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Research Article

IN VITRO ANTI-DIABETIC ACTIVITY *PUNICA GRANATUM* LINN LEAF EXTRACT

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ABSTRACT

Objective: The present investigation deals with the study of *in vitro* anti-diabetic activity by inhibition of intestinal absorption of glucose by alpha-amylase method. *Punica granatum* L plant is a fruit-bearing deciduous shrub. It is used in the treatment of pneumonia as a bitter tonic also used in the treatment of flu, mouth and lip infection, antifungal, and immunosuppressant which used to treat heart disorders, stomach disorders, dental care, anemia, osteoarthritis, and anti-diabetic.

The intestinal digestive enzymes such as alpha-glucosidase and alpha-amylase have played a vital role in carbohydrate digestion that these can be an important approach in managing of blood glucose.

Methods: The air-dried powder of *P. granatum Linn* (leaf part) was extracted using a Soxhlet apparatus with ethanol extract of *Psidium guajava* (EEPG) and water aqueous extract of *P. granatum* (AEPG) as solvent. The extracts were concentrated under reduced pressure. The activities were carried out using the following concentration (10, 20, 30, 40, and 50 µg/mL) and compared with Acarbose as standard drug. It has significant *in vitro* anti-diabetic in alpha-amylase method.

Results: The extract of *P. granatum* possessed significant anti-diabetic property in EEPG than compared to AEPG.

Conclusion: Activity may be due to the presence of chemical profile such as glycosides, flavonoids, and terpenoids. The results of the study have suggested in the use of *P. granatum* Linn. as a potent anti-diabetic in several applications.

Keywords: *Punica granatum* Linn., Anti-diabetic, Alpha-amylase method, Aqueous and ethanol and acarbose.

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INTRODUCTION

In the past few years, there is a incredible growth in the area of herbal medicine. It is becoming popularized in both developing and developed countries due to its natural origin and its lesser side effects. Herbal remedies provide a lot of drugs for the treatment of internal diseases which are considered to be stubborn and incurable by other system of medicines [1].

Diabetes mellitus (DM) is a major endocrine disorder characterized by elevated blood glucose levels resulting from absence of inadequate pancreatic insulin secretion with or without concurrent impairment of insulin action [2]. The main classes of diabetes are type 1 or insulin-dependent DM (IDDM), type 2 or non-insulin-dependent DM (NIDDM), malnutrition-related DM, and gestational DM [3].

Type 1 DM, also known as IDDM, usually begins in childhood and is thought to be a result of autoimmune detection of the pancreatic beta-cells which result in a complete or almost complete loss of insulin production, thereby necessitating insulin injection to maintain blood sugar control [4]. Thus, patients with type 1 DM are characterized by a deficiency of endogenous insulin. From the literature review, it has been revealed that 15–20% of diabetic patients are suffering from type 1 diabetes. Type 2 diabetes, also known as NIDDM, is usually diagnosed after 40 years of age. NIDDM is the most common form in the DM. It is frequently associated with insulin resistance and normal or even elevated levels of insulin, although subnormal insulin levels are also seen in some type 2 diabetics. The epidemic of type 2 diabetes is intricate by the fact that it is a multi-factorial disease, which is associated with a cluster including obesity, hypertriglyceridemia, impaired glucose tolerance, and insulin resistance, collectively referred as the metabolic syndrome [5]. According to the World Health Organization estimates, India is home to about 19% of the world's diabetic population. This is expected to reach 79.4 million by 2010, mainly due to rapid economic, demographic, and lifestyle changes (Chakraborthy *et al.*, 2008). Several causative factors such as heredity, race, lifestyle, nutritional status, stress, infection, altered immune function, altered metabolic/physiological status, drugs, and hormones have been found to be involved in the etiology of the disorder.

METHODS

Drugs and chemicals

All reagents in procured were analytical grade.

Collection and authentication of plant material

Dried leaves of *Punica granatum* were collected from field of JAMBAI near erode and authenticated by Dr. A. Nithya., M.Sc., B.Ed., M. PhilBotanist, PG Assistant, Government higher secondary school, Vangal Road, Manmangalam (TK), Karur district. Voucher specimen (No: SSMCOP/110/47) has been deposited in the Department of Pharmacognosy, SSM College of Pharmacy, Jambai. Tamil Nadu, India. The leaves of *P. granatum* was dried and, then, crushed into fine powder using laboratory Homogenizer then stored for further use.

Preparation of plant extracts

Ethanolic extract of peel of P. granatum

The leaves were dried and powdered using mixer grinder. It was boiled and subjected to Soxhlet extraction with 99% ethanol for 58 h. The mixture was evaporated to dryness in a hot plate and stored in desiccator. The condensed extract was used for preliminary screening of phytochemicals [6].

Aqueous extract of peel of P. granatum

The leaves were dried and powdered using a mixer grinder. It was boiled and subjected to Soxhlet extraction with distilled water for 48 h. The mixture was evaporated to dryness in a hot plate and stored in desiccator. The condensed extract was used for preliminary screening of phytochemicals.

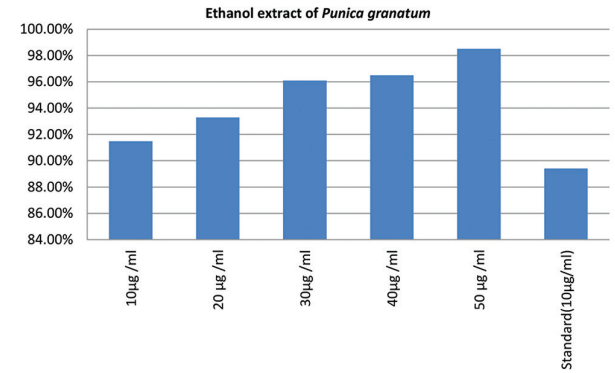
Table 1: Preliminary phytochemical screening of all the leaf extracts of *Punica granatum*

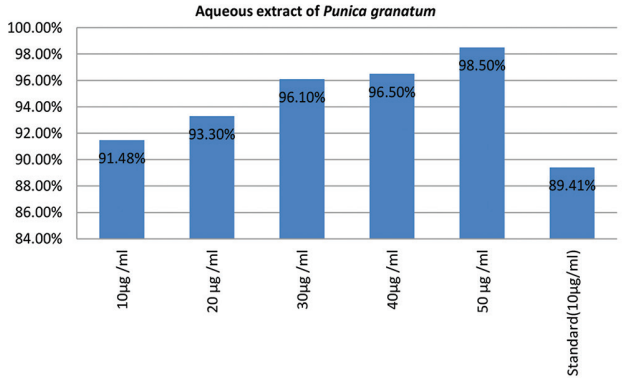
S. No.	Phy to chemical constituents	Leaf extracts	
		Aqueous	Ethanol
1	Alkaloids	−	+
2	Saponins	+	+
3	Glycosides	−	+
4	Reducing sugars	+	+
5	Tannins	−	+
6	Flavonoids	+	+
7	Steroids	−	−
8	Proteins	−	−
9	Terpenoids	+	+
10	Fixed OILS and FATS	−	+

Table 2: Anti-diabetic activity of *Punica granatum* leaf extract by alpha-amylase method

Effect of herbal extracts in different concentration		Absorbance at 590 nm average±SEM	% Inhibition
Control		0.109±0.02	96.3
ACARBOSE (standard) (100 µg/mL)		0.004±0.01	89.41
EEPG	10 µg/mL	0.078±0.02	94.80
	20 µg/mL	0.085±0.08	95.40
	30 µg/mL	0.281±0.01	98.50
	40 µg/mL	0.685±0.01	99.40
	50 µg/mL	0.711±0.01	99.50
AEPG	10 µg/mL	0.047±0.02	91.48
	20 µg/mL	0.060±0.01	93.30
	30 µg/mL	0.105±0.03	96.10
	40 µg/mL	0.116±0.02	96.50
	50 µg/mL	0.269±0.01	98.50

EEPG: Ethanol extract of *Psidium guajava*, AEPG: Aqueous extract of *Psidium guajava*





***In vitro* anti-diabetic activity**

Inhibition of alpha-amylase enzyme

A total of 500 µL (100–500 µg/mL) of test samples and standard drug (100 µg/mL) were added to 500 µL of 0.20 mM phosphate buffer (pH 6.9) containing α-amylase (0.5 mg/mL) solution and it was incubated at 25°C for 10 min. After these, 500 µL of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube. The reaction mixtures were incubated at 25°C for 10 min. The reaction was stopped using 1.0 mL of 3, 5 dinitrosalicylic acid color reagent. The test tubes were then incubated in a boiling water bath for 5 min and cooled to room temperature. Then, it was diluted with 10 mL distilled water before measuring the absorbance at 540 nm. Control represents 100% enzyme activity and was conducted in similar way by replacing extract with vehicle (Thalapaneni 2008, Heidari 2005).

$$\text{Percent inhibition} = 100 - \frac{\text{OD of test solution} - \text{OD of product control}}{\text{OD of test control}} \times 100$$

RESULTS AND DISCUSSION

The preliminary phytochemical screening tests for the ethanol and aqueous extract of *P. granatum* leaves (Table 1) revealed the presence of carbohydrates, alkaloids, flavones, tannins, steroids, phenols, and reducing sugars. Any of these metabolites, singly or in combination with others may be responsible for the anti-diabetic activity. There was a dose-dependent increase in percentage inhibitory activity against alpha-amylase enzyme. At a concentration of 10 µg/mL of plant, extract showed a percentage inhibition 94.5% and for 50 µg/mL plant extract showed inhibition of 99.3% (Table 2). The *P. granatum* ethanol extract revealed a significant inhibitory action of alpha-amylase enzyme than the aqueous extract. The percentage inhibition at 10–50 µg/mL concentrations of *P. granatum* extract showed a dose-dependent increase in percentage inhibition. However, one should try to further figure out extract more as having much better activity in quest of active candidate or chemical molecule that is mainly responsible for this activity through detailed experimentation.

CONCLUSION

From the studies, it can be concluded that peel extract of *P. granatum* showed a significant anti-diabetic activity was proved by alpha-amylase enzyme method.

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AUTHORS' CONTRIBUTIONS

Design of research work, data collection, and drafting of manuscript was done by Muthumanikandan Review and final editing of manuscript was done by Vaijayanthimala P and Sangameswaran B

CONFLICTS OF INTEREST

The authors, hereby, declare that there are no conflicts of interest.

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