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Comparative Study on Anti-Diabetic Activity of *Momordica charantia* and *Psidium guajava*

Pillalamarri Madhavi [a*], D. Niharika [a], B. Niharika [a], Marfat Ali [a], Saidul Islam [a] and C. Ramya [a]

[a] Department of Pharmacology, Pulla Reddy Institute of Pharmacy, Hyderabad-502313, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Diabetes is a metabolic disease characterised by decreased insulin signalling and persistent hyperglycaemia. The most frequent progression of diabetes is known as diabetes mellitus which is defined as persistent hyperglycaemia brought on by either peripheral insulin resistance or impaired pancreatic β cell production of insulin. This condition is caused by the disruptions in the digestion of carbohydrates, fats and proteins which are caused by defects in the production, release and regulation of insulin. The breakdown of carbohydrates is significantly aided by the intestinal digestive enzymes α-glucosidase and α-amylase. One type of antidiabetic treatment is to lower the blood glucose levels after a meal by blocking the enzymes α-glucosidase and α-amylase. This could be a key tactic in blood glucose control. When compared to other commercial pharmaceuticals used to treat diabetes, herbal treatments are thought to be more in harmony with the human body and to have less harmful side effects. This herbal remedy is reasonably priced as well. The aim of the current study is to compare the anti- diabetic activity of the methanolic extracts

**Corresponding author: Email: madhavi.pharma33@gmail.com;*

of *Momordica charantia* and *Psidium guajava*. The methods used in the determination of invitro antidiabetic activity are alpha amylase and alpha glucosidase inhibitory assays. In the current study, when compared the individual inhibition activity of Momordica charantia and Psidium guajava on the intestinal enzymes (alpha amylase and alpha glucosidase), Momordica charantia has the highest inhibition that is 79.83% at 0.8 mL concentration while the Psidium guajava has shown only 70.58% inhibition at 0.8 mL concentration. Which suggests that the presence of bio active compounds may be responsible for the plants many medicinal uses, including the treatment of diabetes.

Keywords: Diabetes mellitus; hyperglycaemia; insulin; resistance; α-glucosidase; α-amylase; herbal remedy.

1. INTRODUCTION

Diabetes mellitus is a complicated and multifaceted set of conditions that impairs how fat, protein and carbohydrates are metabolised. Globally, the number of instances of diabetes mellitus has been rising recently [1]. Diabetes mellitus is brought on by abnormalities in the glucose metabolism, which is connected to low levels of blood insulin of target organ insulin sensitivity. Although there has been significant advancement in the management of diabetes with oral hypoglycaemic medicines, the hunt for novel medications persists due to the numerous drawbacks of the current synthetic therapies [2]. Herbal medications possessing anti diabetic effects have not been developed into modern pharmaceuticals for commercial use, despite being highly regarded in conventional medical systems for their medicinal qualities [3]. Obese people typically develop type-2 diabetes, which is linked to dyslipidaemia and hypertension. Thus, the goal of the treatment is to lower insulin resistance and to increase the release of insulin [4]. Diabetes is a metabolic disease in which the body is unable to make or use insulin, a hormone needed to turn food, sugar and carbs into energy. The hallmark of diabetes mellitus is persistently elevated blood glucose levels. The human body uses glucagon and insulin to keep blood glucose levels within a fairly specific range. The liver releases glucose into the blood stream from its cells in order to produce energy [5]. This is the function of glucagon [6]. Type -1 Diabetes causes the body to be unable to secrete insulin, which lowers the rate at which muscles and adipose tissue absorb glucose. Herbal traditional medicines are employed for the treatment of diabetes in developing nations when the population finds the expense of conventional medications to be prohibitive [7]. Diabetes and its later consequences remain a serious medical issue even with the recent development of hypoglycaemic drugs derived from both the natural and artificial sources. It has been shown that numerous native Indian medicinal herbs can effectively treat diabetes. One of the main benefits is that it has very few adverse effects [8].

2. PLANT TAXONOMY

2.1 *Momordica charantia*

- Botanical name: *Momordica charantia*
- Family: Cucurbitaceae
- Indian name: Bitter melon, bitter gourd
- Habitats: It is a warm season crop grown mainly in sub-tropical and hot-arid regions. They are susceptible to light frost and are provided with partial protection if grown during winter months. Temperature range of 24℃- 27ºC is considered as optimum for the growth of the vines.
- Parts used: fruit
- Phytoconstituents: Tannins, flavonoids, Phenolic compounds, alkaloids, saponins, steroids, cardiac glycosides, phlobatannins, and anthraquinones [9].

2.2 *Psidium guajava*

- Botanical name: *Psidium guajava*
- Family: Myrtaceae
- Indian name: Guava, apple guava, lemon guava
- Habitat: It is an evergreen shrub or small tree native to the Mexico, central America, the Caribbean and the northern south America. It is grown in tropical and subtropical areas world-wide.
- Parts used: Leaf
- Phytoconstituents: Phenolic compounds, iso-flavonoids, gallic acid, catechin, quercetin, epicatechin, rutin, naringenin, kaempferol, caryophyllene oxide, p-selinene, chlorogenic acid, myricetin, avicularin, apigenin, guijaverin, caffeic acid [9].

3. MATERIALS AND METHODS

The bitter melon fruits were purchased from the local market in the Gummadidala, Telangana. Distilled and deionized water was utilized in the studies. The bitter melon fruits were washed, sliced into little pieces, and then oven dried at 50^{O}C. Next, the desiccated sample was finely ground in a grinder and kept in storage at 4^{O}C until needed.

Fresh, green leaves were gathered from the Gummadidala area, allowed to air dried for two to three days, then ground into a powder using a mixer. The powdered material is then stored for further extraction.

3.1 Preparation of Extracts

3.1.1 Soxhlet extraction

Fifty grams of finely grinded sample was weighed into a thimble and was extracted with 70% of methanol for 4hours. The sample residue was removed from the thimble manually. The extract was filtered using Whatman filter paper No.4 and evaporated on water bath to remove methanol. The concentrated extract was stored at 4^{o}C until analysis [10].

4. QUALITATIVE PHYTOCHEMICAL ANALYSIS

Phytochemical screening for *Momordica charantia* and *Psidium guajava*:

4.1 Test for Alkaloids

Wagner's test:1 mL of diluted hydrochloric acid and Wagner's reagent was added to the 3 mL of filtrate, and thoroughly shaken. Formation of reddish-brown precipitate indicated the presence of alkaloids.

Hager's test: A few drops of Hager's reagent was combined with 1 mL of the extract. Appearance of yellow precipitate indicated the presence of alkaloids

Dragendorff's reagent: Two mL of extract was mixed with a mL of dragendorff's reagent. The appearance of orange-red precipitate indicates the presence of alkaloids [11].

4.2 Test for Flavonoids

Alkaline reagent test: Two mL of extract was mixed with three drops of sodium hydroxide. The solution turns into deep yellow colour after adding dilute hydrochloric acid it becomes colourless indicating the presence of flavonoids [11].

4.3 Test for Saponins

Foam test: A test tube was filled with 2 mL of the plant extract. Following a strong 15 seconds of shaking, they were allowed to stand for 15 minutes. The height of the produced foam was measured. The presence of saponins was indicated by foam formation [11].

4.4 Test for Amino Acids

Ninhydrin test: It is essential to produce a 1% solution of the test solution in distilled water. To this solution, a few drops of the 2% ninhydrin solution are needed. It is necessary to submerge the test tube in warm water for around five minutes. Amino acids are present when a rich purple colour is produced [11].

4.5 Test for Anthraquinone Gylocosides

Borntrager's test: One gram of the extract was added to 5 mL of diluted HCl. This solution was boiled in a water bath for ten minutes. Five mL of chloroform along with 1 mL of 10% ammonia were added to this solution. The appearance of a bright pink color indicated the of the anthraquinone moiety [11].

4.6 Test for Polyphenols

Ferric chloride test: A mL of the ectract was mixed with 1 mL of ethanol and 2 mL of distilled water. Subsequently 4 drops of freshly prepared ferric chloride solution was added . The appearance of blue or green colour indicated the presence of polyphenols [11].

5. DRUGS AND CHEMICALS

Acarbose (standard drug), alpha-amylase(intestinal enzyme) , alpha-glucosidase(intestinal enzyme),

6. *In vitro* ANTI-DIABETIC ACTIVITY

6.1 Alpha-Glucosidase Inhibition Assay

200 µL of α-glucosidase enzyme was combined with 100 µL of extract (20 mg/ml) and incubated for 10 minutes at 37°C. Following the preincubation step a 100 µl solution of 5 mM PNPG (4-nitrophenyl α-d-glucopyranoside) was added, and the mixture was incubated for 30 minutes at 37°C. The UV spectrophotometer was used to detect the solution's absorbance every

two minutes at 405 nm. As a control, phosphate buffer was employed. Acarbose's IC50 value served as the positive control. The % inhibition of α-glucosidase activity was computed using the following equation:

Glucosidase (%) inhibition: [Ac-As]/Ac×100

Where, As and Ac stand for the sample and control absorbance curve slopes, respectively [12].

6.2 Alpha-Amylase Inhibition Assay

Quantifying the reducing sugar (maltose equivalent) released under test conditions allowed for the assessment of α-amylase inhibition. A drop in the amount of maltose released per unit was used to quantify the inhibitory activity of the enzyme. To determine the maltose equivalent, a modified dinitro salicylic acid (DNS) technique was used.8 1mL of the chosen plant extracts' aqueous extracts were pre-incubated for 30 minutes with 1 U/mL of α-amylase, and then 1 mL (1% w/v) starch solution was added. The mixture was incubated for an additional 10 minutes at 37°C. After that, the reaction was halted by adding 1 mL of DNS reagent, which was heated for five minutes in a boiling water. Equal amounts of solution (20 mM sodium phosphate buffer with 6.7 mM sodium chloride, pH 6.9 at 20^{o}C) were used tocreate two blanks: one without plant extracts and the other without the amylase enzyme. Measuring the absorbance at 540 nm [13].

7. RESULTS AND DISCUSSION

Phytochemical constituents of *Momordica charantia*:

Momordica charantia fruit was found to contain flavonoids, saponins, alkaloids, glycosides and phenolic compounds were present. This is indicated in the Table 1.

Table 1. Qualitative phytochemical constituents of *Momordica charantia*

Constituent	Methanolic Extract
Alkaloids	+
Flavonoids	+
Anthraquinone glycosides	+
Polyphenol	+
Amino acids	-
Saponins	+

Key: Present (+); Absent (-)

Phytochemical Constituents Of *Psidium guajava*:

Psidium guajava leaves was found to contain alkaloids, flavonoids, saponins and phenolic compounds. This is indicated in the Table-2.

Table 2. Qualitative phytochemical constituents of *Psidium guajava*

Constituent	Methanolic Extract
Alkaloids	+
Flavonoids	+
Anthraquinone glycosides	-
Polyphenol	+
Amino acids	-
Saponins	+

Key: Present (+); Absent (-)

Physicochemical Analysis:

Physicochemical properties of *Momordica charantia*:

Physicochemical properties of *Momordica charantia* was shown to have yellowish green colour,acrid odour, and bitter taste. This indicated in Table-3.

Table 3. Phsicochemical properties of *Momordica Charantia*

Parameter	Observation
Colour	Yellowish-green
Odour	Acrid
Taste	Bitter

Physicochemical properties of *Psidium guajava:*

Physicochemical properties of *Psidium guajava* was shown to have green color, characteristic odour and taste. This indicated in Table 4.

Table 4. Phsicochemical properties of *Psidium guajava*

Parameter	Observation
Colour	Green
Odour	Characteristic
Taste	Characteristic

7.1 *In vitro* Anti-diabetic Activity by Inhibition of Alpha Amylase and Alpha Glucosidase Methods

The invitro anti-diabetic activity by inhibition of alpha amylase and alpha glucosidase methods

are indicated in the Table 5 And Table 6 respectively for *Momordica charantia* and Table7 and table 8 for *Psidium guajava.* Where the highest inhibition was 79.83% at a concentration of 0.8mL for *Momordica charantia* while the highest inhibition was 70.58% at a concentration of 0.8 mL for *Psidium guajava* for alpha glucosidase inhibitory assay and 68.35% at a concentaration of 0.8 mL for *Momordica charantia* and 56.83% at a concentration of 0.8 mL for *Psidium guajava*.

Table 5. Inhibition of alpha glucosidase of methanolic extract of *Momordica charantia*

Serial number	Concentration(ml)	%Inhibition
1	0.2	43.02
2	0.4	63.86
3	0.6	67.22
4	0.8	79.83

Table 6. Inhibition of alpha amylase of methanolic extract of *Momordica charantia*

Serial number	Concentration (ml)	% Inhibition
1	0.2	21.39
2	0.4	46.45
3	0.6	60.50
4	0.8	68.35

Table 7. Inhibition of alpha glucosidase of methanolic extract of *Psidium guajava*

Serial number	Concentration (ml)	% Inhibition
1	0.2	32.94
2	0.4	56.30
3	0.6	63.86
4	0.8	70.58

Table 8. Inhibition of alpha amylase of methanolic extract of *Psidium guajava*

Serial number	Concentration (ml)	% Inhibition
1	0.2	11.77
2	0.4	25.82
3	0.6	47.72
4	0.8	56.83

8. DISCUSSION

Diabetes mellitus is a metabolic disease that is becoming more prevalent globally. A major factor in maintaining glucose homeostasis is insulin. Diabetes self-management without adverse consequences remains a challenge to the medical community. It was suggested that blocking the activity of enzymes like alpha-amylase and alpha-glucosidase would prevent the breakdown of carbohydrates, which would postpone the reduction of glucose absorption and the subsequent raising of postprandial blood glucose levels [14]. The current study aims to assess the phytochemical constituents and perform the invitro anti-diabetic activity.The results from the phytochemical studies of the methanolic extracts of *Momordica charantia* and *Psidium guajava* has shown the presence of several bioactive compounds such as alkaloids flavonoids, polyphenols, saponins and glycosides.The current study also revealed that both Momordica charantia and Psidium guajava has potential to inhibit both of the intestinal enzymes alpha amylase and alpha glucosidase in a dose dependent manner.

9. CONCLUSION

Many herbs have been traditionally used and studied for their potential benefits in managing diabetes. In the current study, when compared the individual inhibition activity of Momordica charantia and Psidium guajava on the intestinal enzymes (alpha amylase and alpha glucosidase), *Momordica charantia* has the highest inhibition that is 79.83% at 0.8 mL concentration while the Psidium guajava has shown only 70.58% inhibition at 0.8 mL concentation. In conclusion, Momordica charantia has slightly increased inhibitory activity when compared to Psidium guajava on anti-diabetic activity, but further studies are needed to understand their mechanisms of action and to determine the optimal dosage forms for therapeutic purposes.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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