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Research Article

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Phytochemical evaluation by GC-MS and *in vitro* antioxidant activity of *Punica granatum* fruit rind extract

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ABSTRACT

The present study was carried out to identify the phytochemicals present in the Punica granatum a medicinally important plant of the Punicaceae family. In the present study the ethanolic rind extract of Punica granatum fruit has been subjected to GC-MS analysis and in vitro antioxidant assay. This analysis revealed that ethanolic extract of P. granatum Linn contains 3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one, Nitroisobutylglycerol, Ethyl .alpha.-d-glucopyranoside, 3H-indole-3-carbaldehyde (4-amino-5-methyl-4H-1,2,4-triazol-3-yl) hydrazone, Maltol etc., justifying the use of this plant to treat many aliments in folk and herbal medicine.

Keywords: *Punica granatum*, GC-MS, phytochemicals, antioxidant activity

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INTRODUCTION

Plants constitute an important source of active natural products which differ widely in terms of structures, biological properties and mechanisms of actions. *Punica granatum* L. (Punicaceae), commonly called pomegranate, is a large deciduous shrub or small tree native to the Mediterranean region and has been used extensively in the form of juice concentrate, canned beverage, jam and jelly, etc. [1]. It is also used medicinally in Europe, Indo-China, the Philippine Islands, North Africa, and South Africa. The plant is used in folklore medicine for the treatment of various diseases, such as ulcer, hepatic damage and snakebite [2]. The rind of the fruit is antihelminthic, useful in dysentery and ulcer, the plant also shows high antioxidant and antiartherogenic activity [3]. The whole plant, but in particular the bark, is antibacterial, antiviral and astringent. A decoction of seed is used to treat syphilis and juice is used to treat jaundice and diarrhea [4]. Tannins such as punicalagins have been identified as the primary components responsible for the reduction of oxidative stress. Pomegranate has been shown to reduce systolic blood pressure by inhibiting serum angiotensin-converting enzyme [5,6]. The peels a powerful astringent and cure for diarrhea and oral aphthae, dried pomegranate peels are decocted in water and employed both internally and externally for numerous problems demanding astringents and/or germicides, especially for aphthae and ulcers [7].

Experimental studies have demonstrated its analgesic, anthelmintic, antibacterial, antidiarroheal, antifertility, antifungal, anti-inflammatory, antimutagenic, antispasmodic, antiviral, hepatoprotective and hypoglycemic activities [8-11]. Pomegranate peel is used for treating the infection of male or female sexual organs, mastitis, acne, folliculitis, piles, allergic dermatitis, tympanitis and for the treatment of oral diseases [12].

In the light of the above information the present investigation was undertaken to evaluate the phytoconstituents by GC-MS and to assess the free radical scavenging property.

EXPERIMENTAL SECTION

Preparation of plant extract

The *Punica granatum* L fruits were obtained at a public market in Tiruchirappalli city. The peel of the fruit was removed and shade dried and powdered with the help of a hand grinding mill. About 200 g of the powder was exhaustively extracted with ethanol. The extract was concentrated to a residue. The crude extract was used for further investigation for its phytochemical compounds and potential antioxidant properties.

Antioxidant assay

Iron chelating activity (FRAP)

The method of Benzie and Strain [13] was adopted for the assay. The principle is based on the formation of *O*-Phenanthroline-Fe2+ complex and its disruption in the presence of chelating agents. The reaction mixture containing 1 ml of 0.05% O-Phenanthroline in methanol, 2 ml ferric chloride (200μM) and 2 ml of various concentrations ranging from 10 to 500μg was incubated at room temperature for 10 min and the absorbance of the same was measured at 510 nm. EDTA was used as a classical metal chelator.

DPPH scavenging assay

DPPH radical scavenging activity of *P. granatum* L fruit rind was determine according to Mensor et al [14]. An aliquot of 0.5 ml of sample solution in methanol was mixed with 2.5 ml of 0.5 mM methanolic solution of DPPH. The mixture was shaken vigorously and incubated for 37 min in the dark at room temperature. The absorbance was measured at 517 nm using UV spectrophotometer. Ascorbic acid was used as a positive control. DPPH free radical scavenging ability (%) was calculated by using the formula.

% of inhibition = absorbance of control – absorbance of sample / absorbance of control ×100.

Column chromatography

10 g of the crude extract was subjected to column chromatography over silica gel (100-200 mesh) and eluted with n-hexane, chloroform, ethanol and methanol respectively. n-Hexane and Chloroform did not elute much of the compounds. The ethanol fraction of the *Punica granatum* fruit rind was taken for GC-MS analysis.

Gas Chromatography- Mass Spectrum Analysis (GC-MS)

GC-MS technique was used in this study to identify the phytocomponents present in the extract.

GC-MS analysis of this extract was performed using GC SHIMADZU QP2010 system and gas chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with Elite-1 fused silica capillary column (Length : 30.0 m, Diameter : 0.25 mm, Film thickness : 0.25 μm Composed of 100% Dimethyl poly siloxane). For GC-MS detection, an electron ionization energy system with ionization energy of 70eV was used. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1.51ml/min and an injection volume of 1μl was employed (split ratio: 10). Injector temperature 240°C; Ion-source temperature 200°C. The oven temperature was programmed from 70°C (isothermal for 3 min.), with an increase of 300°C for 10 min. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds with scan range of 40 – 1000 m/z. Total GC running time was 35 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a GC MS solution ver .2.53.

Identification of components

Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technique (NIST08s), WILEY8 and FAME having more patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST08s, WILEY8 and FAME library. The name, molecular weight, molecular formula and structure of the component of the test material were ascertained.

Statistical analysis

All values are expressed as mean ± S.D. Statistical analysis were performed by Student's *t*-test. The values of p lower than 0.05 were considered significant (p is probability).

Fig.1

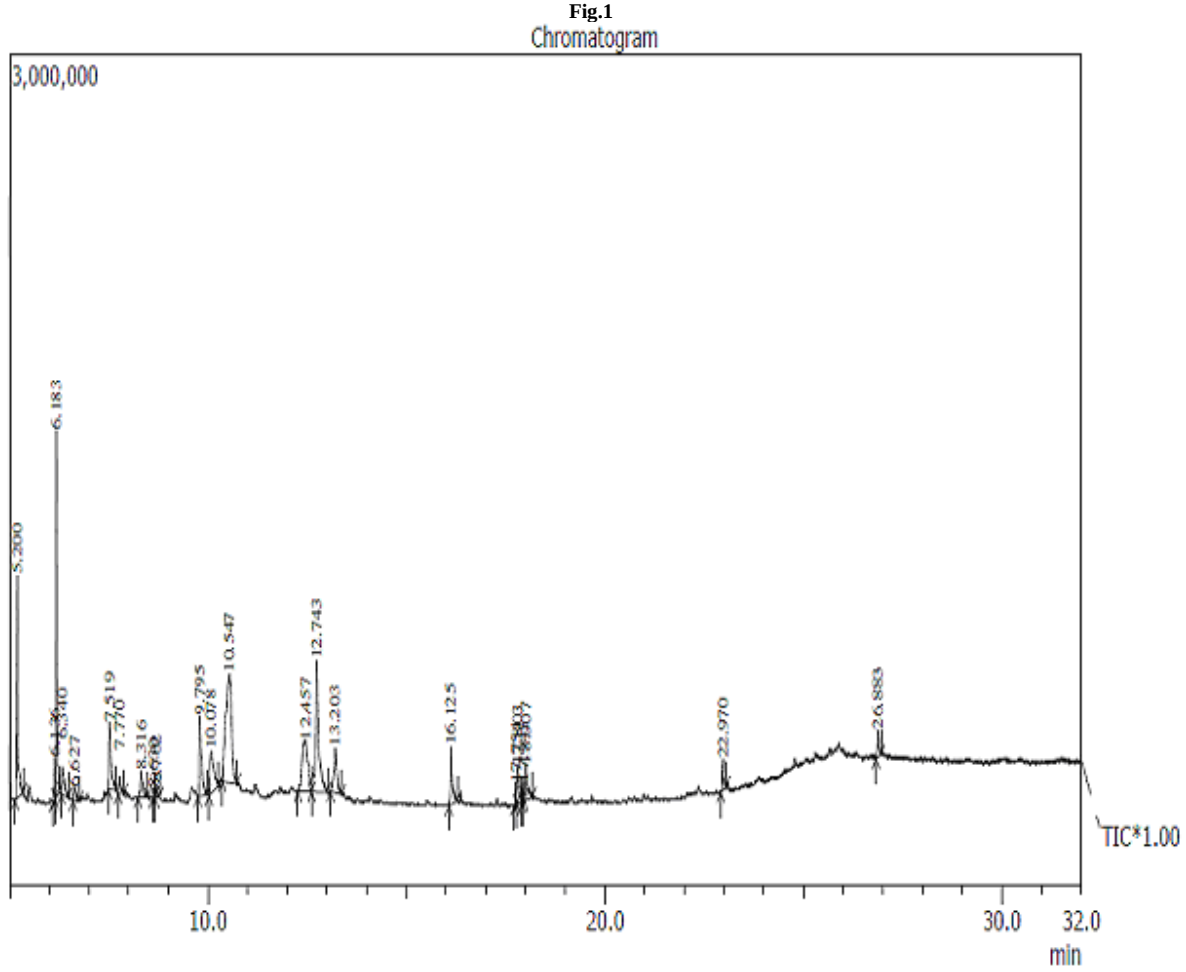


Table 1. Phytochemical components identified in the ethanolic extract of *P. granatum* by GC-MS

RT	Name	Peak area %
5.20	Maltol	9.46
6.136	Isopropylmethylnitrosamine	1.19
6.183	3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	11.83
6.340	Glycerol	2.01
6.627	4-Hydrodihydro-2(3H)-furnanone	0.88
7.519	5-Hydroxymethylfurfural	3.90
7.770	Glycerine monoacetate	0.80
8.316	4-hydroxy-3-methyl-2-butanone	1.93
8.630	3-methyldecanoic acid	0.38
8.702	2-ethtyl-3-nitroso-1,3-oxazainane	0.45
9.795	3H-Indole-3-carbaldehyde (4-amino-5-methyl-4H-1,2,4-triazol-3-yl) hydrazone	5.22
10.078	Pyrogallol	4.64
10.547	Nitroisobutylglycerol	19.02
12.457	4-deuterio-transs-3,4-dihydroxy-cyclopentene	9.41
12.743	Ethyl, alpha-d-glucopyranoside	12.65
13.203	4-O-methylmannose	4.12
16.125	Palmitic acid	3.00
17.754	10,12-hexadecadien-1-ol	0.76
17.803	Oleic acid	2.53
17.945	14-methyl-8-hexadecyn-1-ol	0.79
18.007	Stearic acid	2.38
22.970	9-octadecenamide	1.26
26.883	Gamma sitosterol	1.39

Table 2. Activity of phytocomponents identified in P. granatum rind extract by GC-MS

5.200	Maltol	Anticonvulsant, Antifatigue, Antioxidant, antitumour
6.136	Isopropylmethylnitrosamine	Antioxidant, antihyperglycemic
6.183	3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	Antimicrobial, anti-inflammatory, Antiproliferative.
9.795	3H-Indole-3-carbaldehyde (4-amino-5-methyl-4H-1,2,4-triazol-3-yl) hydrazone	Antioxidant, anticonvulsant, analgesic, Antibacterial, Antifungal
10.078	Pyrogallol	Antibacterial, Antidermatitic, Antimutagenic,Antioxidant,
10.547	Nitroisobutylglycerol	oxytocin-induced activity, Antioxidant, Anti-staphylococcal Activity.
12.457	4-deuterio-trans-3,4-dihydroxy-cyclopentene	antiviral activity, Anticancer Activitiy
12.743	Ethyl .alpha.-d-glucopyranoside	Antituberculous Activity, Antioxidant,alpha amylase inhibitory activity, Hypolipemic activity, Anticonvulsant
13.203	4-O-Methylmannose	Antibacterial activity

Fig 2. Total antioxidant activity of *Punica granatum* rind extracts by FRAP assay

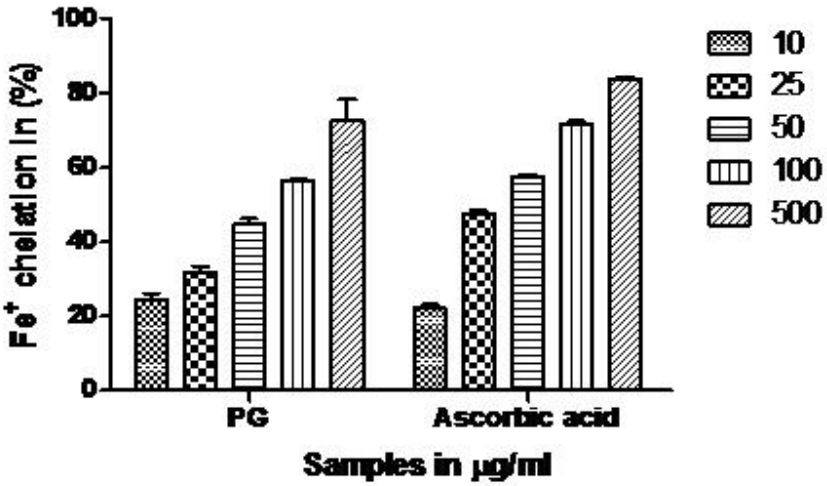
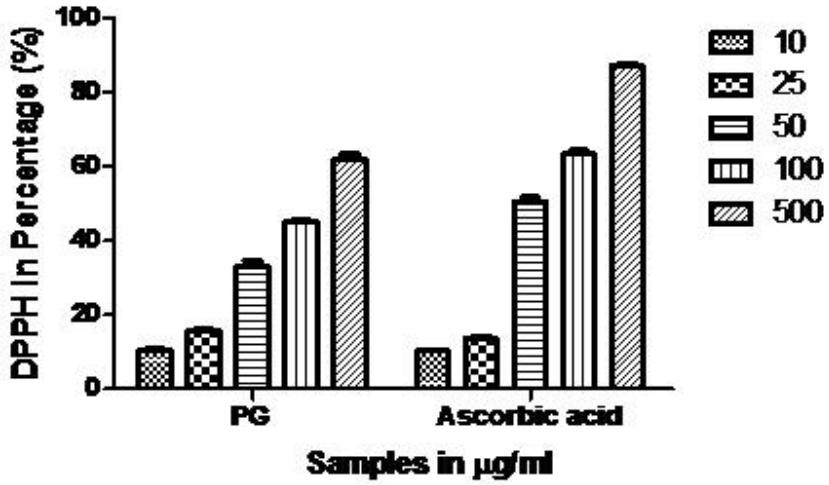


Fig 3: DPPH scavenging activity of *Punica granatum* rind extract



RESULTS AND DISCUSSION

The GC-MS study of *Punica granatum* fruit rind (Figure 1) shows many phytochemicals which contributes to the medicinal activity of the plant. Table 1 shows the major components which present in the fruit rind of *Punica granatum* and in Table 2 is presented the important biological activities of these constituents.

In vitro antioxidant activity of the plant extract was observed by DPPH radical scavenging assay and the FRAP assay.

Metal chelating activity is significant since it reduces the concentration of the catalyzing transition metal in lipid peroxidation [15]. Figure 2 shows concentration dependent chelating effects of the extract of *P. granatum* on Fe^{2+} - ferrozine complex. The ability of the extract was comparable to that of the standard. Iron is a major factor that initiates lipid peroxidation by decomposing lipid hydro-peroxides into peroxyl and alkoxyl radicals that can also be held responsible for lipid peroxidation.

DPPH radical scavenging activity of different concentrations of fruit rind extract of *P. granatum* and ascorbic acid is presented in Fig 2. The extract has significant scavenging effect on DPPH, it was increased with the increasing concentrations from 50 – 250 µg/ml but the scavenging activity of all extracts was lower than that of standard. DPPH radical scavenging test is based on the exchange of hydrogen atoms between the antioxidant and the stable DPPH free radical is reduced to the corresponding hydrazine, a colour change of the solution from violet to yellow is observed and that is monitored spectrophotometrically [16] . The elevated DPPH radical scavenging ability of presence of high concentration of these organic compounds [17].

This study shows that the rind extract of *P. granatum* has phytochemicals which have the very high antioxidant activity which might be helpful in preventing diseases induced by oxidative stress.

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