



## Phytochemical Composition of Methanolic Extract of Pomegranate Flower Petals (*Punica granatum*) by GC-MS Analysis

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### ABSTRACT

Secondary metabolites and different phytochemicals are present in the medicinal plant dried flower petals of *Punica granatum* belonging to family Punicaceae. Scientific evidence related GC/MS analysis of methanolic extract of flower petals of *Punica granatum* is lacking till date during review of literature. The study was aimed to evaluate phytochemical composition of the methanolic extract of dried flower petals of *Punica granatum* by GC/MS. Flower petals of *Punica granatum* were dried under microwave and the methanolic extract was prepared by simple maceration. GC-MS analysis data was provided by IICPT, Thanjavur. Phytochemical investigation was identified in dried flower petals of *Punica granatum* by evaluating retention time in the GC-MS chromatogram. Eight peaks have revealed in the GC-MS spectrum of the methanolic extract of dried flower petals of *Punica granatum*. From the results, squalene and gallic acid are present as major bioactive compounds in methanolic extract of dried flower petals of *Punica granatum*. The data presented the molecular formula and weight of 8 biomolecules. From the results, it is firstly reported that gallic acid derivatives and squalene as major bioactive compounds present in the methanolic extract of pomegranate flower petals. In further investigation isolation of bioactive compounds and their structural elucidation and screening of pharmacological activity in the drug development should be carried out.

**Keywords:** GC/MS analysis, Pomegranate flower petals, *Punica granatum*.

### INTRODUCTION

Pomegranate (*Punica granatum* L.) possesses higher quantities of polyphenolics, flavonoids and anthocyanins. Pomegranate fruit juice and peel or grind extracts are potent antioxidant source which have been exploited in a wide range of studies since many years. Fruits have achieved great attention among peels, leaves, arils for its health benefits. It is popular consumed as fresh juice or converted into beverages<sup>1</sup>. Recently it has reported that ethanolic extract of flowers of *Punica granatum* exhibited antidiabetic and hypolipidaemic effect at dose of 400mg/kg/day<sup>2</sup>. Alcoholic extract of pomegranate flowers has shown its ability to scavenge free radicals like reactive oxygen species (ROS), reactive nitrogen species (RNS), superoxide radicals, hydrogen peroxide radicals and exert hepatoprotective effect<sup>3</sup>.

Till up to date, there is no report regarding GC/MS characterization of pomegranate flower petals carrying a number of secondary metabolites and phytoconstituents. The main objective of study to explore the phytochemicals present in the methanolic extract of pomegranate flower petals by GC/MS characterization.

### MATERIALS AND METHODS

#### Extraction

Flower of pomegranate were collected from the horticulture garden in the month of September 2014. Flower petals were dried in the microwave and powdered into crude form. Three grams of dried petals were dissolved in ten ml volume of methanol macerated for

forty eight hours and filtered. It was evaporated to get dried extract of flower petals and calculated its percentage yield was found to be 0.18%.

#### GC-MS analysis<sup>4</sup>

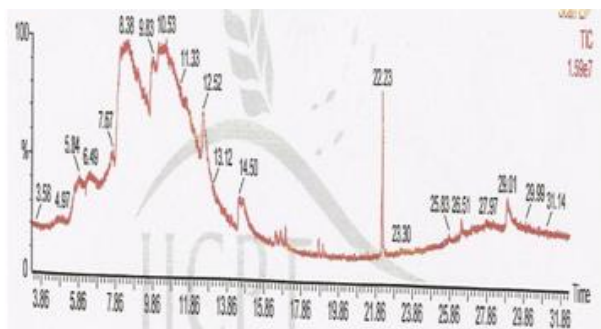
GC/MS analysis of methanolic extract of *Punica granatum* was performed using a GC Clarus 500 Perkin-Elmer system comprising an AOC-20i auto-sampler and a Gas Chromatograph interfaced to a Mass Spectrometer (GC/MS) equipped with a Elite-5MS (5% diphenyl/95% dimethyl poly siloxane) fused a capillary column (30 × 0.25 μm ID × 0.25 μm df). For GC/MS detection, an electron ionization system was operated in electron impact mode with ionization energy of 70 eV. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 ml/min, and an injection volume of 2 μl was employed (a split ratio of 10:1). The injector temperature was maintained at 250°C, the ion-source temperature was 200°C, the oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min to 200°C, then 5 °C/min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 45 to 450 Da. The solvent delay was 0 to 2 min, and the total GC/MS running time was 36 min.

The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The mass-detector used in this analysis was Turbo-Mass Gold-Perkin-Elmer, and the software adopted to handle mass spectra and chromatograms was a Turbo-Mass ver-5.2.



GC/MS mass-spectrum was interpreted by using the database of National Institute Standard and Technology (NIST). Mass spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library. Three major peaks of GC/MS spectrum were ascertained while two phytochemical components were remained unidentified.

## RESULTS AND DISCUSSION



**Figure 1:** GC/MS chromatogram of methanolic extract of *Punica granatum*

### GC/MS analysis

GC/MS chromatogram analysis of the methanolic extract of *Punica granatum* in fig. 1 showed twenty peaks indicating for the presence of phytochemical

constituents. In accordance to reference mass spectra of the constituents available in the NIST library, the peaks of GC-MS spectrum revealed that only eight peaks were identified.

Table 1 shows the list of identified phytoconstituents with peak area. From the results, squalene is present as major bioactive compounds in methanolic extract of dried of *Punica granatum*. Other phytocomponents are found to be fatty acids, lipid, sugar derivatives. 1, 2, 3 Benzenetriol may be indicated the presence of gallic acid derivatives such as hydroxyquinol or phloroglucinol or pyrogallol. Squalene acts as cytotoxic agent<sup>5</sup>, while gallic acid derivatives play an important role in quenching free radicals in the body<sup>6</sup>.

Although it is require to extract these flower petals using different solvents for fractionation which may helpful to explicate isolated phyto-compound and its pharmacological assays. GC-MS has some limitations compared to HPLC so further studies should be investigated to isolate the phytochemicals from flower petals of *Punica granatum* by other chromatographies like HPLC or HPTLC. Also evaluating its antioxidant and free radical scavenging activities of isolated phytochemicals should be carried out.

**Table 1:** Showing phytochemical constituents of methanolic extract of dried flowers of *Punica granatum* by GC/MS

No	RT	Name of the compound	MF	MW	PA %
1	5.84	$\alpha$ -D-Glucopyranoside, methyl 3,6-anhydro-	C <sub>7</sub> H <sub>12</sub> O <sub>5</sub>	176	3.70
2	8.38	2-Furancarboxyaldehyde, 5-(hydroxymethyl)-	C <sub>6</sub> H <sub>6</sub> O <sub>3</sub>	126	33.73
3	10.53	1,2,3 Benzenetriol	C <sub>6</sub> H <sub>6</sub> O <sub>3</sub>	126	48.16
4	12.52	n-Hexadecanoic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256	9.60
5	14.50	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)	C <sub>19</sub> H <sub>32</sub> O <sub>2</sub>	292	1.12
6	22.23	Squalene	C <sub>30</sub> H <sub>50</sub>	410	0.87
7	26.51	1,1-Bicyclohexyl, 2 (1-methylethyl)-trans	C <sub>15</sub> H <sub>28</sub>	208	0.43
8	29.01	9-Octadecenoic acid (Z)-phenylmethyl ester	C <sub>25</sub> H <sub>40</sub> O <sub>2</sub>	372	2.39

## CONCLUSION

The data presented the molecular formula and weight of 8 biomolecules. From the results, it is firstly reported that gallic acid derivatives and squalene as major bioactive compounds present in the methanolic extract of pomegranate flower petals.

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