani and Siddha. India is rich in therapeutic plants. A huge number of medicinal plants are being exploited from the natural plants for the development of drugs. Herbal drugs from plants are established widely, due to their effectiveness, lesser side-effects and comparatively low cost (Rajeshkumar *et al*., 2015; Rajeshkumar *et al*., 2015; Ramesh *et al*., 2015). Diabetes mellitus is a common metabolic disorder causing significant mortality in human life. Diabetes type I do not produce enough insulin or do not make it all and cannot control the blood glucose level (Brownlee, 2005; Kwon *et al*., 2008). Type II Diabetes is non-insulin dependent, and occurs to people that are 40 years of age and older or hereditary. α- amylase and α-glucosidase are the key enzymes involved in carbohydrate metabolism, responsible for carbohydrate digestion and intestinal absorption respectively (Kwon, 2008; Puls *et al*., 1977). *Mauritius papeda* is a small evergreen tree is cultivated throughout India for its fruits and it classified under the family Rutaceae. It is used in folkloric medicine for the treatment of various diseases. It is considered beneficial for cardiac disease, diabetes hyperthyroidism and cancer. The root is considered as a drastic purgative. The plant contains noreorydine and corydine having anticancer activity. The leaves are suppurative and insecticidal and are useful in destroying lice, proctoptosis in children. The leaves are shown to have antidiabetic properties. It is also known for its hepatoprotective powers (Asolkar *et al*., 1992; Vohora *et al*., 1975; Seetharaman, 1985). The aim of the current study was to study the in vitro inhibitory effects of leaf extracts on the activities of α- amylase enzymes.

MATERIALS AND METHODS

Preparation of extracts

The *Mauritius papeda* leaves were collected and shade dried for 3-4 days. The shade dried leaves were ground to a fine powder and stored at room temperature.

Aqueous extraction

50 g of dry powder of each plant sample was soaked in 100 ml of sterile distilled water for 24 h. The extract was filtered using the Whatmann filter paper and preserved at 4°C for further studies.

Methanolic extraction

20 g of dry powder of *Mauritius papeda* leaves are packed in a filter paper and placed in a thimble or extracted in a Soxhlet extractor using 100 ml of methanol solvent at 80ºC for 36 hours. After 36 hours the supernatant was collected and the extracts were left to evaporate in the air at room temperature yielding a concentrated methanol extract which was used for the amylase inhibition studies.

Phytochemical screening of leaves extract

The aqueous and methanolic extract of above mentioned plant leaves was proceeding to preliminary phytochemical analysis. Phytochemical characterization studies are the qualitative chemical analysis used to detect the presence of various groups of phytoconstituents in the plants. The analysis was carrying out the following chemical analysis i.e. Alkaloids, steroids, Flavonoids, Glycosides, triterpenoids, quinone, Tannin, Saponin, Reducing sugar and protein are identified using various reagents (Apostolidis, 2007).

Proximate Analysis

The following quantitative parameters of *Mauritius papeda* leaves sample were determined using standard methods (African pharmacopeia, 1986; Stalh, 1973); Moisture content (water loss on drying), total ash, acid insoluble ash, water soluble ash, alcohol soluble extractive value, and water soluble extractive value.

Determination of total phenolic content

To determine total phenolic content from the methanolic extract of roots *Mauritius papeda*, calibration curve of standard Gallic acid of 20, 40, 60, 80 and 100 mg/ml was prepared in water and 1 mg/ml of methanolic extract of roots of *Mauritius papeda* prepared simultaneously. Each sample was mixed with 0.25 ml of Folinciocalteu reagent and 1.25 ml sodium carbonate solution. The mixtures were allowed to react for 40 minutes at room temperature. After the reaction period the blue color was measured at 725 nm on UV-visible spectrophotometer of LABINDIA 3000+ and calculated the amount of total phenolic content from calibration curve as Gallic acid (Khadbadi *et al*., 2011)

Determination of total flavonoid content

An aliquot (l ml) of standard solution of quercetin (20, 40, 60, 80 and 100 µg/ml) was added to 10 ml volumetric flask containing 4 ml of 5% $NaNO_2$ into it. After 5 minute 0.3 ml of 10% $AlCl_3$ was added. Then 2 ml of 1 M NaOH was added and the total volume was made up to 10 ml with distilled water. Same dilutions were also prepared for the test solution. Blank determination was done by using methanol in place of test or standard solutions. Mixed well and taken the absorbance at 358 nm against blank (Khadbadi, 2011)

Determination of total Triterpenoids

5 g of powder extracted with 50 ml distilled water by heating on water bath for 30 min. then the extract was allow to cool and then filter. 75 ml chloroform and diethyl ether was added in 1:2 concentrations by continuous stirring for 30 min. after 5 gm of sodium carboxyl methyl cellulose was added to forms lumps and sticky mass and then separated. Further marc subjected to extraction with 75 ml chloroform: diethyl ether (1:2) for four times. The obtained residue was dissolved in 50 ml of neutral absolute alcohol. Then the mixture were titrated with 0.1 N NaOH using phenolphthalein as an indicator. Similarly blank readings were taken without addition of sample. Percentage of Triterpenoids content was calculated as per the given factor. Factor for the calculation: each ml of 0.1N NaOH = 48.8 mg of Triterpenoids (Khadbadi, 2011).

In vitro study of α- amylase inhibition activity (Spectrophotometric method)

The α-Amylase inhibition assay was characterized by using spectroscopic method (Apostolidis, 2007). The enzyme

solution was prepared by mixing of 1.0 g of Enzyme powder with pre-chilled 0.02 M sodium phosphates buffer, pH 6.9 with 0.006 M sodium chloride, yielding a clear to hazy solution. Different concentrations of aqueous and methanol extracts (20-100µg/ml) were mixed in 500µl of 0.02 M sodium phosphate buffer, pH 6.9 with 0.006 M sodium chloride containing 0.5 mg/ml. The α-amylase solution was incubated at 25°C for 10 minutes. After incubation, 500 µl of a 1% starch solution in 0.02 M sodium phosphate buffer, pH 6.9 with 0.06 M sodium chloride was added to each tube. The reaction mixtures were then incubated at 25°C for 10 minutes. After that, 1.0 ml of dinitrosalicyclic acid (DNSA) colour reagent was added to stop the reaction and placed water bath at 85°C for 7 minutes. Thereafter the reaction mixture was then diluted with 10 ml of distilled water. The control reaction representing 100% enzyme activity did not contain any plant extract and the absorbance was measured at 546 nm. Acarbose was used as positive control as standard prepared at different concentrations (20-100µg/ml).

RESULTS AND DISCUSSION

Phytochemical screening of *Mauritius papeda* leaf extract

The results in phytochemical analysis of aqueous and methanol solvent extracts of *Mauritius papeda* are represented in table 1. The phytochemical analysis of aqueous extract of leaves showed the presence of glycosides, alkaloids, flavonoids, quinone, and reducing sugars. The methanol extract of leaves shows the presence of glycosides, steroids, alkaloids, flavonoids, tannins, saponins, and protein. The results obtained in this study suggest that the identified phytochemical compounds may be the bioactive compounds and these aqueous and methanol solvent extracts of leaves can be used as potential source of drugs in the treatment of diabetes to inhibit the α-amylase.

Phytochemical analysis of *Mauritius papeda* solvent extracts

The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, steroids etc. The successive extraction leaves of *Mauritius papeda* in aqueous and methanol revealed the presence of flavonoids, triterpenoids, tannins, and quinines. Glycosides and steroids were absent in both extracts. Thus, the preliminary screening tests may be useful in the detection of the bioactive principle and subsequently may lead to the drug discovery and development. From this analysis, methanolic extract of leaves was found to have more constituents compared aqueous extracts of leaves. Since the methanolic extracts were more active than the aqueous extracts because the active molecules were recovered by a less polar solvent than water (Clark *et al*., 1997; Marston *et al*., 1993). The preliminary phytochemical screening tests may be useful to the identification of the bioactive principles which may lead to the discovery and development of new drugs. α- amylase inhibitory assay α-amylase is a key enzyme in the digestive system and catalyses the initial step in starch hydrolysis (Orhan *et al*., 2014) The two plants showed excellent α-amylase inhibitory activity. The present study deals with α-amylase inhibition activity of aqueous, and methanol extracts of *Mauritius papeda* leaves as well as isolated phytochemicals . α- amylase enzyme is responsible for the metabolism of polysaccharides such as starch carbohydrate, etc. The results presented in table 2 show the inhibitory effect of the aqueous and methanol extract of the *Mauritius papeda* leaves tested spectrometrically in this study. The mixture of evaluation concentration of extracts with amylase and starch induced a reduction in the enzyme activity and their IC50 values calculated demonstrate it. The highest inhibitory activity was observed in the methanol extract compared than water extract. The aqueous and methanol extract of leaves extracts tested in vitro showed a varying degree of inhibition of α- amylase activity with IC50 value are 34.10±1.21 µg/ml and 39.11±1.33 µg/ml, respectively which lower than the IC50 of acarbose which equal to 42.11 µg/ml in line with its known α -amylase inhibitory action.

Table 1. Phytochemical analysis of *Mauritius papeda* solvent extracts

Mauritius papeda		
Test	Aqueous Extract	Methanol Extract
Glycosides	+	+
Steroids	-	+
Alkaloids	+	+
Flavanoids	+	+
Triterpenoids	-	-
Tannins	-	+
Saponins	-	+
Quinones	+	-
Protein	-	+
Reducing sugars	+	-
Coumarin	-	-

+ = present
- = absent

Table 2. In vitro α-amylase inhibition activity of aqueous and methanolic extracts of *Mauritius papeda* leaves

% of α- amylase inhibition			
Concentration(µg/ml)	Aqueous extract	Methanol extract	Standard
20	41.01±1.59	44.83 ± 1.29	36.11±1.59
40	50.55±1.37	50.69 ± 1.07	56.33±1.96
60	60.60±2.17	60.61 ± 1.50	66.68±1.11
80	55.21±2.69	85.66 ± 2.64	69.33±1.56
100	77.68±2.22	96.51 ± 1.31	79.88±1.86
IC_{50} value (µg/ml)	34.10±1.21	39.11±1.33	42.11 2.99

Table 3. Detection of inorganic elements

Inorganic elements		
Sl.No	Test	Inference
1	Calcium	+
2	Iron	+
3	Magnesium	-
4	Potassium	+
5	Sulphate	+
6	Phosphate	+
7	Chloride	-
8	Carbonate	+
9	Nitrate	+

(+) present (-) absent

Earlier studies of Komaki *et al*.,(2003) reported that ethanol extract of olive leaves exhibit a high inhibitory effect on human pancreatic α-amylase (IC50 = 0.02mg/ml) compared to hot water extract (IC50=70.2mg/ml). Previous studies related to plant inhibitory potential of α-amylase, as study of Nickavar *et al*. (2011) on Iranian medicinal plants report that olive leaf extract show a weak inhibitory effect on α-amylase (15.84% inhibition at a concentration of 2.30 mg/ml). Regarding to this the most effective plant extract against diabetics, may act by inhibiting the main digestive enzymes are α-amylase and α-

glucosidases responsible for the breakdown of starch and oligosaccharides which converted into glucose as a final product. Plants extract may contain active biomolecules like flavonoids, alkaloids etc which inhibit these functions of enzymes finally reduce its blood glucose level.

Table 4. Percentage (%) values of proximate analysis of *Mauritius papeda* leaves sample

Parameter	Value± SEM (%)
Moisture Content	10.01 ± 1.40
Water extractive index	1.26 ± 0.0710
Total Ash	10.19 ± 0.0360
Acid Insoluble Ash	9.83 ± 0.0259
Alcohol extractive index	0.52± 0.0510

Table 05. Quantitative analysis of phytochemicals (mg/g)

***Mauritius papeda* (leaves)**	
Total flavonoids	85.88 mg/g
Total phenols	69.78 mg/g
Total Triterpenoids	09.22 mg/g

Conclusion

The present study demonstrated that the anti-diabetic activity of selected medicinal plants (*Mauritius papeda*). The anti-diabetic activity of leaves extracts were determined by inhibition of α-amylase using spectrometer studies. The result demonstrated that methanol extract of *Mauritius papeda* leaves have α-amylase inhibitory activities and their IC50 values of plant extracts were much lower with high activity than a positive control i.e. Acarbose. Extract of these plants have alkaloids, flavonoids, steroids, saponins, tannins etc. could help to regulate the fire element in diabetic patients and would result in lowering the blood glucose level.

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