Introduction

Lifestyle of world’s population is influenced with modernization, changed dietary habits and concomitant rise in the stress level. They suffer from many life-threatening diseases like hypertension, cardiac diseases, cancer, diabetes mellitus (DM), obesity, depression and many others (Shomali 2012). DM is characterized by circulating hyperglycemia resulting from either defect in insulin secretion or insulin action or both (Joslin, 2005). According to the international diabetes federation (IDF), it was estimated that more than 425 million people worldwide had diabetes in 2017. Its prevalence is wide increasing rapidly, particularly in developing countries, and is expected to rise to 552 million by 2030 (IDF, 2017).

Currently, antidiabetic therapy other than insulin supplement includes usage of oral hypoglycemic agents viz., thiazolidines, sulfonylureas, meglitinides, biguanides, D-phenylalanine derivatives and α-glucosidase inhibitors along with diet, exercise and weight loss. However, none of them are considered to be ideal, due to prominent side-effects and sometimes diminished response after long term use (Chattopadhyay, 1999), and it has become for an increasing number of people seeking alternative therapies that may have no side-effects.

Abstract

Background: Postprandial hyperglycemia (PPHG) has been proposed as an independent risk factor for cardiovascular diseases (CVD). Therefore, control of PPHG is suggested to be important in the management of diabetes mellitus and related secondary complications. Inhibition of α-amylase and α-glucosidase enzymes can be an important antidiabetic approach for reducing PPHG in type 2 diabetes mellitus. Acarbose, voglibose and miglitol are the typical examples of inhibitors used in the clinical practice. Hence the purpose of the present study is to explore natural and safer α-amylase and α-glucosidase inhibitors. Objectives: To evaluate the in vitro antidiabetic activity of aqueous and methanolic extracts of Feronia elephantum fruit and Paspalum scrobiculatum grains. Materials and methods: Aqueous and methanolic extracts of Feronia elephantum fruit and Paspalum scrobiculatum grains were assayed by α-amylase and α-glucosidase enzyme inhibition assays. Results: All the extracts exhibited a dose-dependent inhibitory activity against α-amylase and α-glucosidase enzymes. The α-amylase IC50 values of methanolic and aqueous extracts of F.elephantum fruit and P.scrobiculatum grains were 119.698, 167.505, 82.107 and 149.886μg/mL respectively which were comparable to that of standard drug acarbose, 109.722μg/mL. The α-glucosidase IC50 values of methanolic and aqueous extracts of F.elephantum fruit and P.scrobiculatum grains were 66.738, 84.548, 47.297 and 118.351μg/mL respectively, with Acarbose showing an IC50 of 52.645μg/mL. Conclusions: Findings of in vitro antidiabetic assay clearly indicate that F.elephantum fruit and P.scrobiculatum grains possesses considerable inhibitory activity against α-amylases and α-glucosidases, and can be of potential use in diabetic patients.

Keywords: α-amylase, α-glucosidase, Feronia limonia, Paspalum scrobiculatum, invitro antidiabetic.
Postprandial hyperglycemia (PPHG) is a condition where there is elevation in blood glucose level after consuming meal, and has been proposed as an independent risk factor for cardiovascular diseases (CVD). Therefore, control of PPHG is suggested to be important in the management of diabetes mellitus and related secondary complications such as CVD, diabetic retinopathy, diabetic neuropathy, etc. (Gin and Rigalleau, 2000).

The enzyme α-amylase, which is a digestive enzyme secreted from salivary gland and pancreas, is responsible for the hydrolysis of 1,4-glycosidic linkages of dietary starch and other oligosaccharides into maltose, maltotriose and other simple sugars prior to absorption (Cazarolli et al., 2008). The enzyme α-glucosidase, is one among a number of glucosidases located in the brush-border surface on the membrane of intestinal cells and, is a key enzyme of carbohydrate digestion. α-glucosidase inhibitors block the action of enzyme in the small intestine, which is rate limiting in the conversion of oligosaccharides to monosaccharides, which is necessary for gastrointestinal absorption of carbohydrates. Thus, inhibition of these enzymes prolong the overall carbohydrate digestion time leading to a marked reduction in the rate of glucose absorption thereby blunting blood glucose level (Rhabasa and Chiasson, 2004), and hence, could be an important antidiabetic approach for reducing postprandial hyperglycemia in type 2 diabetes mellitus (T2DM) patients. Acarbose, oglibose and miglitol are the typical examples of inhibitors used in the clinical practice (Bailey, 2003). However, these drugs have major drawbacks including various gastrointestinal side effects viz., abdominal discomfort, bloating, flatulence and diarrhoea (Cheng and Fantus, 2005). Thus, efforts are needed to explore natural and safer α-amylase and α-glucosidase inhibitors.

Ayurveda and other ancient Indian literature have reported the use of numerous herbal plants and their preparations in the treatment of diabetes (Randive et al., 2016; Grover et al., 2002). Some antidiabetic plants may exert the hypoglycemic action by either stimulating the function or number of pancreatic β-cells thus increasing the insulin release. In some other plants, the effect may be due to decrease in blood glucose synthesis by deferring the activity of enzymes. In other plants, the activity may be to slowdown absorption of carbohydrates and inhibition of glucose transport (Ali et al., 2006). Many plants with bioactive constituents like flavonoids, phenols, saponins etc exhibited potential α-amylase and α-glucosidase inhibitory activity, which is attributed to their antioxidant properties (Rathi Sre et al., 2012).

Feronia elephantum correa (Synonym: Limonia acidissima, Feronia limonia, or Schinus limonia), family Rutaceae, used not only to treat diabetes but also several other clinical conditions by traditional healers in rural areas (Joshi et al., 2009). The fruit (wood apple) contains flavanoids, glycosides, saponins and tannins (Saima, 2000). Some coumarins (Ghosh et al., 1982; Chatterjee et al., 1980) and tyramine derivatives (Parthasarathi et al., 1991) have also been isolated. Fruit pulp is a rich source of Beta carotene, which is a precursor of vitamin-A, and also contains significant amount of vitamins such as riboflavin and thiamine, and it had small quantities of ascorbic acid content (Sachan Kumar et al., 2004).

Paspalum scrobiculatum Linn., family Poaceae, commonly known as ‘Kodo millet’, contains phenolic acids like vanillic acid, cis-ferulic acid, melilotic acid, p-hydroxy benzoic acid, syringic acid, and flavonoids (Sanjay Jain et al., 2010). The grains are styptic, sweet and tonic and are effective in controlling haemorrhages, inflammation, general debility, and hepatopathy (Kiran et al., 2014). Ayurvedic texts like Charak Samhita and Sushruta Samhita, have reported the usefulness of these grains for the management of diabetes mellitus (The Wealth of India, 2003; Murty et al., 1989).

Thus, the present study aimed to evaluate the In vitro antidiabetic activity of aqueous and methanolic extracts of Feronia elephantum fruit and Paspalum scrobiculatum grains by using α-amylase and α-glucosidase enzyme inhibition assays.

**Materials and methods**

**Chemicals and reagents**

3,5-Dinitro Salicylic Acid (DNSA), p-nitrophenyl-α-D-glucopyranoside (pNPG), Acarbose, α-amylase and α-glucosidase enzymes were purchased from Sigma–Aldrich Chemicals Pvt Limited, (Bangalore, India). All other chemicals and reagents used were of high purity analytical grade and purchased from Merck Pvt. Ltd. (India).

**Collection and authentication of plants**

The grains of *P. scrobiculatum* and ripe fruits of *F. elephantum* were collected from a local market in Tirupati, Andhra pradesh, during the month of September 2017. The plants were authenticated by Dr. K. Madhava Chetty, Department of Botany, Sri Venkateswara University (SVU), Tirupathi, Andhra Pradesh, and voucher specimens were deposited at that Institute (Voucher number: 2032 and 1328). The fruits of *F. elephantum* were washed thoroughly and separated into pulp and rind, the fruit pulp with embedded seeds was shade-dried. The grains of *P. scrobiculatum* and fruit pulp of *F. elephantum* were coarsely powdered and furthur used for extraction.

**Preparation of extracts**

The aqueous extracts of *P. scrobiculatum* grains and *F. elephantum* fruit were prepared by cold maceration of 150 g of powdered materials individually in 500 mL of
Method for calculation of α-amylase and α-glucosidase inhibitory concentration (IC₅₀):

The percentage enzyme inhibition of the extracts/standard was calculated using the following formula shown below, and the values were presented as mean±standard error mean of three replicates.

\[
\% \text{ inhibition} = \frac{(A \text{ control} - A \text{ sample})}{A \text{ control}} \times 100
\]

Where “A control” is the absorbance of the control and “A sample” is the absorbance of the sample. The concentration of the extracts required to inhibit 50% of α-amylase and α-glucosidase activity under the assay conditions was defined as the IC₅₀ value (Sium et al., 2017). IC₅₀ was calculated by using the percentage inhibition activities at five different concentrations of the extract, by using probit regression (dose–response curve) using SPSS20.0 software.

Results

Evaluation of in vitro α-amylase inhibitory activity of plant extracts

Four extracts prepared from two plants were screened by in vitro α-amylase inhibition assay, which might be extrapolated to detect their antidiabetic potential. There was a concentration dependent increase in the percentage inhibitory activity against α-amylase enzyme (Table 1). Methanolic extract of F. elephantum fruit (MEFE), Aqueous extract of F. elephantum fruit (AEFE), Methanolic extract of P. scrobiculatum grains (MEPS), Aqueous extract of P. scrobiculatum grains (AEPS) and acarbose at a concentration 100μg/mL showed a percentage inhibition of 39.05±1.02, 26.21±0.75, 50.86±1.03, 29.02±0.64 and 38.72±1.08 respectively, and at a maximum concentration of 250μg/mL it was 86.92±2.32, 72.36±1.93, 92.87±7.96, 79.12±1.72, and 94.67±1.89 respectively (Figure 1). The IC₅₀ values of MEFE, AEFE, MEPS and AEPS were 119.698, 167.505, 82.107 and 149.886μg/mL respectively. The IC₅₀ value of standard drug acarbose was found to be 109.722µg/mL.

Evaluation of in vitro α-glucosidase inhibitory activity

There was a dose-dependent increase in the percentage inhibitory activity against α-glucosidase enzyme. MEFE, AEFE, MEPS and AEPS and acarbose at a concentration 100μg/mL showed a percentage inhibition of 68.41 ± 2.30, 49.45±1.12, 79.27±1.76, 39.45±1.72 and 81.23±1.02 respectively (Table 1). The IC₅₀ values of MEFE, AEFE, MEPS and AEPS were 66.738, 84.548, 47.297 and 118.351μg/mL respectively. The standard positive control, acarbose showed an IC₅₀ of 52.645μg/mL. In this study, significant differences were found between the IC₅₀ values of all extracts (Figure 2). However, the IC₅₀ of acarbose and the methanol extracts of both the plants did not show a significant difference.
Discussion

In diabetic patients, high postprandial blood glucose level is mainly responsible for micro and macro-vascular complications than that of fasting blood glucose levels. It is well known that suppression of starch digestive enzymes reduce elevation of postprandial blood glucose level (Truscheit et al., 1981). Hence, one of the therapeutic approaches for minimizing postprandial (PP) blood glucose level in diabetic patients is to reduce glucose production by impeding its breakdown and intestinal absorption, by inhibiting the enzymes α-amylases and α-glucosidases after food intake.

Table 1. α-Amylase and α-glucosidase inhibition assay of various extracts of F. elephantum and P. scrobiculatum

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration (μg/mL)</th>
<th>% inhibition of α-amylase activity</th>
<th>IC₅₀ (μg/mL)</th>
<th>% inhibition of α-glucosidase activity</th>
<th>IC₅₀ (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanolic extract of F. elephantum (MEFE)</td>
<td>25</td>
<td>9.16±0.61</td>
<td>119,698</td>
<td>21.14±1.75</td>
<td>66.738</td>
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<td></td>
<td>50</td>
<td>17.29±0.59</td>
<td></td>
<td>33.99±1.84</td>
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<tr>
<td></td>
<td>100</td>
<td>39.05±1.02</td>
<td>60.97±1.37</td>
<td>68.41±2.30</td>
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<tr>
<td></td>
<td>200</td>
<td>60.97±1.37</td>
<td>79.04±1.95</td>
<td>92.34±3.31</td>
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<tr>
<td></td>
<td>250</td>
<td>86.92±2.32</td>
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<tr>
<td>Aqueous extract of F. elephantum (AEFE)</td>
<td>25</td>
<td>7.89±0.35</td>
<td>167,505</td>
<td>18.89±0.59</td>
<td>84.548</td>
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<tr>
<td></td>
<td>50</td>
<td>13.91±0.43</td>
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<td>26.50±0.85</td>
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<td></td>
<td>100</td>
<td>26.21±0.75</td>
<td></td>
<td>49.45±1.12</td>
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<tr>
<td></td>
<td>200</td>
<td>51.02±1.09</td>
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<td>75.32±1.29</td>
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<td></td>
<td>250</td>
<td>72.36±1.93</td>
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<td>91.60±0.97</td>
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<tr>
<td>Methanolic extract of P. scrobiculatum (MEPS)</td>
<td>25</td>
<td>15.67±0.84</td>
<td>82,107</td>
<td>28.07±1.03</td>
<td>47.297</td>
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<td></td>
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<td>49.08±1.40</td>
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<tr>
<td></td>
<td>100</td>
<td>50.86±1.03</td>
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<td>79.27±1.76</td>
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<td></td>
<td>200</td>
<td>79.24±1.68</td>
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<td>91.04±1.32</td>
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<td></td>
<td>250</td>
<td>92.87±1.96</td>
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<td>93.65±2.35</td>
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<tr>
<td>Aqueous extract of P. scrobiculatum (AEPS)</td>
<td>25</td>
<td>5.49±1.05</td>
<td>149,886</td>
<td>11.57±0.87</td>
<td>118.351</td>
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<td>19.98±0.93</td>
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<td>29.02±0.64</td>
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<td>250</td>
<td>79.12±1.72</td>
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<td>77.60±1.96</td>
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<tr>
<td>Acarbose</td>
<td>25</td>
<td>17.90±1.05</td>
<td>109,722</td>
<td>22.12±0.93</td>
<td>52.645</td>
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<td>50</td>
<td>20.21±1.24</td>
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<td></td>
<td>100</td>
<td>38.72±1.08</td>
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<td>81.23±1.02</td>
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<td></td>
<td>200</td>
<td>79.93±1.43</td>
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<td>90.64±1.13</td>
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<td></td>
<td>250</td>
<td>94.67±1.89</td>
<td></td>
<td>97.17±1.35</td>
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All determinations were carried out in triplicate manner and values are expressed as the mean ± SEM

Figure 1. α-Amylase inhibitory activity of plant extracts

Figure 2. α-Glucosidase inhibitory activity of plant extracts
The present study demonstrated that both methanolic and aqueous extracts of *F. elephantum* fruit and *P. scrobiculatum* grains had significant α-amylase and α-glucosidase inhibitory potentials. The IC50 values of all the extracts were comparable to that of acarbose, which is a marketed anti-diabetic drug.

The inhibitory potential of the extracts on α-amylase activity may be a possible mechanism by which the extracts exert antidiabetic activity. Since α-amylases play vital role in starch assimilation in human beings and animals, the presence of such inhibitors in foodstuffs or plant extracts may be responsible for impaired starch digestion (Puls et al., 1973). This α-amylase inhibitory activity of the extracts may be of value as novel therapeutic agents.

Similarly, the α-glucosidase inhibitory effect exhibited by all the extracts indicates their potential effectiveness at managing T2DM attributed by reduction in both postprandial glycemic levels and the total range of postprandial glucose levels (Rhabasa and Chiasson, 2004).

*F. elephantum* fruit efficiently inhibits α-amylase and α-glucosidase enzymes *in vitro*, which might be due to the presence of many phenolic compounds, flavonoids, coumarins etc (Kumar and Sachitra, 2004).

*P. scrobiculatum* has been reported for the presence of many phenolic acids, dietary fibres and flavonoids like quercetin. quercetin was found to possess *in vitro* antidiabetic property, by reducing intestinal glucose absorption (Hoda et al., 2017). These phytoconstituents might be responsible for exerting a good inhibitory action against α-amylase and α-glucosidase enzymes, thus preventing diabetes mellitus. Hence these plants can be included as part of dietary management of the disease.

**Conclusion**

Findings of the *in vitro* antidiabetic assay clearly indicate that *F. elephantum* fruit and *P. scrobiculatum* grains possesses considerable inhibitory activity against α-amylases and α-glucosidases, with remarkable activity in the methanolic extracts. This observed antidiabetic activity might be attributed to bioactive compounds like phenols, flavonoids, etc. *F. elephantum* fruit and *P. scrobiculatum* grains could be greatly beneficial in reducing post-prandial blood glucose levels in diabetic patients and thus, can be effectively used in ayurvedic treatments in the management of diabetes. This needs further *in-vivo* investigations in animal models and identification of specific bioactive compounds to develop novel effective anti-diabetic drugs that are free from harmful side effects.

**Conflicts of interest:** None

**References**


