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Research Article

ANTIOXIDANT AND FREE RADICAL SCAVENGING ACTIVITY OF *PUNICA GRANATUM* LEAF EXTRACTS

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ABSTRACT

Objective: The present study was aimed to examine the *in vitro* antioxidant property and free-radical scavenging capacity of *Punica granatum* leaves, which are widely used in the ayurvedic treatment.

Methods: Aqueous and methanol extracts were prepared and to assess for their phytochemical analysis to find out the various compounds and total yield of phenolic and flavonoid contents by spectrophotometer methods. Free-radical scavenging capacity and antioxidant activity were estimated using a number of chemical assays are 2, 2-diphenyl 2-picrylhydrazyl, H_2O_2, FRAP, TRAP, and beta-carotene assay.

Results: It showed that aqueous extract had the greatest antioxidant activity and contained significant levels of total phenolic and flavonoids content expressed as milligram of gallic acid and quercetin equivalent/gram of extracts. The extracts showed antioxidant activity *in vivo* by protecting yeast cells against oxidative stressing agent H_2O_2.

Conclusion: These results specified the *P. granatum* is a good source of natural compounds which might have benefits for health, which can be used in a dietary application with a possible to reduce oxidative stress.

Keywords: *Punica granatum,* Free-radical scavenging capacity, Phytochemical, Antioxidant, Yeast culture.

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INTRODUCTION

Molecules of antioxidant with radical scavenging ability are thought to wield possible protective outcome against the free-radical damage. In the human body, oxidative damage is caused by free-radicals [1], and it is leading to chronic diseases, such as aging, cancer, cardiovascular disease, diabetes, and neurodegenerative [2,3] moreover free-radicals so as to come from the surrounding environment. In human corpse, some biochemical and physiochemical processes are occurring and might generate reactive oxygen species (ROS), such by-products are peroxyl radicals (ROO), hydroxyl radicals (OH), and superoxide radicals (O_2–) [4].

Overproduction of reactive species or free-radicals may cause damage to the biomolecules such as nucleic acids, proteins, carbohydrates, lipid peroxidation in living cells leading to mutagenic changes cell death and tissue damage are occurred [5]. Consequently, antioxidants are important to many health benefits. Compounds of antioxidants thus slow down or delay in the process of oxidation. In the early stage, antioxidant compounds may slow down the formation of free-radicals [6]. Several synthetic drugs were widely used, such as butylated hydroxytoluene and butylated hydroxyanisole are extremely effective in their role-like antioxidants [7]. Although, use in the food products contain failing off due to their instability and promoters of carcinogenesis and they have been reported to cause atherosclerosis, cell damage, inflammation, tissue toxicity in both animals and humans [8,9]. Topical finding clearly illustrates that the plant foods are rich in the natural antioxidants which play an essential role in the prevention of degenerative diseases [10]. Medicinal plants have a capable of synthesizing and storing the biochemical compounds also known as secondary metabolites which can be extracted and used as medium for various diseases [11]. Thus, more than 80,000 species of plants have therapeutic uses [12]. The most of the medicinal plants are studied for their antioxidant activity. Food, which is rich in natural antioxidants when consumed, are associated with lower risks of degenerative diseases, especially cancer and cardiovascular disease. One of the best methods to find out antioxidant compound in the plant is "phytochemical screening" [13].

Medicinal plants have some bioactive compounds which are actively researched for their potential activity. Phenolic compounds and flavonoid contain a therapeutic application. Various researchers are demonstrated the correlation between phenolic compounds and antioxidant activity of plant extracts [10,14].

Punica granatum, commonly known as pomegranate of family Punicaceae, has two species and one genus. It is a shrub or small tree of 5-8 m high (approx.). The leaves are evergreen; stem is short and fruits are filled with fleshy, juicy pulp, 52% of weight of the whole fruit is due to the seeds. The plant is both self- and cross-pollinated. Based on cultivar and season, the size and fertility of the pollen varies [15]. *P. granatum* native from Iran to Himalayas in India, it has been cultivated as ancient times all through the region of Africa, Europe, and Asia (Al-Said *et al.*, 2009). Pomegranates have huge nutritional values and its several health benefits, and they are used for the various treatments such as coughs, throats, skin disorder, digestive disorder and disease [16]. Pomegranate leaf extract showed free-radical scavenging activity. The presence of ellagic acid in the extract makes it a powerful antioxidant [17]. Thus, the aim of this study is to carry out phytochemical screening analysis, *in vitro* and *in vivo* antioxidant activity with methanol and aqueous extract of pomegranate leaves.

METHODS

Sample collection

Based on the morphology, medicinal plant of *P. granatum* leaves was identified and collected from VIT University, Vellore (Fig. 1). The sample was thoroughly washed to remove the impurities using the tap water. After draining off the water, plant material was spread on the paper to remove the excess water. The sample was shade dried at room temperature; finally, the sample was ground and the leaf sample was stored in a refrigerator at 4°C, and it was used for further analysis.

Fig. 1: *Punica granatum* leaves

Extraction processes

Aqueous extract

About 10 g of powdered sample was weighed and dissolved in 100 ml of distilled water and kept in a boiling water bath for 1 hr and slow heat at 70°C. The residue was removed by filtering through of Whatman No. 1 paper; the filtrate was then centrifuged at 3000 g for 10 minutes. The supernatant was collected and further boiled till the volume was reduced to one-fourth of the original volume and stored at 4°C in airtight bottles [18,19].

Methanol extract

About 10 g of powdered sample was dissolved in 100 ml of methanol in a conical flask and plugged with cotton wool. It was allowed to mix well and kept on a rotary shaker at 190-220 rpm for 24 h. The supernatant was collected slowly, and the residue was removed by filtering through of Whatman No. 1 paper. The mix was evaporated in broad mouthed dish at room temperature for 2-3 days and till the volume was reduced. Moreover, final volume was stored at 4°C in airtight container [18]. Stock solutions of the crude extract was used for several assays.

Phytochemical screening analysis

Qualitative and quantitative phytochemical screening analysis of *P. granatum* was performed to identify the bioactive constituents; the study was carried out using standard procedures, extracts were detected by the following tests.

Qualitative analysis

Detection of alkaloids

About 5 mg of powder sample was taken and added with few ml of dilute HCl and filtrate the sample. The alkaloid reagent was carefully added to the test solution.

Mayer's test

To 2 ml of filtrate, two drops of Mayer's reagent were added by the side of the sample tube. A creamy or white precipitate indicates the presence of alkaloids [20].

Detection of carbohydrates

About 2 mg of extract was dissolved in 2 ml of water, and the sample solution was subjected to the following tests [21].

Benedict's test

To a 1 ml of filtrate, added a 0.5 ml of Benedict's reagent and mixed well. The sample mixture was kept in a boiling water bath for 2 minutes. A characteristic colored was found and precipitate indicates the presence of carbohydrates.

Detection of glycosides

Test for cardiac glycoside

To 2 ml of extract, 1 ml of glacial acetic acid, 1 ml ferric chloride and added a few drop of concentration sulfuric acid was added. Brown indicated the presence of glycosides [22].

Detection of saponins

About 2 mg of extract was dissolved with 2 ml of distilled water. The suspension is shaken well for a few minutes. A layer of foam indicates the presence of saponins [23].

Detection of proteins and amino acids

About 2 mg of extract was diluted in 2 ml of distilled water, then filtered the sample extract using Whatman No. 1 filter paper, and then filtrate was tested for proteins and amino acids.

Biuret test

An aliquot of 2 ml of filtrate was treated with one drop of 2% copper sulfate solution. To this, 1 ml of 95% ethanol was added, followed by excess of potassium hydroxide pellets. Pink indicates the presence of protein [24].

Test for tannins

Ferric chloride test

About 0.2 mg of dried powdered samples was mixed with 5 ml of distilled water; the mixture was boiling for few minutes after boiling the mixture get cool and then filtrate sample. A few drops of 0.1% ferric chloride were added to a test sample, of which brownish green indicates the presence of tannins [22].

Detection of phenolic compounds

Lead acetate test

The extract 2 mg is dissolved in 1 ml of distilled water and few ml of 10% lead acetate solution is added to the sample mixture. A bulky white precipitate indicates the presence of phenolic compounds [21].

Detection of steroids

Salkowski test

About 0.2 mg of the extract of sample was mixed with 2 ml of chloroform and 1 ml of concentrated H_2SO_4 was carefully added to form a layer. A reddish brown in the interface indicates the presence of steroid [25].

Detection of flavonoids

Sodium hydroxide test

About 2 mg of extract was dissolved in 2 ml of distilled water; 2 ml of 10% sodium hydroxide solution was added to the test sample, and its turn to yellow, a few drops of dilute hydrochloric acid were added yellow change into colorless indicates the presence of flavonoids [20].

Quantitative analysis

Total phenolic content

Total phenolic content was determined by the Folin-Ciocalteu method [26]. The two different extracts were taken in five different concentrations 20, 40, 60, 80, and 100 µg/ml, and then added 500 µL of Folin-Ciocalteu reagent and 2.5 ml of 2% of sodium carbonate was added and kept in dark for incubation for 30 minutes until the color is developed. Gallic acid was used to make standard calibration curve. The total phenol content was expressed in mg of gallic acid equivalents (GAES) of mg/g extract. The absorbance of the reaction was measured at 725 nm. The total phenolic contents present in the plant leaf extracts were expressed as milligram of GAE mg/g extract using the standard.

Total flavonoid content

The total flavonoid contents were determined using the Change method. The *P. granatum* leaf extracts were taken five different concentrations

via, 20, 40, 60, 80, and 100 µg/ml were mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminum trichloride and added 0.1 mL of 1.0 M potassium acetate, and then added 2.8 mL of distilled water then incubated at room temperature for 30 minutes. The absorbances of all samples are measured at 405 nm. Quercetin was used as positive control. Total flavonoid values are expressed in terms of quercetin equivalents of mg/g extract. This assay was carried out in triplicate, and the mean values were presented [27].

Antioxidant activity

Total antioxidant activity assay

The total antioxidant activity of the extract was determined by phosphor molybdenum method by [28]. 0.1 ml of five different concentrations of extracts was mixed with 1 ml of reagent solution (sulfuric acid [7.45 ml], sodium phosphate [0.99 g], and ammonium molybdate [1.23 g]). The mixture was kept in water bath for 90 minutes at 90°C. Then, it was cooled to room temperature. Absorbance of the solution was measured at 685 nm using the ultraviolet (UV)-spectrophotometer against a blank. Moreover, ascorbic acid was used as a standard.

2, 2-diphenyl 2-picrylhydrazyl (DPPH) free-radical scavenging activity

Determination of the antioxidant activity of *P. granatum* leaf extracts was measured by the bleaching of purple solution of DPPH radical. The method was given by [29]. 1 ml of extract was dissolved in methanol of various concentrations (50, 100, 150, 200, and 250 µg/ml) and treated with 0.5 ml of 0.2 mmol/L DPPH was prepared in methanol and without the extract was used as control. The mixture was then shaken vigorously and allowed to stand in dark for 30 minutes. The scavenging activity was measured as the decrease in the absorbance of DPPH at 517 nm against blank using the UV-VIS spectrophotometer. Ascorbic acid was used as positive control. Moreover, the antioxidant activity was calculated using the following formula:

DPPH radical scavenging activity (%) = $(1-[A_{sample}-A_{sample\ blank}/A_{control}] \times 100)$

Hydrogen peroxide scavenging activity assay

This hydrogen peroxide scavenging activity was assayed by the method given by Nabavi *et al*. [30]. A solution of H_2O_2 (40 mM) was prepared in phosphate buffer with 7.4 pH. Different concentrations of extract (50, 100, 150, 200, and 250 µg/ml) in 1 ml of phosphate buffer were added to 0.6 ml of hydrogen peroxide solution. The solution was left to stand for 10 minutes. Spectrophotometrically, the absorbance of the mixture was observed at 230 nm. Ascorbic acid was used as control.

H_2O_2 radical scavenging activity (%) = ([A0−A1]/A0) × 100

Ferric reducing power assay

Reduction of the ferricyanide complex/Fe3+ to the ferrous form is caused because of the presence of reductions in the solution. Hence, Fe2+ can be monitored by the absorbance at 700 nm. 1 ml of extract was added in five different concentrations such as 50, 100, 150, 200, and 250 ml of in methanol and aqueous solution and then was mixed with 2.5 ml of 200 mmol/L of sodium phosphate buffer of 6.6 pH and 1% 2.5 ml of [K3 Fe (CN) 6]. Then, it was placed in water bath for 20 minutes at 50°C. After incubation, 2.5 ml of 10% trichloroacetic acid was added to the mixture which was then centrifuged for 10 minutes at 10,000 rpm. 2.5 ml of supernatant was then mixed with 2.5 ml of distilled water and 1 ml of 0.1% ferric chloride solution. The absorbance or the intensity of blue green was measured at 700 nm. Ascorbic acid was used as control [31].

Beta-carotene assay

This method is based on the loss of yellow because of the reaction of beta-carotene with radicals formed by the oxidation of linoleic acid in an emulsion. In the presence of antioxidants, the rate of beta-carotene bleaching can be slowed down [32]. A solution of beta-carotene was prepared by dissolving 2 mg of beta-carotene in 10 ml of chloroform. Then, 12 ml of the solution was transferred into a boiling flask containing 20 mg linoleic acid and 200 mg of Tween 40. Chloroform was evaporated using a rotary evaporator and 50 ml of distilled water was slowly added. Aliquots of the emulsion (4.8 ml) were transferred into different test tubes containing 0.2 ml of sample in methanol. Tubes were incubated at 50°C in water bath. As soon as the emulsion was added to each tube, zero time absorbance was measured at 470 nm using the UV-spectrophotometer. Absorbance readings were then recorded after 120 minutes interval of incubation in water bath at 50°C. The antioxidant activity (%) of *P. granatum* can be evaluated using the following formula:

% inhibition = A1−C1/C0−C1 (µg/ml)

Where, A1 and C1 = Absorbance values measured for the test sample and control after incubation of 120 minutes, C0 = Absorbance value measured for control at zero time incubation.

***In vivo* antioxidant activity**

Using eukaryotic yeast cells, the *in vivo* antioxidant activity study was carried out. *Saccharomyces cerevisiae* was exposed to increased concentrations of extract (200, 400, and 800 µg/ml) and this was kept for incubation for 1 hr at 28°C/120 rpm. Then to the cells at exponential phase were introduced with hydrogen peroxide and incubating for 2 hrs at 28°C/120 rpm to give a stress to their growth. After incubation, all the sample cultures of in varying *P. granatum* leaf extract (methanol and aqueous) concentrations were spread in triplicates on yeast peptone dextrose agar (YPDA) plates after diluting properly in sterilized liquid YPD medium. These plates were kept for incubation (48 hrs at 28°C). On comparison with untreated control plates, the colonies were counted and this determines the 100% survival of yeast cells. The evaluation of plant extracts was done by their capacity to prevent (or) minimize the lethal oxidative damage caused by hydrogen peroxide. Tolerance was expressed as percentage or survival [33].

Statistical analysis

All the analyses were performed at least in triplicate by the determination on the different extracts values were then presented as average values along with their standard derivations. Data analyses of phenolic and flavonoid content were performed with one-way analysis of variance. The correlation coefficients (R) were calculated to determine their relationship. A statistically p values <0.05 were consider as a significant difference. Results were produced by the Microsoft Excel (2007).

RESULTS AND DISCUSSION

In recent years, medicinal plants have been widely studied for their antioxidant activity. It is assumed that an increased intake of food rich natural antioxidant is connected with the lower risk of degenerative diseases [34]. Medicinal plants can give a huge scope in the prevention free-radical associated diseases in biology systems. Antioxidant from spicy, aromatic, and medicinal values was studied to expand the natural antioxidant formulations for cosmetics, food, and some other applications [35]. Three main classes of plant chemicals are namely, phenolic, alkaloids, and terpenoids [36]. Along with these groups, phenolic compounds comprise phenolic acids are (hydroxycinnamic acids and hydroxybenzoic acid), polyphenols, tannins, and flavonoids. These compounds were protected the plants, vegetables, and fruits from the oxidative damage and also used as antioxidant by humans. Phytochemical screening is one of the important methods that include used to explore the antioxidant compounds in medicinal plants. Various techniques were followed to recover antioxidant from plants; such methods are Soxhlet extraction, maceration, supercritical fluid extraction, subcritical water extraction, and ultrasound assisted extraction [37].

Although, yield of extraction and antioxidant activity not only depend on the extraction process other than on solvent extraction methods are used. The results of this present studies are agreed with the aqueous and methanol extraction of *P. granatum* leaf it showed the good antioxidant properties.

Phytochemical analysis

The aqueous and methanol leaf extracts of *P. granatum* were subjected to phytochemical screening. Both the extracts showed the presence of phytochemicals such as carbohydrate, protein, tannins, phenols, flavonoids, saponins, glycoside, alkaloids, and steroids is present in the leaf extracts; Secondary metabolites have various therapeutic uses. Directly, they are responsible for the antioxidant property of the leaf extracts. The result of the qualitative analysis of phytochemical is shown in Table 1.

Phytochemical screening analysis in qualitative test

Quantitative analysis

The present study was conducted to identify compounds; the percentage of weight of the sample composition was expressed by phenolic, flavonoids from medicinal plant of *P. granatum*; the sample results were given as a mean with standard deviation values are assurance to the data.

Total phenolic content

P. granatum leaf extracts were tested for total phenolic content; the reaction was based on the Folin-Ciocalteu reagent used to identify the phenolic content. Gallic acid act as a positive control, the tested values were compared with the standard of gallic acid. The linear regression equation was found to be y = 0.176x + 0.162 while the correlation was found to be 0.995. The amount of phenolic content present in the extract in terms mg GAE/g of aqueous extract was found to be 80.227 ± 0.1205 in methanol extract it shows 53.40± using the above linear regression equation it shows in Table 2.

Total flavonoid content

P. granatum extracts the compounds such as flavonoid; it is responsible for the functional group of hydroxyl and considerable to human nutrition. Quercetin acts as a positive control, the sample values were compared with the standard of quercetin. The linear regression equation was found to be y = 0.161x + 0.116, whereas the correlation was found to be 0.991. The amount of flavonoid content present in the *P. granatum* extracts in terms milligram quercetin equivalent/gram of extract was found in Table 3. It showed the values of aqueous extracts 41.80 ± 0.0151 and methanol extract 38.07 ± 0.0261 using the above linear regression equation.

Antioxidant activity

DPPH radical scavenging activity

DPPH is a very stable free-radical, and it has an unpaired electron. In appearance, DPPH is purple (absorbed at 517 nm). When the free-radicals have been scavenged by DPPH, it will generate a purple. This reduction in color is measured with the UV-spectrophotometer. In this assay, among the two extracts, the aqueous extract showed increased antioxidant activity than methanol extract of *P. granatum* leaf. DPPH scavenging activity of extracts of *P. granatum* is summarized in the Fig. 2; Different concentrations (50-250 µg/ml) of plant extracts was also used to determine the antioxidant activity. From the above mentioned concentrations, the concentration of µg/ml showed the highest scavenging activity 86.72% and 78.62% in aqueous extract and methanol, respectively, extracts showing good antioxidant activity when compared to standard ascorbic acid showed 92.46%. Difference in antioxidant activity between the extracts was mostly credited to the presence of various phytochemical compounds.

Hydrogen peroxide scavenging activity

The antioxidant properties of the samples were assessed by the most renowned method as an H_2O_2 scavenging activity. In inside the cell, once hydrogen peroxide cross cell membrane and it can react with Fe2+ and Cu2+ ions to form OH, this may be the basis of various toxic effects. A few enzymes have a feeble oxidizing agent that directly inactivates the hydrogen peroxide, usually by oxidation of essential thiol (– SH) groups. Therefore, removing of H_2O_2 as well as O^{2-} is extremely essential for protection of food system [38]. Whereas medicinal plants have a capacity to reduce H_2O_2 into water the compounds can donate an electron. The electron donation potential and antioxidant activity is directly proportional. From the results, it shows that the H_2O_2 scavenging activity of the sample is significant compared to that of the ascorbic acid Fig. 3. The scavenging activity of aqueous and methanol extracts of *P. granatum* at five different concentrations (50, 100, 150, 200, and 250 µg/ml) on H_2O_2 was expressed like percentage inhibition and compared to the standard of ascorbic acid. Extracts were able to neutralize H_2O_2 in different concentrations. The results showed in the higher concentration of 250 µg/ml 67.07% and 55.25% aqueous and methanol extract, respectively. It was clearly that the extracts showed a significant radical scavenging activity in dose-depended method while compared to the standard of ascorbic acid 86.44%. The compounds which were presents in the extracts; it shows good electron donors and may reduction of H_2O_2 to H_2O.

Table 1: Qualitative screening analysis of *Punica granatum*

S. No.	Phytochemical	Qualitative analysis	Results	
			Aqueous extract	Methanol extract
1	Alkaloids	Mayer's test	+	+
2	Carbohydrates	Benedict's test	++	+
3	Proteins and amino acids	Lead acetate test	+	+
4	Glycoside	Borntrager's test	+	+
5	Saponins	Foam test	++	+
6	Steroids	Salkowski test	+++	++
7	Phenolic	Lead acetate test	+++	++
8	Tannins	Ferric chloride test	+++	++
9	Flavonoids	Sodium hydroxide test	+++	++

+++: Highly present, ++: Moderately present, +: Low. *P. granatum: Punica granatum*

Table 2: Estimation of total phenolic content

Concentration of gallic acid in µg/ml	Absorbance at 765 nm	Amount of total phenolic content in terms mg GAE/g of extract	
		Aqueous extract	Methanol extract
20	0.318±0.0117		
40	0.525±0.0313		
60	0.71±0.0150	80.227±0.1205	53.40±0.0085
80	0.873±0.100		
100	1.024±0.043		

GAE/g: Gallic acid equivalent/g

Table 3: Estimation of total flavonoids

Concentration of quercetin in µg/ml	Absorbance at 405 nm	Amount of total flavonoids content in terms milligram quercetin/gram of extract	
		Aqueous extract	Methanol extract
20	0.318±0.026		
40	0.423±0.0186		
60	0.637±0.066	41.80±0.0151	38.07±0.0261
80	0.778±0.038		
100	0.903±0.030		

Total antioxidant assay is based on the formation of the phosphate complex during the reduction of molybdate VI to V at acidic pH. This was quantified later using a spectrophotometer at 695 nm. The total antioxidant capacity of the *P. granatum* was determined by the method of [28,39] five different concentrations of the sample were absorbed, and it shows increasing concentrations of total antioxidant activities at 50-100 µg/ml. The extracts of *P. granatum* give the increase concentration; it shows in the (Fig. 4) and it compared with the standard of ascorbic acid.

This assay is based on the formation of the phosphomolybdenum complex was calculated by the total antioxidant activity of *P. granatum* leaf extracts. This activity was measured spectrophotometrically at 695 nm. Concentration in 250 µg/ml showed the highest activity 56.26% and 48.43% in aqueous and methanol extracts, respectively. It is showing good antioxidant activity when compared to standard ascorbic acid 63%.

Reducing power

Reducing power of the *P. granatum* extracts serves as a thought of its antioxidant activity; it was determined using customized Fe3+ and Fe2+ reduction method, which can be observed at 700 nm. While the yellow of the sample solution changed to different shades of green and blue, it depending on the reduction power of the extracts. Results showed (Fig. 5) the good antioxidant in the sample, the reason the reduction of Fe3+/ferricyanide complex to form Fe2+. The different extracts show a status of electron-donating capacity in a concentration-dependent method [40]. In the present study, extracts of *P. granatum* leaves give a better reducing power, in which there is a firm increase in reducing the potential of *P. granatum* with increasing concentrations. This result shows in the Fig. 5, reducing the power of samples were found to be range between (50 and 250 µg/ml). The highest values show ranges are (250 µg/ml) 73.34% and 64.90% in aqueous and methanol extracts, respectively. Results showed the good antioxidant activity in the sample and compared to standard of ascorbic acid it shows 85.43%.

Beta-carotene assay

The antioxidant activity of the leaf extracts was estimated with beta-carotene bleaching assay. This method is based on the loss of yellow due to the oxidation of linoleic acid [41,42]. Linoleic acid was attacked the unsaturated beta-carotene, and the presence of antioxidants can hold back the amount of beta-carotene by neutralizing the free-radicals of linoleate, and some other radicals are produced in the scheme. Absorbance is reduced in the samples devoid of antioxidant. While in the presence of antioxidant the color taken for a long time. The capability of *P. granatum* extracts to prevent oxidation of the beta-carotene linoleate Fig. 6. The activity was high in aqueous extract than the methanol extract. Carotenoids are classified along with the essential constituents of the antioxidative effect. Beta-carotene bleaching is able to slow down in the presence of antioxidants. Five different concentrations (50-250 µg/ml) of extracts were used. The concentration of 250 µg/ml showed the highest scavenging activity 93.75% and 84.91% in aqueous extract and methanol, respectively, extracts showing good antioxidant activity. Capability of *P. granatum* extracts to prevent the oxidation of beta-carotene-linoleate.

***In vivo* antioxidant activity**

Extract of *P. granatum* has a significant *in vivo* antioxidant activity by increasing the percentage endurance of yeast cells against a hydrogen peroxide used as a stressing agent [43]. The results show in the Fig. 7 represented the percentage endurance of yeast cells was measured in triplicates. After 48 hrs incubation, the number of colonies was observed. The treated and untreated yeast cells in the presence of stressing agent H_2O_2 was compared to the stressed yeast cells. *P. granatum* leaf extracts showed more percentage survival of the yeast cells when compared to that of negative plate. Hence, the current study resulted in the antioxidant activity of leaf extracts.

CONCLUSION

The antioxidant activity and phytochemical screening analysis of two different extracts of *P. granatum* were evaluated. Different methods of antioxidant activity were effectively applied to evaluate the assays of *P. granatum* extracts; it shows the similar significant radical scavenging

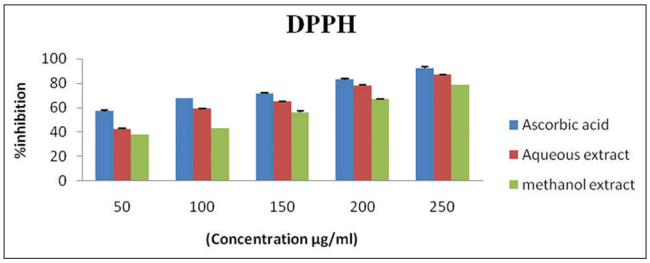

Fig. 2: 2, 2-diphenyl 2-picrylhydrazyl assay with different solvents from *Punica granatum*

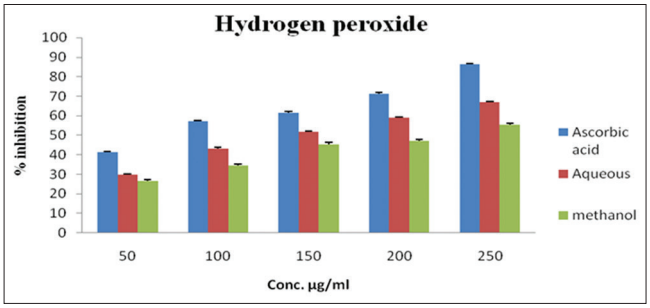

Fig. 3: H_2O_2 radical scavenging activity of different solvent extracts from *Punica granatum*. Total antioxidant activity

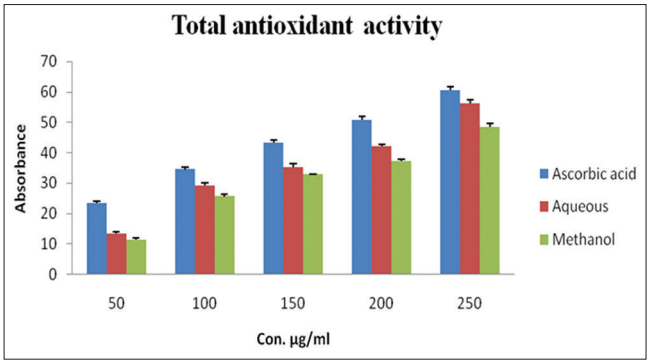

Fig. 4: Total antioxidant activity of leaf extracts of *Punica granatums*

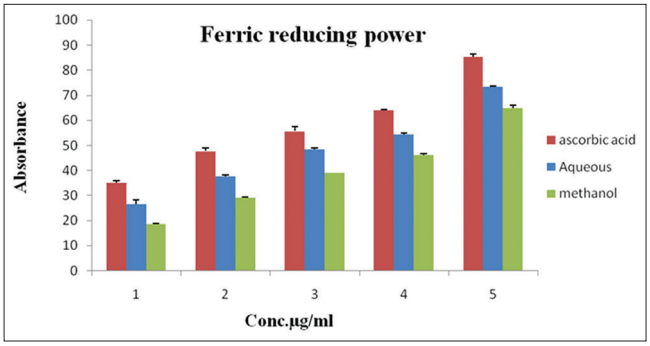

Fig. 5: Ferric reducing power of leaf extracts of *Punica granatum*

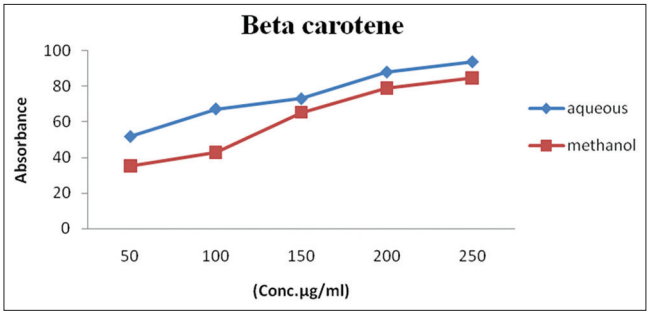

Fig. 6: Beta-carotene assay

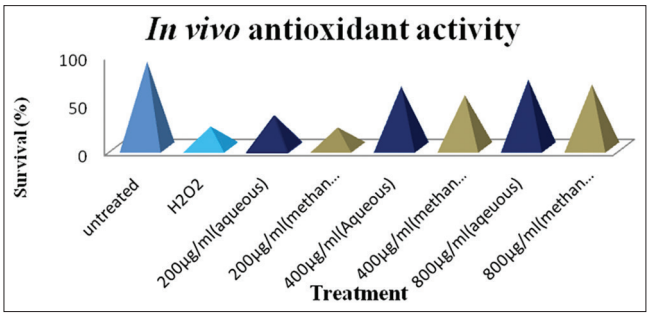

Fig. 7: *In vivo* antioxidant activity of leaf extracts of *Punica granatum*

activity against the hydrogen peroxide stress induced in the culture. *P. granatum* leaves contain a potential antioxidant activity and are capable of scavenging ROS. The results that compared to methanol extract and aqueous extract have a high capability of antioxidants. This may be accredited to the high phenolic content as a linear relation was noticed. The present study may provide a scientific origin for the traditional use of this plant and may endow with the valuable idea for a complete study on the bioactive compound that contributes to these biological properties and also their possible mechanism of action are suggested. A further study on characterization and isolation of bioactive compounds from medicinal plant extracts is being investigated.

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