Research Article

Phytochemical Screening and *"In-Vitro"* Antioxidant Activity of Ethanolic Flower Extracts of *Punica granatum*

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ABSTRACT

The objective of the present study was to evaluate the phytochemical constitutents and antioxidant activity of ethanolic extract of dried flower of medicinally important herb *punica granatum*. Qualitative analysis of phytochemical constituents' Viz. tannins, saponins, flavonoids, steroids, alkaloids, quinines, coumarins, terpenoids, lignins and sugars and quantitative analysis of carbohydrates, protein and phenol was performed by the well-known tests protocol available in the literature. Antioxidant activity was studied through DPPH radical scavenging assay, Reducing power assay, ABTS scavenging assay and Nitric oxide radical scavenging assay. The results suggest that *punica granatum* promising antioxidant activity and could serve as potential source of natural antioxidants.

Keywords: Phytochemical, Antioxidant Activity, *Punica granatum*, DPPH, Nitric oxide, ABTS, Reducing power, Free radical scavenging activity.

INTRODUCTION

Antioxidants are micronutrients that have gained importance in recent years due to their ability to neutralize free radicals or their actions. Reactive oxygen species (ROS) including superoxide radicals, hydroxyl radicals, singlet oxygen and hydrogen peroxide are often generated as byproducts of biological reaction or from exogenous factors.[1]

Free radicals are chemical species which contains one or more unpaired electrons due to which they are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability. Free radicals are continuously produced in the human body, as they are essential for energy supply, detoxification, chemical signaling and immune function.[2]

Ultraviolet light, ionizing radiation, chemical reactions and metabolic process can induce the production of reactive oxygen species (ROS) in the cells.

Free radicals can initiate the oxidation of bio molecules, such as protein, lipid, amino and DNA which will lead to cell injury and can induce numerous diseases.[3] The effects of free radicals on human beings are closely related to toxicity, disease and aging.[4]

An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage and oxidative stress is the main cause of several diseases: cancer, cataracts, age related diseases and Parkinson's disease.

Antioxidants reduce the oxidative stress in cells and are therefore useful in the treatment of many human diseases, including cancer, cardiovascular diseases and inflammatory diseases.

This activity is due to the ability of antioxidants to reduce oxidative stress by neutralizing or scavenging of reactive species by hydrogen donation.[5]

Plants which have one or more of its organ containing substances that can be used for the therapeutic purpose are called medicinal plants.[6] The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of photochemical constituents.[7] Phytochemicals are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defense mechanism and protect from various diseases. Phytochemicals are primary and secondary compounds. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds have terpenoids, alkaloids and phenolic compounds.[8] Medicinal plant parts are commonly rich in phenolic compounds, such as flavonoids, phenolic acids, stilbenes, tannins, coumarins, lignans and lignins. These compounds have multiple biological effects including antioxidant activity.[9] *In vitro* experiments on anti-oxidant compounds in higher plants show how they protect against oxidation damage by inhibiting or quenching free radicals and reactive oxygen species.[10]

Punica granatum L. (Punicaceae), known as pomegranate, is a deciduous small tree, up to 8 m in height with attractive reddish scarlet edible fruits. The species originated in Iran, Afghanistan and Baluchistan, found wild in the warm valleys of the Himalayas and is cultivated throughout India.[11] The dried flowers, known as Gulnar, are efficacious to treat haematuria, haemoptysis, diarrhoea, dysentery, nasal hemorrhage[12] and in Unani literature as a remedy for diabetes.[13,14] Flower juice is recommended as a gargle for sore throat, in leucorrhoea, hemorrhages and ulcers of the uterus and

rectum. The root bark and stem bark of the plant are astringent and used as anthelmintic especially against tapeworms. Fruit rind is valued as an astringent in diarrhea and dysentery. The powdered flower buds are useful in bronchitis. The seeds are reputed as stomachic and the pulp as cardiac and stomachic.

The green leaf paste is applied to relieve conjunctivitis.[15] The aqueous-ethanol (50%, v/v) extract of the flowers leads to significant blood glucose lowering effect in normal, glucose-fed hyperglycemic and alloxan-induced diabetic rats.[16] In Chinese medicine these flower are also used for the treatment of injuries from falls and grey hair of young man.[17]

In addition *Punica granatum* is considered as a pharmacy unto itself in ayurvedic medicine and is used as an antiparasitic agent, a blood tonic, and to ulcers.[18]

The Present investigation was aimed at evaluating the *in-vitro* antioxidant activity of ethanolic extract of the flowers of *Punica granatum (Linn)* by using DPPH radical scavenging assay, ABTS radical scavenging assay, Nitric oxide radical scavenging assay scavenging assay and Reducing power scavenging assay.

MATERIALS AND METHODS

Collection and Authentication of Plant Material

The flowers of *Punica granatum* were collected from in and around the Mannargudi, Thiruvarur DT, Tamil nadu, India. They were identified and authenticated by Dr. S. John Britto, Department of Botany, Rabient Herbarium and center for Modular Systematics, St. Joseph's College, Trichurappalli, Tamil nadu, India.

Preparation and Extraction of Plant Material

Collected plant material were thoroughly washed with distilled water and then dried under shade at room temperature for few days.

The dried plant samples were ground well into a fine powder using blender. The powdered samples were then stored in airtight containers for further use at room temperature. Various organic solvents were used for the extraction of bioactive compounds.

The flower powder (10g) of *Punica granatum* was successfully extracted with Hexane, Chloroform, Ethyl acetate and the water in a Soxhlet apparatus. The extracts obtained were completely evaporated on a water bath.

The concentrated extracts were subjected to qualitative and quantitative test for the identification of various phytochemical. The concentrated extracts were used for in-vitro antioxidant activity.

Organoleptic / Macroscopic Evaluation

In the present study the powder of crude drug was investigated for its macroscopic characteristics i.e. colour, odour, and taste.[19]

Physiochemical Parameters

Foreign Matter Analysis

Foreign matter presence may be due to faulty collection of crude drug or due to deliberated mixing. It was separated from the drug so that results obtained from analysis of the drug gives accuracy. Its percentage in the crude drug was calculated.[20]

Determination of Moisture

10g of accurately weighed fresh flowers of the test plant (without preliminary drying) was taken in a china dish. It was incubated at 105 ˚C for 5 hours. The content of the dish after incubation was weighted and the values were noted. Drying and weighing was continued till the difference between two successive values corresponds to not more that 0.25%. The percentage of total moisture content of the drug was finally calculated.

$$\text{Moisture content (\%)} = \frac{\text{Initial weight sample} - \text{final weight sample}}{\text{weight of sample}} \times 100$$

Determination of Extractive Values

10g of the air dried plant drug was transferred to an extraction thimble, extracted with various solvents in the order of increasing polarity (Hexane, Chloroform, Ethyl acetate) by using Soxhlets extraction apparatus (for 6 hours). The extract was filtered into a tarred evaporating dish and the solvent was evaporated on a water bath. The residue was dried at 105 ˚C to constant weight. The percentage of extractive values for various solvents was calculated with reference to the air-dried drug.

Determination of Alcohol Soluble Extractive Values

5g of the air-dried drug macerated with 100ml of alcohol in a closed flask for 24hrs was frequently shaken during first 6hrs and allowed to stand for 18hrs. It was rapidly filtered taking precautions against loss of solvents. 25ml of filtrate was evaporated to dryness in a tarred flat bottomed china dish and dried at 105 ˚C until constant weight was obtained. The percentage of alcohol soluble extractive was calculated with reference to the air-dried drug.

Determination of Water Soluble Extractive Values

5g of the air-dried drug macerated with 100ml of water in a closed flask for 24hrs was frequently shaken during first 6hrs and allowed to stand for 18hrs. Rapidly filtered and evaporated 25ml of filtrate to dryness in tarred flat-bottomedy china dish and dried at 105 ˚C until constant weight was obtained. The percentage of water-soluble extractive was calculated with reference to the air-dried drug.[21]

Fluorescence Analysis

Fluorescence analysis of the drug was observed under day and UV light using various solvent extracts as well as acids and alkaline treated with solutions of the drug. The powder was treated with neutral solvents like hexane, benzene, chloroform, ethyl acetate, alcohol, acetone and

acids like 1N Hydrochloric acid, 50% Sulphuric acid and alkaline solutions like aqueous and alcoholic 1N NaoH.[22]

Phytochemical Investigation

Preliminary phytochemical screening of drug powder and various extracts were carried out as per the standard procedure.[23] The various extracts of *Punica granatum* qualitatively tested for different phytoconstituents like tannins, saponins, flavonoids, steroids, alkaloids, quinones, coumarins, terpenoids, lignins and sugars and quantitatively for protein, carbohydrates and phenol.

In-Vitro Antioxidant Assay

Determination of DPPH Free-Radical Scavenging Activity

Antioxidant activity can be measured using DPPH radical scavenging assay method of Gyamfi.[24] In this method, free radical scavenging potential of *Punica granatum* extracts was tested against a methanolic solution of DPPH (α, α - diphenyl- β- picryl hydrazyl). Antioxidants react with DPPH and converted it to α, α - diphenyl-β - picryl hydrazine. The degree of discoloration indicates the scavenging potentials of the antioxidant extract. The change in the absorbance produced at 517 nm has been used as a measure of antioxidant activity. To a set of clean and dried test tubes 3ml of methanol and 150 µl of 0.1% DPPH reagent was added and mixed thoroughly; allow the solution to stand for 30 minutes. The initial absorbance of each test tube was measure at 517nm. To these test tubes 1ml of aqueous solution of extracts were added in increasing concentration of 100 to 500 µg/ml. The solution of ascorbic acid (100-500 µg/ml) was taken as a standard. The solution was mixed and allowed to stand for 30 minutes at room temperature and the final absorbance was measured at 517nm using a spectrophotometer. The experiment was performed in triplicate. Free radical scavenging activity was expressed as the inhibition percentage calculated using the formula.

$$\text{DPPH free radical scavenging activity (\%)} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

Determination of ABTS Radical Scavenging Assay

ABTS radical scavenging assay of sample was measured by Re R, Pellegrini N, method.[25] ABTS and ascorbic acid stock solution was prepared. From the stock solution, various dilution were prepared and used for antioxidant study, ABTS solution 2Mm (0.0548g in 50 ml) was prepared in distilled water. Pottassium persulphate solution 70mM (0.0189 g in 1ml) was prepared in distilled water. 200ml of potassium persulphate solution and 50ml of ABTS solution were mixed and used after 2 hrs. This solution is called as ABTS radial cation, which was used for assay. To 500µg of various concentrations of extract 0.3ml of ABTS radical cation and 1.7ml of phosphate buffer was added and the same was performed for the standard ascorbic acid also. The absorbance was measured at 734nm. The experiment was repeated in triplicate. The percentage of reduction in absorbance was calculated from initial and final absorbance at each level. The percentage inhibition was calculated according to the formula:

$$\text{ABTS radical scavenging activity (\%)} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

Determination of Reducing Power Assay

Assay of reducing power was carried out by the method of potassium ferrcoyanide (Oyaizu, 1986).[26] The various concentrations (100 to 500µg) of the plant in corresponding solvents were mixed with phosphate buffer (2.5ml) and potassium ferricyanide (2.5ml). This mixture was kept at 50 ˚C in water bath for 20 minutes. After cooling, 2.5ml of 10% trichloro acetic acid was added and centrifuged at 3000 rpm for 10 minutes whenever necessary. The upper layer of solution (2.5ml) was mixed with distilled water (2.5ml) and a freshly prepared ferric chloride solution (0.5ml). The absorbance was measured at 700nm. Control was prepared in similar manner excluding samples. Ascorbic acid at various concentrations was used as standard. Increased absorbance of the reaction mixture indicates increase in reducing power. Reducing power was measured by varying the concentration of the extract and the contact time. All measurements were made in triplicate. The reducing power was expressed as the inhibition percentage calculated using the formula:

$$\text{Reducing power (\%)} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

Determination of Nitric Oxide Radical Scavenging Activity

Nitric oxide radical scavenging assay was carried out by the method of sodium nitroprusside.[27] This can be determined by the use of the Griess Illosvoy reaction. 3mL of 10mM sodium nitroprusside in 0.2M phosphate buffer saline (pH 7.4) was mixed with 0.5mL of extract at various concentrations (100 to 500µg) and the mixture was incubated at 25 ˚C for 150 minutes.

After incubation 0.5mL of Griess reagent (1% sulfanilamide, 0.1% naphthylethylene diamine dihydrochloride in 2% H3PO4) was added. The absorbance of the chromophore was read at 546nm. The experiment was repeated in triplicate. Percentage of nitric oxide radical scavenging activity of the sample was calculated as follows:

$$\text{NO radical scavenging activity (\%)} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

The sample concentration providing 50% inhibition (IC_{50}) under the assay condition was calculated from the graph of inhibition percentage against sample concentration.

RESULTS AND DISCUSSION

There is increasing evidence that indigenous antioxidants may be useful in preventing the deleterious consequences of oxidative stress and there is increasing interest in the protective biochemical functions of natural antioxidants contained in spices, herbs and medicinal plants.[28,29]

The physicochemical analysis of powder exposed the foreign matter, moisture content (loss on drying), water soluble extractives, chloroform soluble extractives, ethanol extractives, ethyl acetate extractives and hexane soluble extractives are as shown in Table 1. The fluorescence analysis of powdered flower material was subjected to analysis under long ultra violet light after treatment with various chemical and organic reagents. The fluorescence's behavior was noted as in Table 2. Phytochemical evaluation was performed for qualitative detection of various chemical constituents which aid in tracing the presence of active entity that elicit a major pharmacological response. The result proved the presence of alkaloids, flavonoids, saponins, tannins, lignins, quinones, coumarins, terpenoids, steroids and quantitative detection of protein, phenolic compounds and carbohydrates, which were tabulated in Table 3 and 4. Table 4 and Figure 1 showed the availability quantifiable of carbohydrates, protein, and phenol. The ethanolic extract yielded 0.016 mg/g of carbohydrate 0.0005 mg/g of protein and 0.0001 mg/g of phenol. Quantitative phytochemical analysis indicated that the plant contains significant amounts of phenolic compounds. These classes of compounds were responsible for antioxidant and free radical scavenging effect of plant material.[30-32] *In vitro* antioxidant activity of the flower extract of *P. granatum* was investigated in the present study by DPPH radical scavenging, reducing power, ABTS scavenging and nitric oxide radical scavenging assays. In this study, it is evident that the extract of the study species, *P. granatum* possess effective antioxidant activity. (Tables-5, Figure-2)

Table 1: Physicochemical analysis of *Punica granatum*

S. No.	Parameters	% of Concentration
1.	Hexane	0.748
2.	Chloroform	1.872
3.	Ethyl acetate	0.900
4.	Ethanol	36.58
5.	Water	60.7
6.	Moisture	8.51
7.	Foreign matter	1.00

Table 2: Fluorescence Analysis of Flowers of *Punica granatum*

S. No.	Test	0 hours		24 hours		48 hours	
		Day Light	UV Light	Day light	UV light	Day light	UV light
1	Drug + Aqueous	Brown	Brown	Brown	Brown	Brown	Brown
2	Drug + 1N N_aOH	Brown	Dark brown	Red	Reddish brown	Dark brown	Black
3	Drug + Alcoholic 1N N_aOH	Brown	Brown	Ash	Ash	White	Pale green
4	Drug + 1N N_aOH	Dark brown	Red	Orange	Orange	Pale orange	Orange
5	Drug + 50% HCL	Dark brown	Brown	Brown	Brown	Dark brown	Brown
6	Drug + Hexane	Light brown	Brown	Brown	pink	Pale pink	Light brown
7	Drug + CH CL_3	Brown	Light brown	Brown	Brown	Light brown	Brown
8	Drug + Ethyl acetate	Light brown	Brown	Dark brown	Brown	precipitate	Brown
9	Drug + Acetone	Yellow	Yellow	Yellowish brown	Yellow	Yellow	Pale yellow
10	Drug + Benzene	Brown	Brown	Brown	Brown	Brown	Pale green
11	Drug + Alcohol	Yellow	Yellow	Yellowish brown	yellow	Pale yellow	Light brown
12	Drug + water	Orange	Pale orange	Pale orange	orange	Pale orange	Pale orange

Table 3: Phytochemical Analysis of *Punica granatum*

S. No.	Test	Extracts					
		Dry Powder	Hexane	Chloroform	Ethyl acetate	Ethanol	Water
1	Saponins	-	-	-	+	+	-
2	Tannins	-	+	+	+	+	+
3	Steroids	-	-	-	-	-	-
4	Terpenoids	+	+	+	+	+	-
5	Flavonoids	+	-	-	-	-	-
6	Coumarins	-	-	+	+	+	-
7	Quinones	+	-	+	+	-	-
8	Lignins	-	-	-	_	-	-
9	Alkaloids	+	+	+	+	-	+
10	sugars	+	-	-	+	-	+

+ Present **-** Absent

Table 4: Quantitative Analysis of *Punica granatum*

S. No.	Parameters	Amount (g)
1.	Carbohydrate	0.016
2.	Protein	0.0005
3.	Phenol	0.0001

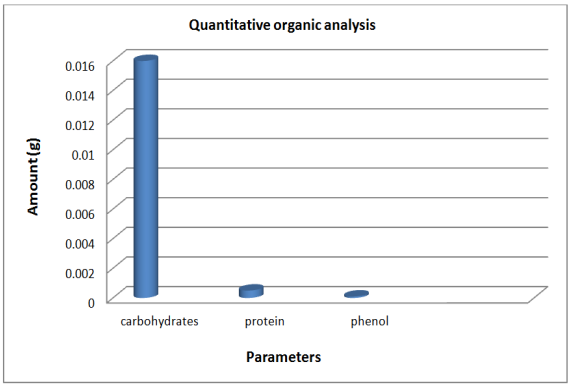

Figure 1: Quantitative Photochemical Analysis of flowers of *Punica granatum*

In-vitro Antioxidant Activity

The various extracts of *Punica granatum* were tested for different phytochemical constituents like tannins, saponins, flavonoids, steroids, alkaloids, quinones, coumarins, terpenoids, lignins, protein, carbohydrates and phenol. The knowledge of the chemical constituents of plant is desirable because such information will be valuable for synthesis of complex chemical substances and to screen for biological activities.[33-35]

The phenolic and flavanoids are widely distributed secondary metabolites in plants having anti-oxidant activity and have wide range of biological activities as anti-apoptosis, anti-aging, anti-carcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities[36,37]

In-vitro antioxidant studies are widely carried to screen various plant containing phenolic and flavanoids constituents. Plant derived antioxidant compounds; flavonoids and phenolic have received considerable attention because of their physiological effect like antioxidant.

Anti-inflammatory, antitumor activities and low toxicity compared with those of synthetic phenolics antioxidant such as BHA (Butylated Hydroxyanisole), BHT (Butylated Hydroxytoluene) and Propyl Gallate (PG).[38,39]

DPPH Radical Scavenging Activity

DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. Antioxidant on interaction with DPPH, transfer electron or hydrogen atom to DPPH and thus neutralizing its free radical character and convert it to 1 – 1 diphenyl – 2 – picryl hydrazine and the degree of discoloration indicates the scavenging activity of the drug.[40] The decrease in absorbance of DPPH radical caused by antioxidants is due to the reaction between antioxidant molecules and radical progress which results in the scavenging of the radical by hydrogen donation. It is visually noticeable as a change in color from purple to yellow.

Hence, DPPH is usually used as a substance to evaluate the antioxidant activity.[41,42] In the present study, the extracts had significant scavenging effect on the DPPH radical which was increasing with the increase in the concentration of the sample 500 µg/mL. Similar trend of DPPH free radical scavenging activity was reported for the species, Lippiaalba[43] and languas galangal.[44]

To evaluate the antioxidant activity of the flower extract, the radical scavenging capacity based on DPPH assay was determined and the results are shown in Table 5 and Figure 2 for the species, *P. granatum*. The percentage of scavenging effect on the DPPH radical was increased with the increase in the concentrations of the extract from 100-500 µg/mL. The percentage of inhibition of the DPPH radical was varying from 2.7 % (in 100 µg/mL of the extract) to 91.6% (in 500 µg/mL of extract). The IC_{50} value of the flower extract of this species was determined to be 12 µg/mL. The extracts in all concentrations showed higher percentage of inhibition of free radicals than the standard drug, ascorbic acid (100 µg/mL - 2.7%, 200 µg/mL - 25.04%, 300 µg/mL - 75.01%, 400 µg/mL - 83.3%, 500 µg/mL - 91.6%).

ABTS Radical Scavenging Activity

ABTS assay is an excellent tool for determining the antioxidant activity of hydrogen–donating antioxidants and of chain-breaking antioxidants.[45] The extract scavenged ABTS radicals generated by the reaction between 2, 2'-azinobis (3-ethylbenzothiazolin 6-sulphonic acid) (ABTS) and ammonium persulfate. The activity was found to be increased in a dose-dependent manner from 23.07% to 80.76% at concentrations of 100-500 µg/mL (Table 5 and Figure 2). The extract exhibited an IC_{50} value of 260 µg/mL. Therefore, the ABTS radical scavenging activity of ethyl acetate extract of *P. granatum*, flower indicates its ability to scavenge free radicals, thereby preventing lipid oxidation via a chain-breaking reaction.

Table 5: *In vitro* antioxidant activity of flowers of *Punica granatum*

S. No.	Antioxidant activity	Concentration(µg/ml)					IC_{50} Value (µg/ml)
		100	200	300	400	500	
		(%) Inhibition					
1	DPPH	2.7	25.03	75.01	83.3	91.6	12
2	ABTS	23.07	42.30	57.69	69.23	80.76	260
3	Reducing Power	7.54	10.90	31.94	37.97	47.87	300
4	NO	50.01	60.17	70.23	75.40	85.09	100

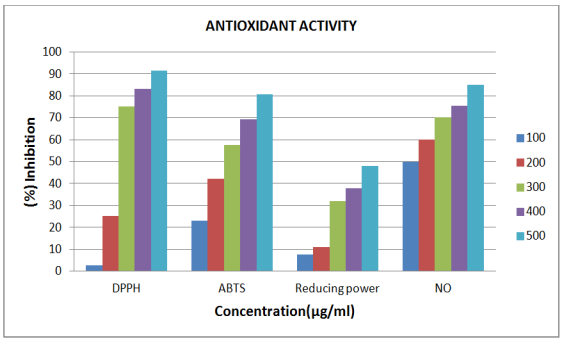

Figure 2: *In vitro* antioxidant activity of flowers of *Punica granatum*

Reducing Power Assay

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. In this assay, the yellow color of the test solution changes to various shades of green and blue depending on the reducing power of each compound. Presence of reducers causes the conversion of the Fe3+/ferricyanide complex used in this method to ferrous form. By measuring the formation of Pearl's Prussian blue at 700 nm, it is possible to determine the concentration of ferrous ions. Figure 5 presents the reductive capabilities of the ethanol extract of *P. granatum* flower. In the concentration range investigated, the extracts demonstrated reducing power that increased linearly with concentration. At 100, 200, 300, 400 and 500 µg/mL, reducing power of *P. granatum* extract was found to be 7.54, 10.90, 31.94, 37.97 and 47.87 respectively. The IC_{50} value was found to be 300 µg/mL (Table-5). The reducing power of the extract might be do their hydrogen-donating ability. Increased absorbance of the reaction mixture indicated increased reducing power of the extracts. The reducing power of the extracts increased with the increase in their concentration. Possibly, *P. granatum* flower contain high amounts of phenolic compounds which could react with radicals to stabilize and terminate radical chain reactions.

Nitric Oxide (NO) Radical Scavenging Assay

NO is a very unstable species and reacting with oxygen molecule producing stable nitrate and nitrite which can be estimated by using Griess reagent. In the presence of a scavenging test compound, the amount of nitrous acid will decrease which can be measured at 546 nm. Ethanol extract of *P. granatum* flower has oxide scavenging activity. The extract exhibited an IC_{50} value of 100 µg/mL. NO is a potent pleiotropic inhibitor of physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical that plays many roles as an effectors molecule in system including neuronal messenger, vasodilatation and antimicrobial and anti-tumor activities.[46] However, excess production of NO is associated with several diseases.[47]

Suppression of released NO may be partially attributed to direct NO scavenging as the extract of *P. granatum* flower decreased the amount of nitrite generated from the decomposition of SNP *in-vitro*. The scavenging of NO by the extract was increased in dose dependent manner. However, a maximum inhibition was achieved at a higher concentration of 500 µg/mL. Figure-2 and Table-5 illustrates a significant decrease in the NO radical due to the scavenging ability of extract.

CONCLUSION

The findings of this study support the view, that the ethanol extract of plants are promising sources of potential antioxidant and may be efficient as preventive agents in some diseases. The phytochemical analysis of the medicinal plants are also important and have commercial interest in both research institutes and pharmaceuticals companies for the manufacturing of the new drugs for treatment of various diseases. Further detailed studies on isolation of phytoconstituents of the plant extracts are essential to characterize them as biological antioxidants. Medicinal plants have a promising future because there are about half million plants around the world, and most of them their medical activities have not investigate yet, and their medical activities could be decisive in the treatment of present or future studies.

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