INTRODUCTION

Diabetes mellitus is the most common endocrine disease worldwide and about 173 million people suffer worldwide. Diabetes causes about 5% of all deaths globally each year and 80% of the people with diabetes live in low and middle income countries. Diabetes deaths are likely to increase by more than 50% in the next 10 years unless urgent action is taken. Diabetes mellitus (DM) is a metabolic disorder resulting from deficiency in insulin secretion, insulin action, or both, promoting disturbance of carbohydrate, fat and protein metabolism. Long term complications of diabetes mellitus include retinopathy, nephropathy, neuropathy, micro-angiopathy and increased risk of cardiovascular disease.

Management of diabetes without any side effects is still a challenge to the medical community. There is continuous search for alternative drugs. Therefore, it is prudent to look for options in herbal medicine for diabetes as well. One therapeutic approach for treating diabetes is to decrease the postprandial hyperglycemia. This is done by retarding the absorption of glucose through the inhibition of the carbohydrate hydrolyzing enzymes α-glucosidase in the digestive tract. Inhibitors of these enzymes delay carbohydrate digestion, causing a reduction in the rate of glucose absorption and consequently blunting the postprandial plasma glucose rise. Inhibitors of intestinal α-glucosidase have been used in the treatment of noninsulin-dependent diabetes mellitus (NIDDM) and represented at the huge proportion of antidiabetic drug market.

Psidium guajava L. is a small medicinal tree and belonging to the family Myrtaceae, is commonly known as guava. It has been used traditionally as a medicinal plant throughout the world for a number of ailments. The main constituents of guava leaves are phenolic compounds, isoflavonoids, gallic acid, catechin, epicathechin, rutin, naringenin, kaempferol. Extraction from guajava leaves mostly essential oil, tannins, flavonoids, phenol compounds, carotenoids and vitamin C. Flavonoids particularly rich in quercetin, saponins, alkeloids, cardiac glycosides, phlobatannis and anthraquinones.

Guava leaf extracts introduces many biological activities i.e. Antibacterial, antioxidant, and analgesic, anti inflammatory, antimicrobial, phytotoxic, hepatoprotection, and anti hyperglycaemic and anti cancer activities. In our recent results demonstrated the ability of the guavanoic acid from P. guajava mediated gold nanoparticles stability and its antidiabetic activity by PTP 1B inhibition represents a significant advance in nanomaterial with realistic implications. More recently we showed that the the methanolic extract showed a significant inhibitory effect on glucose diffusion in vitro. It has therefore, been suggested that it will be an added advantage for plant-based medicines for diabetes which will also have antidiabetic activity through in vitro study by the inhibition of α-glucosidase and α-amylase.

MATERIALS AND METHODS

Preparation of leaf extracts

Apparently healthy plant leaves of guava were collected from Vellore,
authenticated, washed and allowed to dry at room temperature. The leaves were separated, shade dried and coarsely powdered. The coarse powder was subjected to extraction with methanol by soxhlet apparatus and the extract was concentrated to dryness in vacuum.

Inhibition assay of α-glucosidase activity

α-Glucosidase (0.075 units) was premixed with the aqueous and methanol of P. guajava extract at various concentrations (50-150 μg/mL). 3 mM p-nitrophenyl glucopyranoside (pNPG) as a substrate was added to the reaction mixture to start the reaction. The reaction was incubated at 37 ºC for 30 min and stopped by adding 2 mL of Na₂CO₃. The α-glucosidase activity was determined by measuring the p-nitrophenol release from pNPG at 400 nm. The IC₅₀ value was defined as the concentration of α-glucosidase inhibitor to inhibit 50% of its activity under the assay conditions.

Inhibition assay α-amylase activity

α-Amylase was premixed with the aqueous extract and methanol of P. guajava at various concentrations (50-150 μg/mL) and starch as a substrate was added as a 0.5% starch solution to start the reaction. This was carried out at 37 ºC for 5 min and terminated by addition of 2 mL of DNS (3,5-dinitrosalicylic acid) reagent. The reaction mixture was heated for 15 min at 100 ºC and diluted with 10 mL of distilled water in an ice bath. α-Amylase activity was determined by measuring spectrum at 540 nm. The IC₅₀ value was defined as the concentration of α-amylase inhibitor to inhibit 50% of its activity under the assay conditions.

\[ \% \text{ Inhibition} = \frac{A_{540 \text{ Control}} - A_{540 \text{ Exp.}}}{A_{540 \text{ Control}}} \times 100 \]

Determination of total phenol content

Total phenol was determined according to Gupta and Prakash (2009) by using folin-cioicaleau (analytical grade, Merck) reaction in basic condition and the absorbance measured spectrophotometrically at 765 nm. Gallic acid was used as a reference. Total phenol content of the extract was expressed as μg Gallic Acid Equivalent (GAE)/mL.

Statistical Analysis

For the essential oils, standard compounds and positive control, three samples were prepared for each assay. The data was presented as mean ± standard deviation of three experiments.

RESULTS AND DISCUSSION

In vitro α-glucosidase inhibition study

Table 1 reports the inhibitory activity of α-amylase using MEPG and AEPG. The maximum inhibition of MEPG was 90.7% at a concentration 150 μg/mL. The percentage inhibition ranged from 90.7% - 62.3%. AEPG produced a maximum inhibition of 79.8% at a concentration 150 μg/mL. At the lowest concentration 50 μg/mL, there was about 58.1% inhibition. MEPG showed a higher inhibitory potential than AEPG. The IC₅₀ values are tabulated. The ethanolic extract from guava (Psidium guajava Linn.) leaves was extracted fractions showed high inhibitory activity against α-amylase. This revealed that the MEPG have more active components to inhibitory activity a-amylase enzyme than AEPG.

Total Phenol Contents

Table 3 shows the total phenol contents of MEPG and AEPG extracts. This finding was similar to results reported for other Psidium guajava extract products has been reported that guava leaf contains some polyphenols, such as pedunculagin, casuarin and isositrictinin and further found that phenolic compounds in Psidium guajava extract are gallic acid, vanillic acid, caffeic acid, epicatechin and coumaric. Enhancement of total phenolic soluble in methanol was higher than that of soluble in water. The phenolic soluble in methanol produced during extraction possibly contribute to the α-glucosidase and α-amylase inhibition activity.
Table 3. Total phenol contents of methanolic and aqueous extracts of *P. guajava*

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total phenol content (µg GAE/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEPG</td>
<td>456.3</td>
</tr>
<tr>
<td>AEPG</td>
<td>301.2</td>
</tr>
</tbody>
</table>

**CONCLUSION**

In this study, the chemical composition, *in vitro* α-glucosidase and α-amylase inhibitory activity of both methanolic and aqueous extracts are reported. The results indicated that both MEPG and AEPG possess in vitro antidiabetic property and can potentially play an important role in controlling diabetic. Methanolic extracts have higher α-glucosidase and α-amylase inhibition activity than that of aqueous extracts. MEPG and AEPG extracts have α-glucosidase and α-amylase inhibition activity with IC$_{50}$ of 187.2141µg/mL, 142.0141µg/mL and 199.1µg/mL and 151.41µg/mL respectively. Additional studies are warranted with both these MEPG and AEPG to further evaluate their potential in control of sugar levels in diabetic patients. However, an in vivo study is further needed to confirm the efficiency of the essential oils for the treatment of diabetes.

**ACKNOWLEDGEMENT**

The authors are grateful to Auxilium College Management for providing necessary facilities for the experimental work.

**REFERENCES**


**Source of support:** Nil, **Conflict of interest:** None Declared