

EFFECT OF COMBINATION OF AQUEOUS LEAF EXTRACTS OF *PSIDIUM GUAJAVA* LINN AND *MORINGA OLEIFERA* LAM ON DIABETES MELLITUS

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ABSTRACT

Objective: To determine the anti-diabetic activity of combined aqueous extracts (1:1 mixture) of dry leaves of *Psidium guajava linn* and *Moringa oleifera lam* as well as to compare the anti-diabetic activity of these plants by *in vitro* methods.

Methods: *In vitro alpha amylase* inhibitory assay was performed on porcine *alpha amylase* and the absorbance was measured at 540 nm using a microplate reader and glucose diffusion inhibitory assay using dialysis membrane. Acarbose was used as the standard in the above mentioned methods.

Results: The mixture (1:1) of aqueous plant extracts (at a concentration of 100µg/ml) of *Psidium guajava linn* and *Moringa oleifera lam* exhibited 72.08333% inhibition with IC₅₀ value of 10.9µg/ml. The leaf extracts of *Psidium guajava* (at a concentration 100µg/ml) exhibited 71.23288% of α amylase inhibitory activity with an IC₅₀ values 19.883µg/ml whereas the leaf extracts of *Moringa oleifera* (at a concentration of 100µg/ml) exhibited 70.58824% of α amylase inhibitory activity with an IC₅₀ value of 27.974 µg/ml. The Acarbose (standard drug) at a concentration of 100µg/ml showed 72.09302% inhibitory effect on the α amylase activity with an IC₅₀ value 8.9µg/ml. In glucose diffusion inhibition assay the mixture of plant extracts exhibited 76.57% inhibition at 150 min which produces more effects than the two plants. The aqueous extract of *Psidium guajava* leaves exhibited maximum glucose diffusion inhibition (75.32%) at 150 min as well as *Moringa oleifera* leaf extract showed the maximum inhibition of 73.70% at the same time interval. For acarbose the percentage was 82.74 at 150 min. The interpretation of the results was done by one-way anova method.

Conclusion: The combined extract of the leaves of the 2 plants was found to be more effective than individual plant extracts against diabetes. On comparison of two plants *Psidium guajava* was found to be more active against diabetes than *Moringa oleifera*. Also the potentiation effect shown by the combination of extract may be due to synergistic effect of the phytochemical constituents. As the 1:1 mixture of the aqueous extract is found to be more active, the combination of the two plants can be used to formulate drugs for treating diabetes.

Keywords: *Alpha amylase* inhibitory assay, Glucose diffusion inhibition assay method, *Psidium guajava*, *Moringa oleifera*, and IC₅₀

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INTRODUCTION

Psidium guajava Linn is a fruit-bearing tree commonly known as guava, goiaba, guayaba, goavier, perala which belongs to the family Myrtaceae (fig. 1). The leaves and bark of the tree have long history of medicinal uses. It is now widely cultivated, distributed and the fruits enrich the diets of millions of people in the tropics of the world. It is a genus of about 133 genera and more than 3800 species of tropical shrubs and a small tree of 10m height with spreading branches which can survive on all kinds of soils. It is also known as 'poor man's apple of the tropics' has a long history of traditional use [1, 2]. A number of metabolites in good yield and some have been shown to possess useful biological activities belonging mainly to phenolic, flavonoid, carotenoid, terpenoid and triterpene. *P. guajava* is antispasmodic and antimicrobial properties in the treatment of diarrhoea and dysentery, has also been used extensively as a hypoglycemic agent. Many pharmacological studies have demonstrated the ability of this plant to exhibit antioxidant, hepatoprotection, anti-allergy, antimicrobial, antigenotoxic, antiplasmodial, cytotoxic, antispasmodic, cardio active, anticough, antidiabetic, anti-inflammatory and antinociceptive activities, supporting its traditional uses. Suggest a wide range of clinical applications for the treatment of infantile Rota viral enteritis, diarrhoea and diabetes [3].

Moringa oleifera Lam is the most widely cultivated species in the genus *Moringa*, the only genus in the plant family *Moringaceae* (fig. 2). The common names include *Moringa*, Drumstick tree, Horse raddish tree and Ben oil tree or Benzoi tree. *Moringa* has been used from long ago in different parts around the globe as a traditional

culture in the herbal medicine reported for effective ailments ranging from inflammations to various gout and fevers. *Moringa* leaves rubbed against any temple may relieve severe headaches. There is an anti-inflammatory and anti-bacterial effect when applied to insect bites or wounds. Apply just a poultice of fresh *Moringa* leaves to stop bleeding from any kinds of shallow cut. *Moringa* extracts may be used against fungal or bacterial skin problems. Eating food products of *Moringa* are good for the people who are suffering from malnutrition due to the high fiber and protein content. *Moringa* leaves used as an antidiabetic agent. *Moringa* leaves treat bronchitis, fevers, ear and eye infections, and painful mucus membrane inflammation [4, 5].

Diabetes mellitus (DM), commonly referred to as diabetes, is a group of metabolic disorders in which there are high blood sugar levels over a prolonged period. Symptoms of high blood sugar include frequent urination, increased thirst, and increased hunger. If left untreated, diabetes can cause many complications. Acute complications can include diabetic ketoacidosis, hyperosmolar hyperglycemic state, or death. Serious long-term complications include cardiovascular disease, stroke, chronic kidney disease, foot ulcers, and damage to the eyes.

Prevention and treatment involve maintaining a healthy diet, regular physical exercise, a normal body weight, and avoiding use of tobacco. Control of blood pressure and maintaining proper foot care are important for people with the disease. Type 1 DM must be managed with insulin injections. Type 2 DM may be treated with medications with or without insulin. Effective measure in Gestational diabetes usually resolves after the birth of the baby.

The aim of the present study is to compare the anti-diabetic activity of aqueous leaf extracts of *Psidium guajava* and *Moringa oleifera* and to investigate the anti-diabetic activity of the combined extract of both the plants. The objective of the study is to perform the Glucose diffusion inhibition method and alpha amylase inhibitory assay of the plants under study individually and as well as a combination of aqueous extracts of both the plants.



Fig. 1: Leaves and fruits of *Psidium guajava*

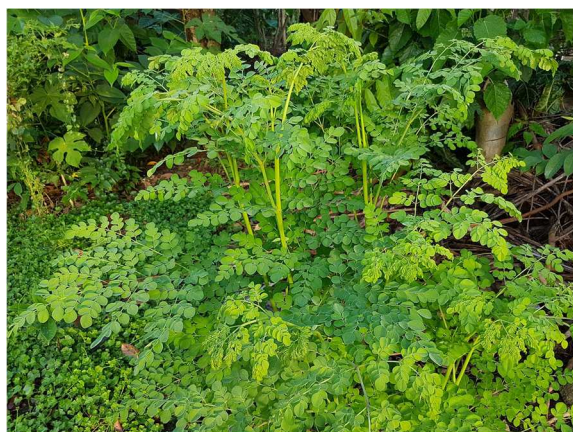


Fig. 2: Leaves of *Moringa oleifera*

MATERIALS AND METHODS

Collection and identification of plant material

The leaves of both plants were collected from the hilly regions of Ernakulum District, and were authenticated by Dr. Sr. Tessy Joseph, Professor, Department of Botany, Nirmala College, and Muvattupuzha. Voucher specimen was deposited in the herbarium of Nirmala College, Muvattupuzha, Kerala, India, <https://www.latlong.net/c/?lat=9.958500&long=76.627937> with a Voucher no: NCB 3932.

Preparation of extracts

The mature fresh leaves of *Moringa oleifera* and *Psidium guajava* were thoroughly washed with water and allowed to dry under shade. Dried leaves were finely powdered and used for extraction. The plant extract is prepared by decoction. The crude drug is boiled in a specified volume of water for defined time. It is then cooled and strained or filtered. This procedure is suitable for the extracting water soluble, heat stable constituents. The starting ratio crude drug to water is fixed, e. g. 1:4, 1:6. The volume is then brought down to one fourth its original volume by boiling during the extraction procedure. The extract is filtered and used as such or processed further concentrated.

In vitro alpha-amylase inhibition assay

Materials required

Enzyme; (0.5 mg/ml) α -amylase in 0.02 Phosphate Buffer (Type VI B: From porcine pancreas, 500000 U) [15.8 U/mg solid at pH 6.9]- Stored at 2-8 °C, USA (A3176) Substrate: Starch 1%w/v, Sodium dihydrogen orthophosphate ($\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$), Disodium hydrogen phosphate ($\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$), Indicator: Iodine solution 1% w/v, NaCl solution (0.006 M).

Instruments

UV-Visible Spectrometer (Shimadzu UV-1800), Electronic balance-Citizen CY104.

Procedure

Alpha-amylase activity was carried out by starch-iodine method. The concentrations 25 $\mu\text{g/ml}$, 50 $\mu\text{g/ml}$, 75 $\mu\text{g/ml}$ and 100 $\mu\text{g/ml}$ of both plant extract were prepared by dissolving in distilled water. 500 μl of plant extract and 500 μl of 0.02M sodium phosphate buffer (pH 7 with 0.006 M NaCl) containing α -amylase solution (0.5 mg/ml) should be taken in each test tube. It is then incubated at 37 °C for 10 min, 500 μl of starch solution (1%) was added, and the mixture was re-incubated for 1h. Next, 0.1 ml of 1% iodine solution was added, and after adding 5 ml distilled water, the absorbance was taken at 565 nm. Sample, substrate and α -amylase blank determinations were carried out under the same reaction conditions [10-12].

Inhibition of enzyme activity was calculated as (%) = $(A - C) \times 100 / (B - C)$

Where, A= absorbance of the sample, B= absorbance of blank (without α -amylase), C= absorbance of control (without sample).

Glucose diffusion inhibitory assay

Materials required: 0.15 M sodium chloride solution, Glucose dialysis membrane.

Instruments: Electronic balance-Citizen CY104, Magnetic stirrer-Rotek UV spectrophotometer (Shimadzu UV 1800)

Procedure: 1.1 ml of extract was placed in a dialysis membrane along with glucose solution (0.22 mmol in 0.15 M sodium chloride). Tied the both ends of membrane with the thread and it was then immersed in water containing a beaker of 40 ml 0.15 M NaCl solution and 10 ml distilled water. The beakers were placed in magnetic stirrer and kept at room temperature. 4.5 ml of solution was pipetted out at 30 min. Interval for 2.5 h from beaker and absorbance of the solution was determined at 248 nm by using UV spectrophotometry. On each withdrawal 5 ml solution from beaker the volume was compensated by replacing it with fresh solution similarly prepared. A controlled determination was also done by taking 1 ml of 0.15M NaCl containing 0.22 mmol glucose and 1 ml distilled water in dialysis membrane. The procedure was repeated for each plant extract and to study combined effect of mixture of extract [13-15].

Statistical analysis: The values obtained are analysed by one-way anova method. Results are expressed as mean \pm SEM.

RESULTS AND DISCUSSION

The mixture (1:1) of aqueous plant extracts (at a concentration of 100 $\mu\text{g/ml}$) of *Psidium guajava* linn and *Moringa oleifera* lam exhibited 72.08333% inhibition with IC_{50} value of 10.9 $\mu\text{g/ml}$. The leaf extracts of *Psidium guajava* (at a concentration 100 $\mu\text{g/ml}$) exhibited 71.23288% of a α amylase inhibitory activity with an IC_{50} values 19.883 $\mu\text{g/ml}$ whereas the leaf extracts of *Moringa oleifera* (at a concentration of 100 $\mu\text{g/ml}$) exhibited 70.58824% of α amylase inhibitory activity with an IC_{50} value of 27.974 $\mu\text{g/ml}$. The acarbose (standard drug) at a concentration of 100 $\mu\text{g/ml}$ showed 72.09302% inhibitory effect on the α -amylase activity with an IC_{50} value 8.9 $\mu\text{g/ml}$. The values are tabulated in table 1. The mixture showed more inhibitory effects than the individual plant extracts. By comparing the both plant extracts *Psidium guajava* leaves showed more inhibitory effect than the leaves of *Moringa oleifera*.

Table 1: *Alpha amylase* inhibitory activity of standard drug acarbose, aqueous extract of *Psidium guajava* (Pg), *Moringa oleifera* (Mo) and the 1:1 mixture of aqueous extracts of *Psidium guajava* and *Moringa oleifera* at 25 µg/ml, 50 µg/ml, 75 µg/ml and 100 µg/ml with respect

to control. Results are expressed as mean±SEM. The percentage inhibition was compared with standard acarbose and the IC₅₀ values are 19.883, 27.974 and 10.9 respectively. The mixture has least difference from the standard acarbose, when compared with others.

Table 1: *Alpha amylase* inhibitory activity of different concentrations of acarbose and the plant extracts on porcine *alpha amylase*. Results are expressed as mean±SEM. n = 3

S. No.	Sample	Concentration (µg/ml)	Absorbance at 565 nm mean±SEM	Control mean±SEM	Percentage of inhibition	IC ₅₀
1	Acarbose (Standard)	25	0.172±0.0012	0.048±0.0008	53.21888	8.9
		50	0.191±0.0017	0.051±0.0005	60.86957	
		75	0.216±0.0005	0.057±0.0003	70.98214	
		100	0.221±0.0006	0.066±0.0014	72.09302	
		Blank	0.281	-	-	
2	Aqueous extract of <i>Psidium guajava</i> (Pg)	25	0.160±0.0001	0.046±0.00088	49.13793	19.883
		50	0.189±0.0012	0.048±0.00033	61.30435	
		75	0.206±0.0008	0.051±0.00033	68.28194	
		100	0.215±0.0011	0.059±0.00153	71.23288	
		Blank	0.278	-	-	
3	Aqueous extract of <i>Moringa oleifera</i> (Mo)	25	0.147±0.00033	0.039±0.000333	49.76959	27.974
		50	0.159±0.00115	0.042±0.002309	54.6729	
		75	0.184±0.00088	0.048±0.000577	65.38462	
		100	0.196±0.00088	0.0523±0.000667	70.58824	
		Blank	0.256	-	-	
4	1:1 mixture of Pg and Mo	25	0.181±0.00577	0.049±0.002028	51.76471	10.9
		50	0.211±0.0003	0.053±0.000577	62.94821	
		75	0.222±0.001528	0.061±0.000333	66.25514	
		100	0.237±0.00145	0.064±0.001202	72.08333	
		Blank	0.304	-	-	

The *invitro* glucose diffusion inhibition assay of both the plant extracts was carried out to determine the percentage inhibition of glucose diffusion at different time intervals 30, 60, 90, 120 and 150 min. The values are tabulated in table 2. The aqueous extract of *Psidium guajava* leaves exhibited maximum glucose diffusion inhibition (75.32%) at 150 min as well as *Moringa oleifera* leaf extract showed the maximum inhibition of 73.70% at the same time interval. By comparing both the plant extracts, *Psidium guajava* exhibited more inhibitory effects than *Moringa oleifera* leaves. The mixture of plant extracts exhibited 76.57% inhibition at 150 min which produces more effects than the two plants. The *invitro* glucose diffusion inhibition assay was found to increase with time from 30 to 150 min and both plants extracts demonstrated significant inhibitory effects on the movement of glucose into external solution across dialysis membrane as compared to control. The retardation of glucose diffusion by *Psidium guajava* extract was significantly higher

than *Moringa oleifera*. The hypoglycemic effect inhibited by the extract of *Psidium guajava* and *Moringa oleifera* is mediated by increasing glucose absorption or by decreasing glucose diffusion rate. At the same time both plant extracts showed significant inhibitory effect produced as compared to standard drug. The combination of the plant extract showed a synergistic effect with higher glucose diffusion inhibitory effect.

Table 2: The *invitro* glucose diffusion inhibition assay of standard drug acarbose, aqueous extract of *Psidium guajava* (Pg), *Moringa oleifera* (Mo) and the 1:1 mixture of aqueous extracts of *Psidium guajava* and *Moringa oleifera* at different time intervals 30,60,90,120,and 150 min was performed and percentage of inhibition was calculated with respect to test and control. Results are expressed as mean±SEM. The percentage inhibition was compared with standard acarbose. The mixture has least difference from the standard acarbose, when compared with others.

Table 2: Percentage of inhibition of various plant extracts in Glucose diffusion inhibitory assay at different time intervals using dialysis membrane, results are expressed as mean±SEM, n = 3

S. No.	Sample	Time min	Control mean±SEM	Test mean±SEM	Percentage of inhibition
1	Acarbose (Standard)	30	0.491±0.00057	0.143±0.000577	77.32
		60	0.569±0.00057	0.135±0.000453	78.03
		90	0.578±0.00175	0.131±0.000333	78.71
		120	0.598±0.00057	0.123±0.00033	80.10
		150	0.620±0.00023	0.115±0.00088	82.74
2	Aqueous extract of <i>Psidium guajava</i> (Pg)	30	0.491±0.00057	0.160±0.00033	67.41
		60	0.569±0.00057	0.159±0.00033	72.05
		90	0.578±0.00175	0.158±0.00033	72.66
		120	0.598±0.00057	0.156±0.000577	73.91
		150	0.620±0.00023	0.153±0.00882	75.32
3	Aqueous extract of <i>Moringa oleifera</i> (Mo)	30	0.491±0.00057	0.175±0.00033	64.35
		60	0.569±0.00057	0.172±0.000577	69.77
		90	0.578±0.00175	0.169±0.000333	70.76
		120	0.598±0.00057	0.167±0.000333	72.07
		150	0.620±0.00023	0.163±0.000333	73.70
4	1:1 mixture of Pg and Mo	30	0.491±0.00057	0.129±0.00045	70.87
		60	0.569±0.00057	0.125±0.000667	72.50
		90	0.578±0.00175	0.123±0.000333	73.31
		120	0.598±0.00057	0.119±0.000667	74.94
		150	0.620±0.00023	0.107±0.002028	76.57

Insulin resistance, which is the reduced response of target tissues such as the skeletal muscle, liver, and adipocytes, to insulin, plays a major role in the pathogenesis of Type II Diabetes. Skeletal muscle is the predominant site of insulin-mediated glucose uptake in the postprandial state. Normal glucose homeostasis depends on a well-balanced interaction between tissue (muscle, liver, and fat) sensitivity to insulin and insulin secretion [16]. The present study showed that, the ability of the combined aqueous extract of *Psidium guajava* and *Moringa oleifera* to increase the percentage glucose uptake is comparable to that of the standard drug Acarbose, showing its ability to reduce, post prandial blood sugar level.

Type II Diabetes mellitus can be treated by reducing post prandial hyperglycemias. The intestinal digestive enzyme, *alpha amylase* is a carbohydrate hydrolyzing enzyme. Alpha amylase inhibitors prevent break down of polysaccharide in to mono and disaccharide. Thus Alpha amylase inhibitors can prevent the postprandial hyperglycaemia by preventing glucose release from starch and delaying carbohydrate metabolism [17]. This research demonstrated the, better antidiabetic potential of the 1:1 mixture of aqueous extract of *Psidium guajava* and *Moringa oleifera* leaves, by its ability to inhibit Alpha amylase more effectively than either of the individual plant extracts of *Psidium guajava* and *Moringa oleifera* leaves.

The results of the Alpha amylase activity and glucose diffusion inhibition assay demonstrated that even though the individual aqueous plant extracts of *Psidium guajava* and *Moringa oleifera*, could increase the glucose uptake and inhibit the Alpha amylase activity, the combined aqueous extracts of both the plants increased the glucose uptake and inhibited the alpha amylase more efficiently than individual plants. So the combined aqueous extract (1:1 mixture) of *Psidium guajava* and *Moringa oleifera* can act as a better antidiabetic agent. Plants containing various phytochemicals that exhibit additive and synergistic interaction in antidiabetic properties which is a beneficial effect, which may help to formulate more potent antidiabetic drugs in combination [18].

CONCLUSION

The Antidiabetic properties of *Psidium guajava* and *Moringa oleifera* was evaluated *in vitro* by glucose diffusion inhibition assay and alpha amylase inhibition assay. The results of our study indicates that aqueous extract of *Psidium guajava* and *Moringa oleifera* inhibit the breakdown of complex carbohydrate as well as reduces the rate of absorption of glucose. Also the potentiation effect shown by the combination of extract may be due to synergistic effect of the phytochemical constituents. Further studies are recommended to find the mechanism behind this synergistic or additive effect. This study also suggests that the active compounds isolated from these plants can be used as lead compound for designing potent anti-diabetic drugs. Based on the future investigations the plants can be utilised as the components of polyherbal formulations for treating diabetes.

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AUTHOR CONTRIBUTIONS

Mathew Anu Jayamol and Reji Ashly conducted the experiment and prepared the manuscript. Johns Nithya designed the experiment

and Shamsudeen Shijina contributed in experimental part of the work.

CONFLIT OF INTERESTS

The authors confirm that this article content has no conflict of interest.

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