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Research Article

EVALUATION OF IN VITRO ANTIOXIDANT AND ANTI-DIABETIC POTENTIAL OF KASHMIRI POMEGRANATE (*PUNICA GRANATUM* LINN.) FLOWER EXTRACT

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ABSTRACT

Aim: The aim of the present study was to find the effect of two extracts namely, aqueous and methanolic of *Punica granatum* L. Flowers on *in vitro* anti-oxidant and anti-diabetic potential Methods: The antioxidant activity of extracts was monitored using DPPH assay, Reducing power, Nitric oxide radical inhibition assay whereas, phenolic content was estimated using Folin-Ciocalteu,s reagent. *In vitro* anti diabetic activity was evaluated by their effect on alpha-amylase and non-enzymatic glycosylation of haemoglobin. Alpha-Amylase inhibitory activity of *Punica granatum* L. Flower extracts were evaluated using Porcine pancreatic alpha-amylase (PPA) with starch as a substrate, whereas, in non-enzymatic glycosylation of haemoglobin Glucose (2%), haemoglobin (0.06%) and Gentamycin (0.02%) solution were prepared in phosphate buffer 0.01 M, pH 7.4, 1 ml each of above solution was mixed. 1 ml of each concentration was added to above mixture. Mixture was incubated in dark at room temperature for 72 hrs. The degree of glycosylation of haemoglobin was measured colorimetrically at 520 nm in which Ascorbic acid was used as standard drug. Results: There was significant anti-oxidant activity. Methanolic extract showed the highest inhibitory activity than aqueous extract. Both the extracts were strong inhibitors of alpha-amylase and non-enzymatic glycosylation of haemoglobin. Discussion: The alpha-amylase and non-enzymatic glycosylation of haemoglobin inhibitory activities of *Punica granatum* L. Flowers correlated to antioxidant activity and phenolic content of extracts. Conclusion: *Punica granatum* L. flowers can be effective in treatment of diabetes not only through inhibition of alpha-amylase and non-enzymatic glycosylation of haemoglobin inhibitory activities, but also by its antioxidant effect.

Key Words: *Punica granatum* L. Flowers, anti-diabetic, antioxidant

INTRODUCTION

Diabetes mellitus is a heterogeneous group of diseases characterized by chronic elevation of glucose in the blood. It arises because the body is unable to produce enough insulin for its own needs, either because of impaired insulin secretion, impaired insulin action, or both. Diabetes affects some 300 million people world-wide and is on the increase. Chronic exposure to high blood glucose is a leading cause of renal failure, visual loss and a range of other types of tissue damage. Diabetes also predisposes to arterial disease, not least because it is often accompanied by hypertension, lipid disorders and obesity [1-5]. Many cases of diabetes and all of its unwanted long-term consequences are potentially avoidable, but this will require intervention at a societal as well as at a medical level. Currently, there are conventional anti-diabetic drugs; however, these drugs are associated with various side-effects. Hence there is urgent need to identify and explore natural sources with fewer side-effects[6-8]

Although, majority of natural drugs are derived from plant and animal origins, one of the fruits is Pomegranate (*Punica granatum* L.) and with the passage of time, more people have started acknowledging the importance of consuming pomegranates. It has a number of traditional uses. There are advantages, like pomegranate reduces the likelihood of having premature infants. It is a good natural aphrodisiac and improves sperm count and semen quality. The astringent features of the flower juice, rind and tree bark are considered valuable for a wide range of purposes, such as stopping nose bleeds and gum bleeds, toning skin (after mixing with mustard oil) firming-up sagging breasts and treating hemorrhoid. Pomegranate seed (of specific fruit strains) is also used as eye drops as it is believed to slow the development of cataracts. Pomegranate is used as a gargle for a sore throat, and it is applied to the epidermis to cure hemorrhoid flare-ups. It cleanses and clarifies oral cavity, throat, esophagus stomach and chest. New therapies for preventing cancer may be on their way as scientists have identified components in pomegranate juice that inhibit the movement of cancer cells. Use of pomegranate flower extract showed significant reduction in wound area and increased the well-organized bands of collagen, fibroblasts, and few inflammatory cells [9-20]. Therefore, with the reference to traditional uses, the present study was undertaken to investigate the effect of two extracts, methanolic and aqueous extracts of the flowers of the fruit on *in vitro* anti-oxidant and anti-diabetic potential and give a scientific rational for its use.

MATERIALS AND METHODS

Collection and Identification of Plant Material

The flowers of *Punica granatum* L. were collected from Chandilora, Tangmarg, Kashmir in the month of May and were identified and authenticated by Mr. Akhtar, Curator, at the Centre for Biodiversity and Taxonomy, Department of Botany, University of Kashmir. The Voucher specimen has been retained in the herbarium of The Centre for Biodiversity and Taxonomy, Department of Botany, University of Kashmir for future reference under herbarium number: **2428 (KASH).**The flower material was cleaned, reduced to small fragments, air dried under shade at

room temperature and coarsely powdered in a grinding mill. The powdered material was stored or taken up for extraction process.

Preparation of Plant Extracts

The powdered plant material was successively extracted in 250ml of methanol and distilled water by using Soxhlet extractor. The plant material was suspended in the main chamber of Soxhlet extractor which was then placed onto a flask containing the extraction solvent. The Soxhlet was then equipped with a condenser. The flask was heated; the solvent evaporated and moved up into the condenser where it was converted into a liquid that trickled into the extraction chamber containing the sample. The chamber containing the solid material was slowly filled with warm solvent. When the Soxhlet chamber was almost full, the chamber was automatically emptied, with the solvent running back down to the distillation flask. This cycle was repeated many times, over hours or a few days, until the colour of the solvent in the siphon of the Soxhlet faded away. At the end of the hot extraction process each extract was filtered. The filtrate was concentrated, and the solvent was recovered using rotary evaporator. The extracts were then kept in desiccators to remove remaining moisture, if present, and finally stored in air tight containers at 4ºC for further use.

***In vitro* Antioxidant Activity** [21-32]

For evaluation of antioxidant activity of *Punica granatum* L. flower extracts, different methods followed were as:

Determination of 2, 2, diphenyl-1-1-picryhydrazyl (DPPH) free radical scavenging

The free radical scavenging capacity of methanolic and aqueous flower extracts of *Punica granatum* L. was determined using DPPH. Freshly prepared DPPH (2,2-diphenyl-1- picrylhydrazyl), solution was taken in test tubes and extracts were added followed by serial dilutions (50µg/ml to 250µg/ml) to every test tube so that the final volume was 3 ml and after 30 min, the absorbance was read at 517 nm using a spectrophotometer. Ascorbic acid was used as standard. Control sample was prepared containing the same volume without any extract and standard and the absorbance was read at 517 nm using a spectrophotometer. Methanol was served as blank. The capability of scavenging DPPH radical was calculated by following equation.

$$\text{Scavenging effect (\%)} = [\text{Abs of control} - \text{Abs of sample}] / \text{Abs of control} \times 100$$

Determination of reducing power

The reductive capability of *Punica granatum* L. flower extracts were quantified by the following method. One ml of (extract) of different concentrations of methanolic and aqueous extracts was mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide [K_3 Fe (CN) $_6$]. Similar concentrations of standard ascorbic acid were used as standard. The mixture was incubated at 50°C for 20 min. Then, the reaction was terminated by adding 2.5 ml of 10% trichloroacetic acid. The upper layer of solution (2.5 ml) was mixed withFeCl3. Ascorbic acid (1mg/ml) was used as standard and same concentrations of ascorbic acid were mixed with the above reagents without extracts. The absorbance was measured at 700 nm in a spectrophotometer against a blank sample. Increased absorbance of the reaction mixture indicated greater reducing power.

Nitric oxide radical inhibition assay

Nitric oxide radical inhibition can be estimated by the use of Griess Illosvoy reaction. In this assay, Griess Illosvoy reagent was modified by using naphthyl ethylene diamine dihydrochloride (0.1% w/v) instead of 1-napthylamine (5%). The reaction mixture (3 ml) containing sodium nitroprusside (10 mM, 2 ml), phosphate buffer saline (0.5 ml) and *Punica granatum* L. flower extracts (50 to 250 µg/ml) or standard solution (rutin) was incubated at 25°C for 150 min. After incubation, 0.5 ml of the reaction mixture mixed with 1 ml of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for completing diazotization. Then, 1 ml of naphthyl ethylene diamine dihydrochloride was added, mixed and allowed to stand for 30 min at 25 °C. A pink coloured chromophore is formed in diffused light. The absorbance of these solutions was measured at 540 nm against the corresponding blank solutions. Rutin was used as a standard.

Determination of the Total Phenolic and Flavonoid content

The concentration of phenolics for *Punica granatum* L. extracts were determined using standard method**.** Methanolic and aqueous flower extracts of *Punica granatum* L. were dissolved in the concentration of 1mg/ml. The reaction mixture was prepared by mixing 0.5 ml of methanol solution of extracts, 2.5 ml of 10% Folin's-Ciocalteu's reagent dissolved in water and 2.5 ml of 7.5% NaHCO3. Blank was concomitantly prepared, containing 0.5ml methanol 2.5 ml of 10% Folin's-Ciocalteu's reagent dissolved in water and 2.5 ml of 7.5% NaHCO3. The samples were then incubated for 45 mins at a temperature of 45 °C. Absorbance was measured at 765 nm. The samples were prepared in triplicates for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for standard solution of Gallic acid and for control all reagents except extract was used**.** The content of flavonoids in the flower extract was determined using standard procedure. The sample contained 1ml of methanol solution of the extract in the concentration of 1mg/ml and 1ml of 2% AlCl3 solution dissolved in methanol. The samples were incubated for an hour at room temperature. The absorbance was determined using spectrophotometer at 415nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The content of flavonoids in *Punica granatum* L. flower extracts were expressed in terms of rutin equivalent (mg of RU/g of extract)**.**

***In vitro* Antidiabetic activity** [33-34]

Alpha-amylase inhibition assay

Alpha-amylase is an enzyme that hydrolyses alpha linked polysaccharide such as glycogen starch to yield glucose and maltose. Alpha amylase inhibitory activity on based on the starch iodine method. In alpha amylase inhibition method 1ml substrate-potato starch (1%w/v), 1ml of drug solution (voglibose Std drug) of five different concentration such as 100, 200, 300, 400, and500µg/ml, 1ml of alpha amylase enzyme (1%w/v) and 2ml of acetate buffer. The above mixture was incubated for 1hr. Then 0.1ml iodine-iodine indicator (635 mg Iodine and 1 gm potassium iodide in 250 ml distilled Water) was added in the mixture. Absorbance was taken at 565 nm in UV-Visible spectroscopy. Percentage inhibition was calculated as:

$$([\text{Abs}_{\text{sample}} - \text{Abs}_{\text{control}}] / \text{Abs}_{\text{sample}}) \times 100$$

All the tests were performed in triplicate.

Non-enzymatic glycosylation of Haemoglobin assay

Antidiabetic activity of flower extracts of *Punica granatum* L. were investigated by estimating degree of non-enzymatic haemoglobin glycosylation measured colorimetrically at 520 nm. Glucose (2%), haemoglobin (0.06%) and Gentamycin (0.02%) solution were prepared in phosphate buffer 0.01 M, pH 7.4, 1 ml each of above solution was mixed. 1 ml of each concentration was added to above mixture. Mixture was incubated in dark at room temperature for 72 hrs. The degree of glycosylation of haemoglobin was measured colorimetrically at 520 nm. Ascorbic acid was used as standard drug for assay.

Percentage inhibition was calculated as:

$$([Abs_{sample} - Abs_{control}]/ Abs_{samples}) \times 100$$

All the tests were performed in triplicate.

Statistical Analysis

All the determinations were done in triplicate. Means, standard deviations and IC_{50} values were calculated using a Microsoft Excel program.

RESULTS AND DISCUSSION

Table 1: Antioxidant activity of DPPH Radical Scavenging Assay

S.N.	Type of extract	Conc. (µg/ml)	Percentage Scavenging activity of extracts triplicate			Average percentage scavenging
1	Methanolic	100	40.56	42.24	41.25	41.35±0.4875
		200	51.12	52.19	51.05	51.45±0.3689
		300	65.40	65.94	65.07	65.47±0.2536
		400	71.24	72.34	71.45	71.67±0.3372
		500	76.94	76.99	76.75	76.89±0.07311
2	Aqueous	100	31.95	31.72	31.22	31.37±0.2155
		200	35.42	36.28	36.10	35.93±0.2619
		300	41.85	42.61	41.12	41.86±0.4302
		400	54.42	55.73	55.85	55.33±0.4580
		500	68.61	69.88	69.21	69.23±0.3668
3	Standard (Ascorbic acid)	100	47.49	48.24	47.22	47.65±0.3051
		200	58.15	58.05	58.21	58.13±0.04667
		300	69.19	69.87	69.72	68.59±0.2063
		400	76.78	77.62	76.78	77.06±0.2800
		500	88.08	87.49	87.70	87.75±0.1727

*Percentage inhibition is expressed as mean ± SEM of triplicate experiments.

Table 2: IC_{50} values of ascorbic acid, methanolic and aqueous extracts of *Punica granatum* L. flowers in DPPH radical scavenging activity

DPPH radical scavenging activity	IC_{50} values (µg/ml)
Ascorbic acid	130.65
Methanolic extract	175.50
Aqueous extract	334.30

Table 3: Result of antioxidant activity of reducing power

S. No	Concentration µg/ml	Absorbance measured at 700 nm *		
		Methanolic extract	Aqueous extract	Standard (Ascorbic acid)
1	100	0.291±0.0012	0.165±0.0015	0.382±0.001
2	200	0.413±0.0015	0.235±0.0017	0.470±0.002
3	300	0.588±0.0014	0.286±0.0014	0.634±0.001
4	400	0.681±0.0012	0.343±0.0012	0.754±0.002
5	500	0.714±0.0014	0.354±0.0011	0.862±0.001

*Absorbance is expressed as mean ±SEM of triplicate experiments.

Table 4: Result of antioxidant activity of Nitric Oxide Scavenging Activity

S.N.	Type of extract	Conc. (µg/ml)	Percentage Scavenging activity of extracts triplicate			Average percentage scavenging
1	Methanolic	100	26.31	26.34	26.29	26.31±0.01453
		200	37.12	38.09	38.26	37.82±0.03551
		300	46.57	46.67	46.57	46.60±0.03333
		400	61.11	61.85	60.12	61.02±0.3830
		500	74.26	74.06	73.95	74.09±0.09074
2	Aqueous	100	24.77	24.49	24.41	24.55±0.1091
		200	35.51	34.12	35.49	35.04±0.4600
		300	46.74	46.52	46.18	46.48±0.1629
		400	58.12	58.18	57.53	57.94±0.2074
		500	69.27	68.36	68.67	68.76±0.2633
3	Standard (Ascorbic acid)	100	52.31	52.04	52.48	52.26±0.1281
		200	61.85	62.67	61.58	62.03±0.3277
		300	71.5	71.25	72.20	71.65±0.2843
		400	80.9	79.99	79.70	80.19±0.3615
		500	88.5	87.87	87.73	88.03±0.2567

*Percentage inhibition is expressed as mean ± SEM of triplicate experiments

Table 5: IC_{50} values of NO inhibition assay of methanolic and aqueous extracts of *Punica granatum* L flowers and ascorbic acid as standard

Nitric Oxide Scavenging Activity	IC_{50} values (µg/ml)
Rutin	67.75
Methanolic extract	306.90
Aqueous extract	331.01

Determination of Total Phenolics

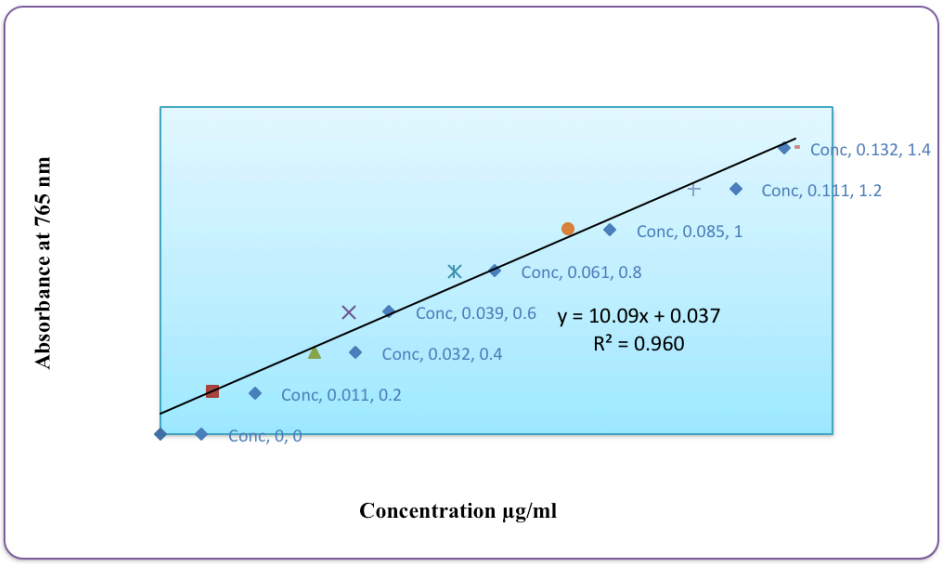

Fig 1: Calibration curve of standard gallic acid for determination of Total Phenolic Content.

Table 6: Gallic acid equivalent in mg/g of methanolic and aqueous extract of *Punica granatum* L. flowers.

Extract	Total phenolics in GAE mg/g (value ± SEM)
Methanolic	190.50±0.510
Aqueous	103.81±3.983

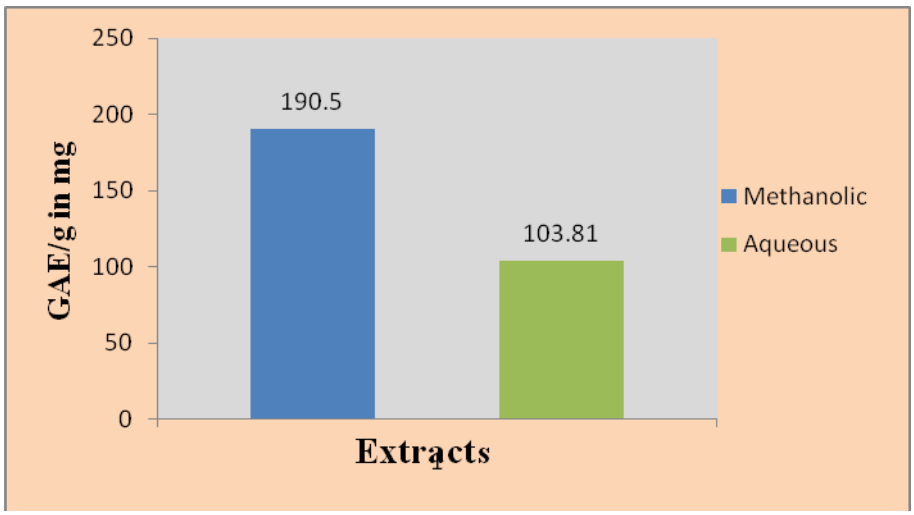

Fig. 2: Total Phenolic content of methanolic and aqueous extracts of *Punica granatum* L. flowers.

Determination of Total Flavonoids

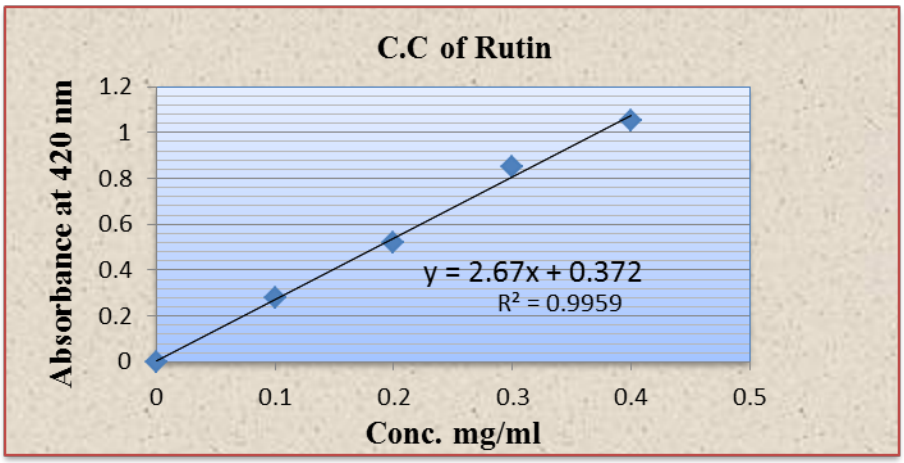

Fig. 3: Estimation of flavonoids in methanolic and aqueous extracts obtained from *Punica granatum* L. flowers

Table 7: Total flavonoids in mg of RU/g of methanolic and aqueous extract of *Punica granatum* L. Flowers

Extract	Total phenolics in GAE mg/g (value ± SEM)
Methanolic	154.63±4.163
Aqueous	60.83±0.589

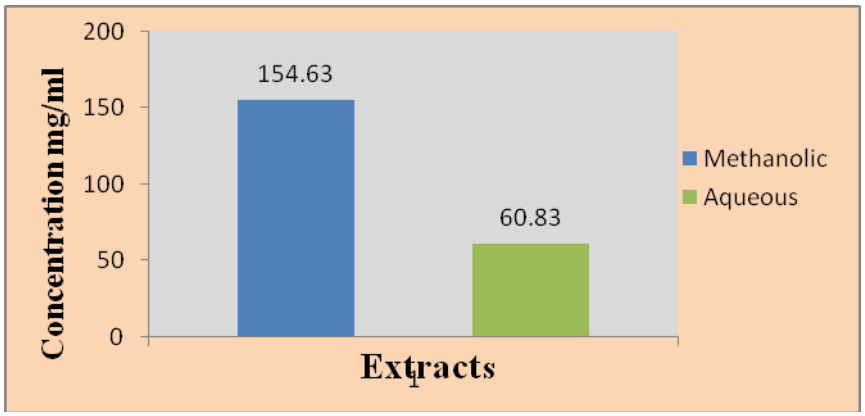

Fig. 4: Total Flavonoid Content (mg/g) in methanolic and aqueous extracts obtained from *Punica granatum* L. flowers.

Table 8: Results of *In vitro* Anti-diabetic Activity using α-amylase inhibition assay

S.No.	Conc. µg/ml	Standard	Methanolic Extract	Aqueous Extract
		% Inhibition*	% Inhibition*	% Inhibition*
1.	100	46.46±0.2431	40.90±0.2895	23.77±0.1717
2.	200	57.66±0.3502	51.52±0.2854	31.60±0.2696
3.	300	69.22±0.2631	62.51±0.1861	42.02±0.2949
4.	400	77.39±0.2809	71.35±0.2524	47.91±0.2749
5.	500	87.04±0.2809	79.09±0.4517	56.53±0.1922
IC_{50} mg/ml		125.99	184.91	417.88

*Percentage inhibition is expressed as mean ± SEM of triplicate experiments.

Table 9: IC_{50} values of α-amylase inhibition assay of methanolic and aqueous extract of *Punica granatum* L flowers as compared to standard antidiabetic drug voglibose

Alpha amylase inhibition assay	IC_{50} values (µg/ml)
Voglibose	125.99
Methanolic extract	184.91
Aqueous extract	417.88

Table 10: Results of *In vitro* Anti-diabetic Activity-non-enzymatic glycosylation of Haemoglobin assay

S.NO.	Conc. µg/ml	Standard	Methanolic Extract	Aqueous Extract
		% Inhibition*	% Inhibition*	% Inhibition*
1.	100	47.96±0.4574	37.46±0.2783	15.94±0.3334
2.	200	57.88±0.4268	48.92±0.2949	28.05±0.02082
3.	300	69.57±0.1308	57.74±0.03180	43.37±0.2857
4.	400	78.23±0.1986	67.62±0.2950	48.52±0.3218
5.	500	84.49±0.02333	74.28±0.1819	57.62±0.2918
IC_{50} mg/ml		111.31	222.08	408.94

*Percentage inhibition is expressed as mean ± SEM of triplicate experiments.

Table 11: IC_{50} values of Non-enzymatic glycosylation of hemoglobin assay of methanolic and aqueous extract of *Punica granatum* L. flowers as compared to standard antidiabetic drug voglibose

Non enzymatic glycosylation of Hb assay	IC_{50} values (µg/ml)
Voglibose	111.31
Methanolic extract	222.08
Aqueous extract	408.94

DISCUSSION

Antioxidant is a substance that inhibits oxidation, especially one used to counteract the deterioration of stored food products. A substance such as vitamin C or E that removes potentially damaging oxidizing agents in a living organism.

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 [35]. In the present study, flower extracts (Methanolic and Aqueous) of *Punica granatum* L. were subjected to extraction for evaluation of anti-oxidant and anti-diabetic activities using i*n-vitro* methods.

From the result, it was noted that DPPH scavenging activity increased with increase in concentration for both standard and different flower extracts of *Punica granatum* L. Percentage scavenging activity at 100 µg/ml concentrations of standard ascorbic acid was found to be 47.65% which increased up to 87.75% at 500 µg/ml.

DPPH scavenging activity was highest in methanolic extract, which was 41.35% and increased up to 76.89% at same concentration as that of standard. DPPH assay is based on the ability of 2, 2-diphenyl-1-picrylhydrazyl, a stable free radical to decolorize from purple to yellow color in presence of antioxidants. The DPPH contains an odd electron, which is responsible for the absorbance at 517nm and also for a visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized. The highest percentage scavenging activity of methanolic and aqueous extract of flowers of *Punica granatum* L. was measured to be 76.89% and 69.23% respectively. The IC_{50} value calculated for both methanolic and aqueous extract was found to be 175.50 µg/ml, 334.30 µg/ml respectively whereas for standard ascorbic acid it was found to be 130.65 µg /ml. This activity of extract can be attributed to the presence of compounds having hydrogen donating ability. From the reducing power assay, absorbance increased with increase in concentration for both standard and sample extracts. Among the extracts used methanolic extract showed the highest scavenging activity followed by aqueous extract.

Reducing power was found to be less for all the two extracts when compared with the standard. Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants

Nitric oxide radical scavenging capacity of flowers of *Punica granatum* L. extracts and ascorbic acid was also in a dose dependent manner with IC_{50} values 306.90 µg/ml, 331.01 µg/ml and 68.31 µg/ml for methanolic, aqueous extract and ascorbic acid respectively, suggesting good activity of extract for nitric oxide scavenging assay. This behavior of methanolic extract can be thought due to presence of antioxidant compounds.

Total phenolic content was observed to be maximum for methanolic extract among the two extracts tested. The phenolic content measured for methanolic and aqueous extracts was 190.50 and 103.81 mg GAE/g. The Folin-Ciocalteu method gives a crude estimation of the total phenolic compounds present in a sample. It is not specific to polyphenols, but many interfering compounds may react with the reagent, giving elevated apparent phenolic concentrations. Polyphenols have been reported to be responsible for the antioxidant activities of botanical extracts. The antioxidant activity of phenolic compounds is due to their ability to scavenge free radicals, donate hydrogen atoms or electron, or chelate metal cations.

Flavonoids as one of the most diverse and widespread group of natural compounds are the most important natural phenols. These compounds possess a broad spectrum of chemical and biological activities including radical scavenging properties. Total flavonoid content in the methanolic and aqueous extract was found to be 154.63 and 60.83 mg /g respectively, and again it was observed to be maximum in methanolic extract in comparison with aqueous extract. This indicates that methanolic extract of *Punica granatum* L. has the highest total phenolic and flavonoid content when compared to the aqueous extract**.**

***In vitro*, α-amylase inhibitory activity** of *Punica granatum* L. flower extracts was tested. Concentrations of the range 100mg, 200mg, 300and 400 mg/mL and 500 mg/mL of the methanolic and aqueous extracts of flowers was tested. The methanolic extract showed a significant inhibitory activity of 79.09% at a concentration of 500mg/mL while aqueous shows 69.70% at the same concentration. For a similar concentration the standard drug (voglibose - α-amylase inhibitor) showed 87.04% inhibition. IC_{50} of the *Punica granatum* L. flowers of methanolic and aqueous extracts was found to be 184.91 mg/mL and 417.88mg/mL respectively.

From the results, it is evident that the methanolic extract of *Punica granatum* L. flowers had potent α-amylase inhibitory

activity comparable to the standard drug voglibose. α-amylase begins the process of starch digestion. It takes starch chains and breaks them into small pieces with two or three glucose units. The α-amylase inhibitory action of *Punica granatum* L. flowers might play a role in diabetic treatment.

***In vitro* non-enzymatic glycosylation of haemoglobin assay, i**ncrease in the concentration of globulin blood leads to its binding to haemoglobin which results in formation of reactive oxygen species. The methanolic extract of *Punica granatum* L. flowers shows higher inhibition of glycosylation for the concentration of 100 ug/ml and 200 ug/ml as compared to standard drug voglibose. But in further concentration the inhibition of haemoglobin glycosylation the extracts do not show much effect. Hence the lower concentration of plant extract may helpful for decreasing the formation of the glucose haemoblogin complex and thus increase the amount of free haemoglobin.

CONCLUSION

The *Punica granatum* L. flower extracts showed positive result for antidiabetic activity with maximum antioxidant activities. The present finding reveals that the compound efficiently inhibits alpha amylase and enzyme in a dose dependent manner. It was proposed that inhibition of the activity of alpha-amylase would delay the degradation of carbohydrate, which would in turn cause a decrease in the absorption of glucose, as result the reduction of postprandial blood glucose level elevation. The assay results suggested that the presence of bioactive compounds could be responsible for the versatile medicinal properties of this plant including diabetes. The antidiabetic action of compounds isolated from *Punica granatum* L. flower extracts can also be attributed due to the presence of polyphenolic compounds in *Punica granatum* L. flowers and may have a potentially important role in managing diabetes via the inhibition of α-amylase and non-enzymatic glycosylation of haemoglobin. In this present study, we evaluated *In-vitro* alpha amylase inhibition assay and non-enzymatic glycosylation of haemoglobin assay. It was suggested that further studies are required to elucidate the mechanism of antidiabetic potential. Thus, the current study provided concrete evidence for the antioxidant and antidiabetic activity of methanolic and aqueous extracts of flowers of *Punica granatum* L. However, methanolic extract proved to be more effective than the aqueous extract in both the pharmacological activities. Further, the bio active compounds can be isolated from these extracts and can be studied for their toxic effects, if they are found safe, they may be used as formulations associated with free radical scavenging activity. The compound may also be chemically modified so as to enhance its pharmacological activity and suppress toxicological effects (if any), thus it can serve as a lead molecule.

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