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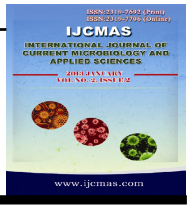

Original Research Article

Phytochemical and in vitro anti-diabetic activity of methanolic extract of *Psidium guajava* leaves

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Keywords

Psidium guajava; Phytochemical; alpha-glucosidase; alpha-amylase

A B S T R A C T

The intestinal digestive enzymes alpha-glucosidase and alpha-amylase are plays a vital role in the carbohydrate digestion. One antidiabetic therapeutic approach reduces the post prandial glucose level in blood by the inhibition of alpha-glucosidase and alpha-amylase enzymes. These can be an important strategy in management of blood glucose. The aim of the present study was to investigate the phytochemical bioactive compounds of the methanolic extract of *Psidium guajava leaves*, its *in vitro* anti-diabetic activity. The assay results suggests that the presence of bioactive compounds, could be responsible for the versatile medicinal properties of this plant including diabetes, the extract exhibit the dose-dependent increase in inhibitory effect on alpha-glucosidase enzyme (upto 89.4%), and alpha-amylase enzyme (upto 96.3%). The current study proves that the antidiabetic activity of methanolic extract of *Psidium guajava* leaves by *in vitro* studies.

Introduction

Diabetes mellitus is a complex and a diverse group of disorders that disturbs the metabolism of carbohydrate, fat and protein. The number of diabetes mellitus cases has been increasing worldwide in recent years. In 2000, the world health organization estimated a total of 171 million of people with diabetes mellitus from the global population, and this report projected to increase to 366 million by 2030 (Wild *et al.*, 2004). With a long course and serious complications often resulting in high death-rate, the treatment of diabetes spent vast amount of resources including medicines, diets, physical training and so on in all countries. Thus searching for a new class of compounds is essential to overcome diabetic problems. There is continuous search for alternative drugs (Syamsudin, 2010).

Psidium guajava Linn. (Myrtaceae) is commonly known as 'Peru' or 'Guava' in English. The *Psidium guajava* leaves are used in the treatment of diarrhoea, cough, stomachache, dysentery and decoction of the leaves for cholera patients, toothache and gum boils. The leaves showed hypoglycemic, cardioprotective, myocardial depressant, antimicrobial, antispasmodic actions (Ross, 1999). Inhibition of alpha-amylase and alpha-glucosidase enzymes can be an important strategy in management of post prandial blood glucose level in type 2 diabetes patient (Ali *et al.,* 2006). Thus, objective of the present study is to investigate the phyto chemical, invitro antidiabetic activity of methanolic extract of *Psidium guajava* leaves.

Materials and Methods

Plant material and extraction

The fresh leaves of *Psidium guajava* were collected locally and authenticated by the department of Botany, St. Joseph College, Trichy. The shade dried *Psidium guajava* leaves were powdered mechanically and stored in an air tight container. The extraction was carried out by hot percolation method using Soxhlet apparatus. The solvent used was methanol. About 100 gm of powder was extracted with 600 ml of methanol. The extract was concentrated to dryness under controlled temperature 40-50°C. The percentage yield was found to be 10.15%. The extract was preserved in refrigerator till further use.

Phytochemical Screening

For preliminary phytochemical analysis the freshly prepared crude methanolic extracts of leaves were tested for the presence or absence of phytoconstituents such as reducing sugar, tannins, flavonoids, steroids and alkaloids by using standard phytochemical procedures (Evans, W.C and Evans, T. 2003).

In vitro methods employed in antidiabetic studies

Inhibition of alpha-amylase enzyme

A starch solution (0.1% w/v) was obtained by stirring 0.1g of potato starch in 100 ml of 16 mM of sodium acetate buffer. The enzyme solution was prepared by mixing 27.5 mg of alpha-amylase in 100 ml of distilled water. The colorimetric reagent is prepared by mixing sodium potassium tartarate solution and 3, 5 di nitro salicylic acid solution 96 mM. Both control and plant extracts were added with starch solution and left to react with alpha- amylase solution under alkaline conditions at 25ºC. The reaction was measured over 3 minutes. The generation of maltose was quantified by the reduction of 3, 5 dinitro salicylic acid to 3-amino-5- nitro salicylic acid. This reaction is detectable at 540 nm (Malik and Singh, 1980).

Inhibition of alpha-glucosidase enzyme

The inhibitory activity was determined by incubating a solution of starch substrate (2 % w/v maltose or sucrose) 1 ml with 0.2 M Tris buffer pH 8.0 and various concentration of plant extract for 5 min at 37°C. The reaction was initiated by adding 1 ml of alpha-glucosidase enzyme (1U/ml) to it followed by incubation for 40 min at 35°C. Then the reaction was terminated by the addition of 2 ml of 6N HCl. Then the intensity of the colour was measured at 540nm (Krishnaveni *et al.,* 1984).

Calculation of 50% Inhibitory Concentration (IC_{50})

The concentration of the 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 extract. Percentage inhibition (I %) was calculated by

I % = (Ac-As)/Ac X 100, (Shai *et al.*, 2010).

where Ac is the absorbance of the control and As is the absorbance of the sample.

Results

The preliminary phytochemical screening tests for the methanol extract of *Psidium guajava* leaves (Table 1) revealed the presence of carbohydrates, alkaloids, flavones, tannins, steroidal glycosides, phenols and coumarin. Any of these secondary metabolites, singly or in combination with others could be responsible for the anti-diabetic activity of the plant.

There was a dose-dependent increase in percentage inhibitory activity against alpha-amylase enzyme. At a concentration of 0.2 ml of plant extract showed a percentage inhibition 27.8% and for 1.0 ml plant extract showed inhibition of 96.3% (Table 2).

The *Psidium guajava* methanol extract revealed a significant inhibitory action of alpha-glucosidase enzyme. The percentage inhibition at 0.2-1.0 ml concentrations of *Psidium guajava* extract showed a dose dependent increase in percentage inhibition. The percentage inhibition varied from 89.4% - 31.7% for highest concentration to the lowest concentration (Table 3).

Table 1 Phytochemical analysis of methanolic extract of *Psidium guajava* leaves

S. No.	Phytochemical Constituents	Name of the Test	Methanolic Extract
1	Alkaloids	Mayer's test Dragondraff test Wagner Test	+ + +
2	Carbohydrates	Molish Test Fehling Test Benedicts Test	+ + +
3	Tannins	Lead Acetate	+
4	Pseudo tannins	Ferric chloride.	Condensed Tannin
5	Chlorogenic acid	Ammonia	-
6	Steroidal Glycosides	Salkowaski	+
7	Anthocyanin	H_2So_4	-
8	Steroidal Glycosides	Liebermann's Burchard Test	-
9	Saponins glycosides	H_2So_4	+
10	Flavonoids	Ammonia	-
11	Flavones	Shinoda's Test	+
12	Phenols	Ferric chloride	+
13	Coumarin	Sodium chloride	+
14	Phytochemical constituents	Borntrager's test	–

+++= High; ++ = Moderate;

+ = Present; - = Absent

Table. 2 In vitro antidiabetic activity of alpha-amylase method

S. No	Concentration of Sample (ml)	% of Inhibition
1	0.2	27.8
2	0.4	48.9
3	0.6	62.5
4	0.8	87.2
5	1.0	96.3

Table 3 *In vitro* antidiabetic activity of alpha glucosidase method

S. No	Concentration of Sample (ml)	% Of Inhibition
1	0.2	31.7
2	0.4	53.9
3	0.6	68.2
4	0.8	76.5
5	1.0	89.4

Discussion

Diabetes mellitus is a metabolic disorder with increasing incidence throughout the world. Insulin is a key player in the control of glucose homeostasis. Lack of insulin affects carbohydrate, fat and protein metabolism (Rajiv Gandhi and Sasikumar, 2012). Management of diabetes without side effects is still challenge to the medical community. It was proposed that inhibition of the activity of such alpha-amylase and alpha-glucosidase would delay the degradation of carbohydrate, which would in turn cause a decrease in the absorption of glucose, as a result the reduction of postprandial blood glucose level elevation (Rhabaso–Lhoret and Chiasson, 2004). In the present study, research has been carried out to evaluate the preliminary phytochemical investigation and the potential of methanol extract of *Psidium guajava* leaf in inhibiting alpha-glucosidase and alpha-amylase.

The present finding of Phytochemical screening of the plant extract confirmed the presence of several bioactive compounds like alkaloids, flavones, tannins and phenols which could be responsible for the versatile medicinal properties of this plant.

The present finding reveals that *Psidium guajava* efficiently inhibits both alpha-amylase and alpha-glucosidase enzymes *in vitro* in a dose dependent manner. The aqueous extracts from *Syzygium cumini* seeds and *Psidium guajava* leaves both showed a dose dependent inhibitory effect on alpha-amylase activity (Karthic *et al.*, 2008). The antidiabetic action of *Psidium guajava* can also be attributed to the intestinal alpha-amylase and alpha-glucosidase inhibitory activity. Further studies are required to elucidate whether *Psidium guajava* have antidiabetic potential by *in vivo* for validating the traditional claim of the plant.

In this present study we evaluated *in vitro* alpha amylase and alpha glucosidase activity of crude methanol extract of *Psidium guajava leaves*. The plant showed significant inhibition activity, so further the compound isolation, purification and characterization which is responsible for inhibiting activity, has to be done for the usage of antidiabetic agent.

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