Determination of Bioactive Components of Ethyl Acetate Fraction of *Punica granatum* Rind Extract

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**ABSTRACT**

*Punica granatum* belongs to a Punicaceae family. The *Punica granatum* is valued as a powerful medicinal plant and used in folk medicines. Hence the present investigation was carried out to determine the possible chemical components from ethyl acetate fraction of *Punica granatum* rind extract by GC-MS Technique. This analysis revealed that ethyl acetate fraction of *Punica granatum* rind extract contain Pyrogallol (41.88%), 5-Hydroxymethylfurfural (14.10%), D-Allose (9.17%), 2-Methoxy-1, 4-Benzenediol (8.34%) and 2, 3 Dimethylfumaric acid (3.96%) justifying the use of this plant to treat many disease in folk and herbal medicine.

**Keywords:** *Punica granatum*, GC-MS Technique, Pyrogallol, Herbal medicine.

**INTRODUCTION**

Plants constitute an important source of active natural products which differ widely in terms of structures, biological properties and mechanisms of actions. Various phytochemical components, especially polyphenols (such as flavonoids, phenyl propanoids, phenolic acids, tannins, etc) are known to be responsible for the free radical scavenging and antioxidant activities of plants.

A Pomegranate (*Punica granatum*) is a fruit-bearing deciduous shrub or small tree growing between five and eight meters tall. The pomegranate is mostly native to the Iranian Plateau and the Himalayas in Northern India. Powder prepared from rind is used as a tooth powder and also as medicine a cosmetic industries. Rind powder is excellent source of beta-carotene, potassium, phosphorous and calcium. The pomegranate rind exploited in folk medicine is their strong astringency, making them a popular remedy throughout the world, in the form of an aqueous decoction (i.e., boiling the hulls in water for 10-40 minutes), for dysentery and diarrhea, and also for stomatitis. [1-3] The decoction can be drunk, used as a mouthwash, douche or enema.

Pure drugs that are technologically produced or isolated from plants may be chosen for their high activity against a human disease, but they have disadvantages. They rarely have the same level of activity as the crude extract at parallel dose or concentrations of the active component. [4] This phenomenon is attributed to the absence of interacting substances present in the extract. Furthermore, many plants contain substances that inhibit multi-drug resistance (MDR). A further disadvantage is that pure drugs are often more expensive to produce and distribute, and so are often unavailable and/or unaffordable to the poorest populations in remote areas who need them most. In contrast, herbal medicines can sometimes be grown and produced locally, at lower cost, by or close to those who need them. [5] Since there is no report on the phytoconstituents of ethyl acetate fraction of *Punica granatum* rind extract it was chosen as the subject of this study. The aim of this paper is to determine the organic compounds present in the active fraction of *Punica granatum* rind extract with the aid of GC-MS Technique, which may provide an insight in its use in folklore medicine.

**MATERIALS AND METHODS**

**Plant material**

Ganesh variety of plant specimen for the proposed study was collected from local fruit market. Care was taken to select healthy fruits. It was identified as *P. granatum* Linn having yellowish red rind (Ganesh variety) belongs to Punicaceae family. The required fruit rind were cut and removed from the fruit and fixed and cast in to paraffin blocks. It was authenticated by Dr. P. Jayaraman, Director of National Institute of Herbal Science, Plant Anatomy Research Centre, Chennai. A voucher specimen is maintained in plant anatomy research centre, Chennai (PARC/2009/459). The fresh rind of the fruit was used for the microscopic studies.

**Preparation of plant extract**

*Punica granatum* rind was collected from local market. They were dried in shade and powdered mechanically. 30 g of
powdered *Punica granatum* rind was macerated with methanol and stored for 72 hours in ice cold condition for the extraction of phytochemicals. At the end of the third day extract was filtered using whatmann No. 1 filter paper and the organic layer was allowed to evaporate. The residue settled was used for further analysis.

**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, ethyl acetate and alcohol respectively. n-Hexane and Chloroform did not elute much of the compounds. The ethyl acetate fraction of the *Punica granatum* 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 technique was carried out at Sargam laboratory, Chennai, Tamil Nadu. 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 2µl was employed (split ratio: 20). Injector temperature 200°C; Ion-source temperature 200°C. The oven temperature was programmed from 70°C (isothermal for 2 min.), with an increase of 30°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.
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Fig 2: Molecular formula, M.W and Molecular structure of the first compound

Line # 1   R.Time: 5.975(Scan#385) MassPeaks:34
Raw Mode: Averaged 5.967-5.983(357-359) BasePeak: 126.05(20001)
BG Mode:Calc. from Peak

Hit#1 Entry: 12662 Library: NIST08.LIB
St: 84 Formula: C6H8O4 CA: S00-00-0 M.W. Weight: 144 RetIndex: 1293
CompName: 2,3-Dimethyllumeric acid

Fig 3: Molecular formula, M.W and Molecular structure of the second compound

Line # 2   R.Time: 6.122(Scan#384) MassPeaks:45
Raw Mode: Averaged 6.183-6.200(383-385) BasePeak: 68.00(9874)
BG Mode:Calc. from Peak

Hit#1 Entry: 6350 Library: NIST08.LIB
St: 84 Formula: C6H10O3 CA: S7112-31-5 M.W. Weight: 126 RetIndex: 993
CompName: Levoglucosenone $\mathcal{S}$ 6,9-Dioxabicyclo[3.2.1]oct-2-en-4-one $\mathcal{S}$

Fig 4: Molecular formula, M.W and Molecular structure of the third compound

Line # 3   R.Time: 7.223(Scan#508) MassPeaks:30
Raw Mode: Averaged 7.217-7.233(507-509) BasePeak: 56.00(19761)
BG Mode:Calc. from Peak

Hit#1 Entry: 4841 Library: NIST08.LIB
St: 89 Formula: C4H6O4 CA: S0-0-0 M.W. Weight: 118 RetIndex: 1203
CompName: 2-Furanone, 3,4-dihydroxy trihydro

Fig 5: Molecular formula, M.W and Molecular structure of the fourth compound

Line # 4   R.Time: 7.358(Scan#524) MassPeaks:31
Raw Mode: Averaged 7.350-7.367(523-525) BasePeak: 43.00(11331)
BG Mode:Calc. from Peak

Hit#2 Entry: 13485 Library: WILEY02.LIB
St: 81 Formula: C7H14O CA: S117-7-7 M.W. Weight: 114 RetIndex: 0
CompName: HEPTANAL $\mathcal{S}$ ENANTHALDEHYDE $\mathcal{S}$ 1-HEPTALDEHYDE $\mathcal{S}$ 1-HEPTANAL $\mathcal{S}$ 207720-40-1 HEPT

Fig. 5: Molecular formula, M.W and Molecular structure of the fourth compound

Fig 6: Molecular formula, M.W and Molecular structure of the fifth compound

Fig 7: Molecular formula, M.W and Molecular structure of the sixth compound

Fig 8: Molecular formula, M.W and Molecular structure of the seventh compound

Fig 9: Molecular formula, M.W and Molecular structure of the eighth compound
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Fig 10: Molecular formula, M.W and Molecular structure of the ninth compound

Fig 11: Molecular formula, M.W and Molecular structure of the tenth compound

Fig 12: Molecular formula, M.W and Molecular structure of the eleventh compound

Fig 13: Molecular formula, M.W and Molecular structure of the twelfth compound
Fig 14: Molecular formula, M.W and Molecular structure of the thirteenth compound

Fig 15: Molecular formula, M.W and Molecular structure of the fourteenth compound

Fig 16: Molecular formula, M.W and Molecular structure of the fifteenth compound

Fig 17: Molecular formula, M.W and Molecular structure of the sixteenth compound
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 was ascertained.

RESULT AND DISCUSSION
Eighteen compounds were identified in ethyl acetate fraction of *Punica granatum* rind extract by GC-MS analysis. The chromatogram obtained by ethyl acetate fraction of *Punica granatum* rind was shown in Fig. 1. The active principle, area of the peak, Concentration (%) and Retention Time (RT) are presented in Table 1. The Molecular Weight, Molecular Formula and its structure of the compounds are presented in Fig. 2-19. The prevailing compounds were Pyrogallol (41.88%), 5-Hydroxymethylfurfural (14.10%), D-Allose (9.17%), 2-Methoxy-1, 4-Benzenediol (8.34%) and 2, 3-Dimethylfumaric acid (3.96%).

The present investigation clearly indicates the highest percentage of Pyrogallol compound in ethyl acetate fraction of *Punica granatum* rind extract. The Pyrogallol as various biological activity like Allelochemic; Antibacterial; Abortifacient; Anticlastogen; Antidermatitic; Antilupus; Antimitogenic; Antioxidant; Antipsoriac; Antiseptic; CNS-Active; Candidicide; Cardiovascular; Dye; Ecobic; Fungicide; Insulin-Sparing; Irritant; Nephrotoxic; Nigrifacient; Pesticide; Prostaglandin-Synthesis-Inhibitor from Dr. Duke's Phytochemical and Ethnobotanical Database. So the compounds present in the active fraction are responsible for the activity of the rind extract.

ACKNOWLEDGEMENTS
I would like to thank wholeheartedly Mr. P. Gopal, Technical Manager of Sargam Laboratory, Chennai for his support in carrying out the analysis.

REFERENCES