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Research Article

DETERMINATION OF ANTIOXIDANT CAPACITY AND GALLIC ACID CONTENT IN ETHANOLIC EXTRACT OF *PUNICA GRANATUM L.* LEAF

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ABSTRACT

Objective: The present research work was carried out to evaluate the antioxidant potential of ethanolic extract of *Punica granatum* leaf (EPGL) that belongs to the family of *Punicaceae* and determine its gallic acid (GA) content using chromatography method.

Methods: Six complementary test systems, namely, 1,1-diphenyl 2-picryl hydrazine (DPPH), hydrogen peroxide (H_2O_2), superoxide (SO), nitric oxide (NO), hydroxyl (OH) radical scavenging, and reducing power activities were analyzed for determining antioxidant activity of EPGL. The simple and novel chromatography techniques such as thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) were used for the detection and quantification of GA in EPGL.

Results: IC_{50} values of EPGL were found to be 136 µg/ml for DPPH, 88.5 µg/ml for H_2O_2, 16.8 µg/ml for SO, 96.5 µg/ml for NO, and 143 µg/ml for OH. The ascorbic acid (AA) and GA were used as standard compounds. The absorbance of EPGL in reducing power assay was found to be 0.18 at 100 µg/ml, while AA and GA absorbance was found to be 0.24 and 0.4 at the same concentration. The amount of GA in EPGL was found to be 1.189 mg/g.

Conclusion: These findings suggested that EPGL could be a potential source of natural antioxidant, and HPLC method used for the determination of GA is simple, precise, accurate, and suitable for routine analysis of GA in EPGL.

Keywords: *Punica granatum*, Free radical scavenging, Oxidative stress, Chromatography techniques, Gallic acid.

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INTRODUCTION

Free radicals are molecules containing one or more unpaired electrons in atomic or molecular orbitals. There is increasing evidence that the abnormal production of free radicals leads to increased oxidative stress on cellular structures which causes a change in molecular pathways that pave the pathogenesis of several important diseases such as cardiovascular diseases, neurological diseases, and cancer and in the process of physiological aging [1]. All human cells protect themselves against free radical damage by enzymes such as superoxide dismutase and catalase or compounds such as ascorbic acid (AA), tocopherols, and glutathione [2]. Sometimes, these mechanisms are disrupted by various pathological processes, and therefore, antioxidant supplements are vital to combat oxidative damage. In recent years, natural antioxidants, particularly, those present in fruits and vegetables have gained increasing interest among consumers and in the scientific community. Epidemiological studies have demonstrated that frequent intake of fruits and vegetables is associated with a lower risk of age-related diseases such as coronary heart diseases and cancer [3,4]. Recently, much attention has been directed toward the development of ethnomedicines with strong antioxidant properties but low cytotoxicity. One important aspect of the quality control of herbal medicinal products is the determination of the chemical marker, which consists of a constituent (or groups of constituents) that is chemically defined and present in the plant product. These constituents may or may not be related to their pharmacological activity [5]. While several different analytical techniques can be used to identify and quantify these substances, we used high-performance liquid chromatography (HPLC), which is the most commonly used method [6].

Punica granatum L., commonly known as pomegranate, is a fruit-bearing deciduous shrub or small tree, native to Asia, and belongs to the family *Lythraceae* [7]. Different parts of the plant such as bark, leaves, immature fruits, and fruit rind have medicinal significance [8]. *P. granatum* has been extensively used as a traditional medicine in many countries for the treatment of dysentery, diarrhea, helminthiasis, acidosis, hemorrhage, and respiratory pathologies [9]. In addition, this plant is reported to have excellent antibacterial, antifungal, and antiprotozoal activity [10-12].

We have reported the phytochemical content of *P. granatum* leaf extract [13]. Therefore, the present study was undertaken with the objectives to assess the antioxidant potential of the ethanolic extract of *P. granatum* L. Leaf (EPGL) using different standard methods and to develop and validate an analytical method using thin-layer chromatography (TLC) and high performance liquid chromatography for the identification and quantification of the chemical marker, namely, gallic acid (GA) in EPGL.

METHODS

Collection and identification of plant materials

P. granatum (*Punicaceae*) leaves were collected on August 2016 from around the Sathyavedu village, Andhra Pradesh, India, and was identified by Dr. P. Jayaraman, Director of National Institute of Herbal science, Plant anatomy research center, Chennai (PARC/2017/3381).

Preparation of leaf extracts

The leaf samples were shade dried at room temperature and then ground to a fine powder in a mechanical grinder. The powdered material was then extracted using a solvent ethanol in the ratio 1:10 using Soxhlet apparatus. After extracting all coloring material, the solvent was removed by evaporating in a water bath, which gives rise to a solid mass of the extract. The percentage yield of the extract was found to be 12%.

Chemicals

GA was purchased from Sigma-Aldrich (Chennai, India). HPLC grade water, methanol, acetonitrile, and orthophosphoric acid from Merck Specialities Private Ltd. (Mumbai) were used in this study. All other chemicals and reagents used were commercially available purity.

Preparation of standards

In vitro antioxidant activity of ethanolic extract of *P. granatum* Leaf (EPGL) was studied by comparing with the standards - AA and GA. A stock solution of AA and GA was prepared at a concentration of 1 mg/ml. From the stock solution, required concentrations of AA and GA were prepared.

Statistical analysis

Data were expressed as mean±standard deviation for three parallel measurements. The 50% inhibitory concentration (IC_{50}) was calculated from the dose-response curve (Graph Pad Prism Version 6.0) by plotting the percentage of inhibition versus concentrations.

Percentage of inhibition=Absorbance of control−Absorbance of test sample÷Absorbance of control×100

The IC_{50} value is defined as the concentration (µg/ml) of dry extract that inhibits the formation of free radicals by 50%.

1,1-diphenyl 2-picryl hydrazine (DPPH) radical scavenging assay

Method of Singh *et al.* [14] was adopted for the determination of DPPH free radical scavenging activity. DPPH (7.886 mg) was accurately weighed and dissolved in 100 ml of methanol to obtain 200 µmol solutions of DPPH. EPGL was prepared with five concentrations, namely, 50, 100, 150, 200, and 250 µg/ml. 2ml of methanol solution of DPPH shaken vigorously with 2 ml of sample solution and allowed to stand at room temperature for 15 min in the dark. The absorbance of the reaction mixture was measured spectrophotometrically at 517 nm. Different concentrations of AA and GA (20, 40, 50, 80, and 100 µg/ml) were used as reference compounds. The percentage of DPPH scavenging of both the EPGL and standard compounds (AA and GA) was calculated.

Hydrogen peroxide radical (H_2O_2) scavenging assay

H_2O_2 scavenging ability of EPGL was determined according to the method of Ebrahimzadeh *et al.* [15]. Standard AA and GA were prepared at concentrations of 20, 40, 50, 80, and100 µg/ml, whereas EPGL was prepared at concentrations of 50, 100, 150, 200, and 250 µg/ml. A solution of H_2O_2 (40 millimol) was prepared in phosphate buffer (pH 7.4). To 1 ml of standards and 1 ml of EPGL solution at various concentrations, 3.4 ml of phosphate buffer and 0.6 ml of H_2O_2 were added. The absorbance of H_2O_2 at 230 nm was determined after 10 min against the blank solution containing the phosphate buffer without H_2O_2. 1 ml of phosphate buffer and 0.6 ml of H_2O_2 were used as a control. Reactions were carried out in triplicate spectrophotometrically. The percentage of H_2O_2 scavenging of both the EPGL and standard compounds (AA and GA) was calculated.

Superoxide (SO) radical scavenging assay

The SO radical scavenging activity of the EPGL was studied using the method of Liu *et al.*[16]. SO radicals are generated in phenazine methosulfate-reduced nicotinamide adenine dinucleotide (PMS-NADH) systems by oxidation of NADH and assayed by the reduction of nitro blue tetrazolium (NBT). About 200 µl of EPGL at different concentrations were taken in a series of test tubes. SO radicals were generated by 1 ml of Tris-hydrochloride (Tris-HCl) buffer (16 millimol, pH-8), 1 ml of NBT (50 µmol), 1 ml of NADH (78 µmol) solution, and 1 ml of PMS (10 µmol). The reaction mixture was incubated at 25°C for 5 min and the absorbance was measured at 560 nm. A control tube containing Tris-HCl buffer was also processed in the same way without EPGL and standards. Different concentrations of AA and GA were used as reference compounds. The inhibition percentage of SO anion generation was calculated.

Nitric oxide (NO) radical scavenging assay

NO radical scavenging activity was measured spectrophotometrically according to the method described by Govindharajan *et al.*[17]. When sodium nitroprusside was mixed with an aqueous solution at physiological pH, suddenly it generates NO, which reacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. NO scavengers compete with oxygen, which leads to reduced production of nitrite ions. About 1 ml of sodium nitroprusside (5 millimol) in phosphate buffer (pH 7.4, 0.1 mol) was mixed with different concentrations (50, 100, 150, 200, and 250 µg/ml) of the EPGL in phosphate buffer (pH 7.4, 0.1 mol). The tubes were then incubated at 25°C for 2 h. After incubation, 1.5 ml of the reaction mixture was taken and diluted with 1.5 ml of Griess reagent (1% sulfanilamide, 2% O-phosphoric acid, and 0.1% of N-[1- naphthyl ethylenediamine dihydrochloride]). The absorbance of the chromophore formed during diazotization of the nitrite with sulfanilamide and subsequent coupling with N-(1- naphthyl ethylenediamine dihydrochloride) was measured spectrophotometrically at 546 nm. Control tube was maintained with all chemicals excluding EPGL and standards. The AA and GA were used as reference standards. The percentage inhibition of NO anion generation was calculated.

Hydroxyl (OH) radical scavenging assay

The OH radical scavenging activity of EPGL was measured according to the method of Halliwell *et al.*[18]. Stock solutions of ethylenediaminetetraacetic acid (EDTA) (1 millimol) were prepared in dimethyl sulfoxide, whereas ferric chloride ($FeCl_3$) (10 millimol), AA (1 millimol), H_2O_2 (10 millimol), and deoxyribose (10 millimol) were prepared in deionized water. The method was carried out by adding 100 µl of EDTA, 10 µl of $FeCl_3$, 100 µl of H_2O_2, 360 µl of deoxyribose, 1000 µl of the EPGL (50–250 µg/ml), 330 µl of phosphate buffer (50 millimol, pH 7.4), and 100 µl of AA. This mixture was then incubated at 37°C for 1 h. About 1 ml of incubated mixture was mixed with 1 ml of 10% trichloroacetic acid (TCA) and 1 ml of 0.5% thiobarbituric acid (TBA) (in 0.025 mol sodium hydroxide containing 0.025% butylated OH anisole), and the development of pink chromogen was measured spectrophotometrically at 532 nm. The AA and GA were used as standards. The inhibition percentage of OH anion was calculated.

Reducing power assay method

The reducing power of EPGL was determined by the method of Oyaizu [19]. Different concentrations of EPGL (50, 100, 150, 200, and 250 µg/ml) and standards (20, 40, 50, 80, and 100 µg/ml) were prepared, and 1 ml of each solution was mixed with 2.5 ml of phosphate buffer (0.2 mol, pH 6.8) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min. To this mixture, 2.5 ml of 10% TCA was added and then centrifuged at 3000 rotation per minute for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and 0.1% $FeCl_3$ (0.5 ml), and the absorbance was measured at 700 nm. The AA and GA were used as standards.

TLC and HPLC Profiling of PGLE

TLC

Chromatography was performed as described by Marston *et al.*[20] on 10×10 cm precoated silica gel 60F 254 TLC plate and 5 µl of standard and test solution (concentration 1 mg/ml) at 25 mm distance to the TLC plate was applied. Thereafter, the plate was developed using optimized solvent system chloroform, methanol, and formic acid (FA) (7:2:1) as mobile phase in a glass chamber which was previously saturated for 10 min. The plate was developed up to 8 cm and development time was 50 min. Then, the developed plate was dried at 60°C for 15 min and sprayed with 1% methanolic $FeCl_3$ reagent. Identification of GA in EPGL and comparison of retardation factor value with the standard were noted.

HPLC

Quantification of GA was carried out using Shimadzu 20 AD, HPLC system (Shimadzu, Japan) consisted of ultraviolet (UV) detector, a binary pump, a 20 µl injection loop, and a RP-18 column of dimensions 4.6×250 mm. The mobile phase used for GA was water (80%) and acetonitrile (20%) with pH is maintained at 3.0 using O-phosphoric acid at a flow rate of 1.0 ml/min. The eluted samples were detected by UV detector at 272 nm. Stock solutions of standard GA and EPGL were prepared in methanol as 50 µg/ml and 1 mg/ml, respectively. EPGL and GA as well as the mobile phase were degassed and filtered through a membrane filter (0.45 millipore). The amount of GA in EPGL was determined by comparing the retention time with the standard GA [21].

RESULTS AND DISCUSSIONS

In living systems, free radicals are constantly generated and they can cause extensive damage to tissues and biomolecules leading to various disease conditions, especially degenerative diseases and extensive lysis [22]. Many synthetic drugs protect against oxidative damage, but they have diverse side effects. An alternative solution to the problem is to consume natural antioxidants from food supplements and traditional medicines [23]. Plants rich in secondary metabolites, including phenols, flavonoids, and carotenoids, have antioxidant activity due to their redox properties and chemical structures [24,25].

DPPH free radical scavenging activity

The 1,1-diphenyl 2-picryl hydrazine (DPPH) scavenging ability of EPGL was 14.8±0.4 to 77.7±0.4% at concentrations of 50–250 µg/ml. The scavenging activity of AA and GA was 36.2±2 to 86.9±0.8% and 29.6±0.25 to 75.86±0.25% at concentrations of 20–100 µg/ml which were used as reference compounds. Fig. 1 shows the dose-dependent DPPH scavenging activity of EPGL, AA, and GA with an IC_{50} value of 136 µg/ml, 30.5 µg/ml, and 38.5 µg/ml. Our results (IC_{50}=136 µg/ml) were similar to the reports given by Zhang *et al.*(IC_{50}=0.14 mg/ml) and Gheith and El-Mahmoudy (IC_{50}=113 µg/ml) [26,27]. DPPH is usually used as a reagent to evaluate free radical and accepts an electron or hydrogen to become a stable diamagnetic molecule [28]. The decrease in absorbance of DPPH radical scavenging caused by antioxidant was due to the scavenging of the radical by hydrogen donation. It is visually noticeable as a color change from purple to yellow.

H_2O_2 scavenging assay

The ability of the EPGL to scavenge H_2O_2 is shown in Fig. 2 and compared with that of AA and GA as reference compounds. The IC_{50} value of the EPGL (88.5 µg/ml) was more than that of the reference compounds, AA (16.5 µg/ml) and GA (42.5 µg/ml). At 250 µg/ml, EPGL showed 67±0.2% of inhibition, whereas Vinodhini *et al.* reported methanolic extract of PGL 55.25% of inhibition at the same concentration [29]. Biological systems can produce H_2O_2. H_2O_2 can be formed *in vivo* by several oxidizing enzymes such as SOD. It can cross membranes and may slowly oxidize a number of compounds. It is used in the respiratory burst of activated phagocytes [30]. It is therefore biologically advantageous for cells to control the amount of H_2O_2 that is allowed to accumulate. The ability of EPGL to scavenge H_2O_2 may be attributed to the presence of phenols and tannins, which could donate electrons, thereby neutralizing it into water.

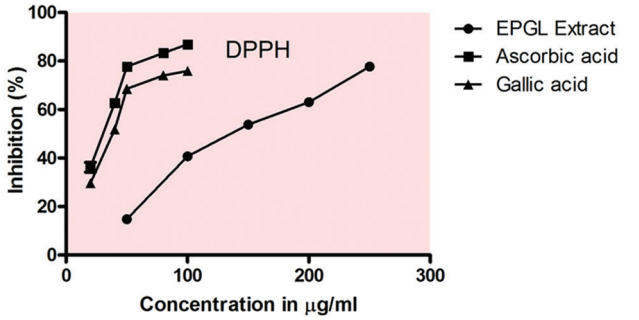

Fig. 1: 1,1-diphenyl -2-picrylhydrazyl radical scavenging activity of ethanolic extracts of *Punica granatum* Leaf, ascorbic acid, and gallic acid. The values are expressed in mean±standard deviation (n=3)

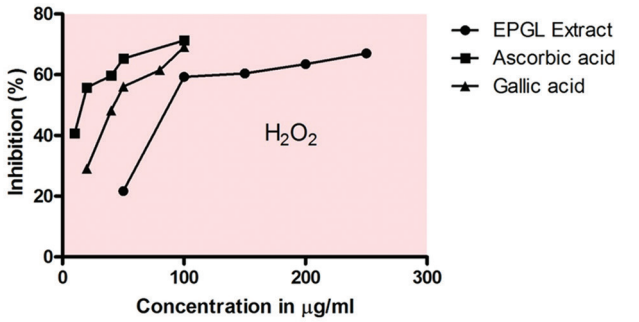

Fig. 2: Hydrogen peroxide scavenging activity of ethanolic extracts of *Punica granatum* Leaf ascorbic acid, and gallic acid. The values are expressed in mean±standard deviation (n=3)

SO radical scavenging activity

The SO scavenging activity of EPGL (IC_{50} - 16.8 µg/ml) was more than AA (IC_{50} - 23.8 µg/ml) but less than GA (IC_{50} - 3.25 µg/ml). However, Rummun *et al*. proposed IC_{50} value of methanolic extract of *P. granatum* leaves as 0.072±0.001 mg/ml which is more than we value [31]. The EPGL at a concentration of 5 µg/ml showed 17.7%, whereas in 100 µg/ml concentration, the percentage of inhibition was recorded as 86.7% (Fig. 3). This clearly indicates the inhibition activity was dose dependent. SO anion plays an important role in the formation of other reactive oxygen species such as H_2O_2, OH radical (OH), and singlet oxygen, which induce oxidative damage in lipids, proteins, and deoxyribonucleic acid [32]. It has been reported that antioxidant properties of some flavonoids are effective mainly through scavenging of the SO anion radical [33]. As shown in Fig. 3, the decrease in absorbance by antioxidants indicates consumption of SO anion in the reaction mixture. The results suggested that the EPGL is a more potent scavenger of SO radical than the standard AA but less than GA.

NO scavenging activity

The NO scavenging activity of the EPGL was 31.5±3% of the minimum concentration of 50 µg/ml, whereas maximum activity was 75.6±1.5% at 250 µg/ml. Our IC_{50} value (96.5 µg/ml) of EPGL was in near agreement with IC_{50} values (55.65±0.72 µg/ml) given by Hossain *et al.* [34], whereas IC_{50} values of AA and GA were 29.5 µg/ml and 5.5 µg/ml, respectively. Fig.4 shows that the percentage of inhibition was increased with increasing concentration of the EPGL. However, the activity of AA and GA was more pronounced than that of the EPGL. *In vitro* inhibition of NO (NO) radical is a measure of antioxidant activity of plant drugs. NO plays an important role in various inflammatory processes, but the overproduction of NO contributes to various diseases. The toxicity of NO increases greatly when it reacts with SO radical, forming the highly reactive peroxynitrite anion [35]. EPGL effectively reduced the generation of NO from sodium nitroprusside. The degree of inhibition of the NO free radicals was found to be increased with increasing concentrations of the EPGL extracts, and this indicates that the EPGL may contain compounds capable of inhibiting the generation of NO and offers scientific evidence for the use of EPGL in the treatment of various diseases.

OH radical scavenging activity

Fig. 5 showed the abilities of the EPGL and standards (AA and GA) to inhibit OH radical-mediated deoxyribose degradation of ferric

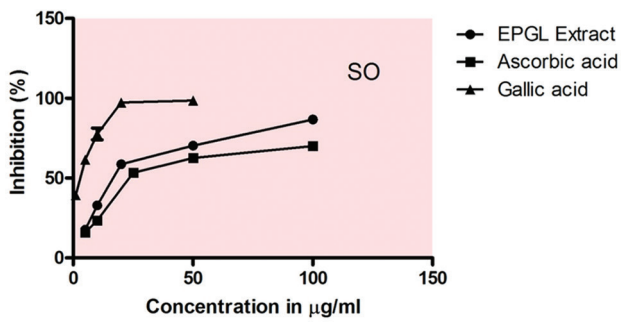

Fig. 3: Superoxide scavenging activity of ethanolic extracts of *Punica granatum* Leaf, ascorbic acid, and gallic acid. The values are expressed in mean±standard deviation (n=3)

(Fe^{3+}) -EDTA - AA and H_2O_2 reaction mixture. The IC_{50} values of the EPGL, AA, and GA in this assay were 143 µg/ml, 32.5 µg/ml, and 8.5 µg/ml, respectively. However, Gheith and Mahmoudy reported IC_{50} of hydromethanolic extract of PGL as 79.67±0.03 µg/ml [27]. At 100 µg/ml, the inhibition percentage values were 33.6%, 78.6%, and 99.2% for EPGL, AA, and GA, respectively. The OH radical is regarded as a detrimental species in pathophysiological processes and capable of damaging any molecule of the biological system and thus contributes to carcinogenesis, mutagenesis, and cytotoxicity [36]. As mentioned in the methods section, OH radicals were produced by incubating ferric-EDTA with AA and H_2O_2 at pH 7.4 and reacted with 2-deoxy-2-ribose to generate a malondialdehyde (MDA) like product. This compound forms a pink chromogen on heating with TBA at low pH. When EPGL was added to the reaction mixture, it removed the OH radicals from the sugar and prevented the reaction. The IC_{50} indicates that the plant extract has good OH radical scavenging ability, but lesser than the standard AA and GA.

Reducing power assay

An increase in absorbance indicates an increase in the antioxidant activity. The EPGL showed increased absorbance as the concentration increased. At 100 µg/ml, EPGL showed 0.18 absorbance, while AA and GA showed 0.24 and 0.4 absorbance at the same concentration (Table 1).

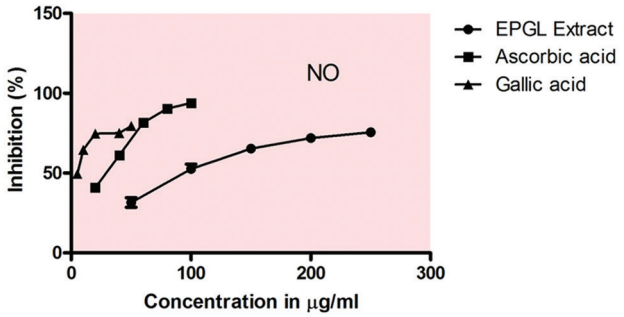

Fig. 4: Nitric oxide activity of ethanolic extracts of *Punica granatum* Leaf, ascorbic acid, and gallic acid. The values are expressed in mean±standard deviation (n=3)

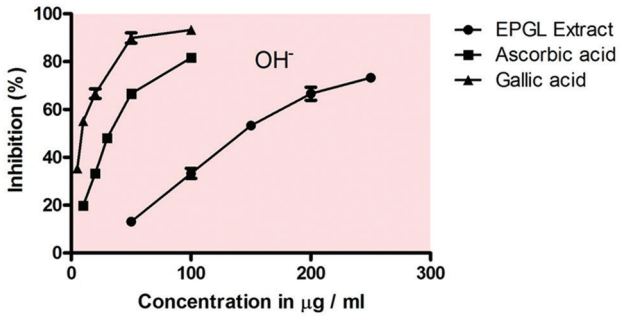

Fig. 5: Hydroxyl radical scavenging activity of ethanolic extracts of *Punica granatum* Leaf, ascorbic acid, and gallic acid. The values are expressed in mean±standard deviation (n=3).

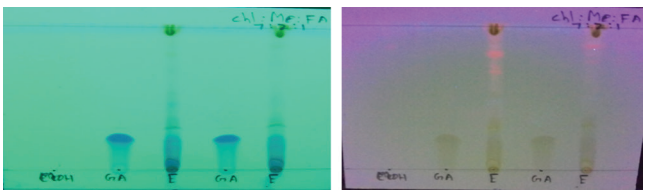

Fig. 6: Thin-layer chromatography of gallic acid and ethanolic extract of *Punica granatum* Leaf in short ultraviolet (UV) (254 nm) and long UV (365n m), respectively, with the mobile phase composition chloroform:methanol:Formic acid =7:2:1

However, Hossain *et al*. proposed that EPGL showed 2.015±0.09 absorbance at 100 µg/ml which was much more than our absorbance [34]. Compounds by reducing power can reduce the oxidized intermediates of lipid peroxidation processes so that they can act as primary and secondary antioxidants [37]. In the reducing power assay, the presence of antioxidants in the samples would result in the reduction of ferric ($Fe3^+$) to ferrous (Fe^{2+}) by donating an electron. Amount of Fe^{2+} complex can be monitored by measuring the formation of Prussian blue at 700 nm indicating an increase in reducing power [38]. Therefore, by measuring the formation of Perl's Prussian Blue at 700 nm, we can monitor the Fe^{2+} concentration. A high absorbance value of the sample indicates its strong antioxidant activity [39]. In the present study, the reducing power of EPGL was found to be excellent and steadily increased in direct proportion to the increasing concentrations of the EPGL.

From the above observation, we concluded that, except DPPH scavenging activity, other antioxidant activities of EPGL showed the difference when compared to other works. This difference may be due to the method and solvent adopted for extraction and in addition harvest time.

TLC and HPLC analysis

The result of TLC analysis using chloroform, methanol, and FA solvent mixture confirmed the presence of GA in EPGL (Fig. 6). The HPLC analysis of EPGL showed the presence of GA and other important peaks of phenols and flavonoids, which are reported to have antioxidant activity and these findings, are in concordance with our results. Quantitative HPLC studies show that the EPGL contained GA (1.189 mg/g) in good quantity with retention time in minute (*Rt* value) recorded was compared to standard GA (Figs. 7 and 8). The precision as well as the reproducibility of this method was satisfactory.

CONCLUSION

Nowadays, our knowledge about the role played by the free radicals in metabolic diseases such as diabetes, liver disease, and hypertension is significantly increased. The present study indicates that the EPGL possesses antioxidant properties and could serve as free radical inhibitor or scavenger or act as primary antioxidant. By measuring the antioxidant activity, we noticed that EPGL exhibited very high SO scavenging activity reaching over 86.7% at 100 µg/ml. The findings of the present study suggested that EPGL is a potential source of natural antioxidant that could have great importance as therapeutic agent in preventing or slowing the progress of aging and age-associated oxidative stress-related

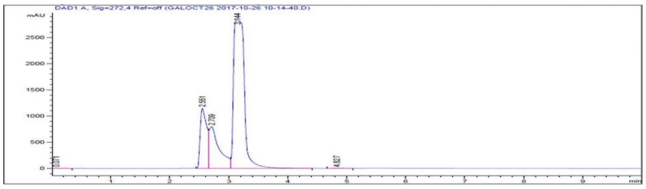

Fig. 7: Chromatogram of gallic acid was analyzed using high-performance chromatography with mobile phase composition 80%water:20% acetonitrile at pH 3

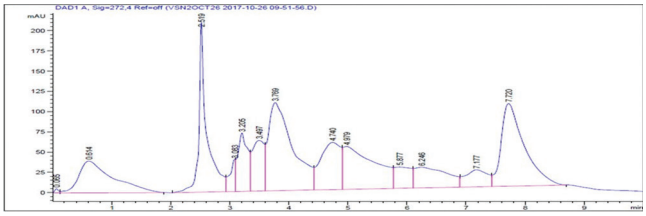

Fig. 8: Chromatogram of ethanolic extract of *Punica granatum* Leaf L. was analyzed using high-performance liquid chromatography with mobile phase composition 80% water:20% acetonitrile at pH 3.

Table 1: Reducing power of EPGL, AA, and GA

EPGL	Concentration in µg/ml	50	100	150	200	250
	Absorbance at 700 nm	0.09±0.01	0.18±0.015	0.24±0.005	0.29±0.02	0.33±0.01
	Concentration in µg/ml	20	40	50	80	100
AA	Absorbance at 700 nm	0.06±0.005	0.13±0.01	0.20±0.01	0.21±0.01	0.24±0.01
GA	Absorbance at 700 nm	0.10±0.01	0.19±0.01	0.28±0.02	0.33±0.01	0.40±0.01

Values are expressed in mean±standard deviation (n=3). EPGL: Ethanolic extract of *Punica granatum* Leaf, AA: Ascorbic acid, GA: Gallic acid

Table 2: Chromatography result of GA and EPGL

Parameter	GA	EPGL
Retention time (min)	3.14	3.205
Peak area	35949.5	854.9606
Area (%)	65.96	6.5756
Response factor (mAU*S)	718.99	1.10535
Amount of GA in EPGL=1.189 mg/g of extract		

EPGL: Ethanolic extract of *Punica granatum* Leaf, GA: Gallic acid, min: Minutes, mAU*s: Milli absorbance units per second.

degenerative diseases. Further, a simple, accurate, and reliable method was developed to determine the GA content in EPGL using HPLC. Thus, it is suitable for the quantification of GA in herbal medicine.

AUTHORS CONTRIBUTION

Mrs. P. Sreedevi (Research Scholar) carried out the experiments and has written manuscript. Dr. K. Vijayalakshmi (Principal Investigator) designed the work, proofread and validated the manuscript.

CONFLICT OF INTEREST

Declared None.

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