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GC-MS Analysis of Bio-Active Compounds in Methanolic Extract of *Punica Granatum* L. Root

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ABSTRACT

There have been reports of phytochemical constituents from *Punica granatum* L.species. There are currently no reports on phytochemical constituents or biological activity of root extracts. The objective of this study was to identify the bioactive substances in methanol extract of root of *Punica granatum* L. Several phytochemical constitutions were found in the root part of *Punica granatum* L. The methanol extract of root of *Punica granatum L.* was exposed to a gas chromatography-mass spectrometry (GC-MS) analysis using GC-MS equipment. The methanolic extract of *Punica granatum* contains a variety of phytochemical compounds, according to the GC-MS analysis. 16 compounds in all, composition of the methanolic extract, were identified. It is clear from the data that root of *Punica granatum* is a plant of phytopharmaceutical significance and contains a variety of phytocomponents. Additional research is required to identify the active components of the extract and to clarify their precise mechanisms of action in different disorders.

Keywords: *Punica granatum L.* Root, Secondary metabolites, GC-MS

1. INTRODUCTION

Pomegranate is regarded as one of the oldest known edible fruits, referenced in sacred texts such as the Bible, the Koran, the Jewish Torah, and the Babylonian Talmud, where it is called the 'Food of Gods'(Aviram et al., 2000; Seeram *et al.*, 2006). It has also been mentioned in ancient Greek myths and by Chinese alchemical practitioners. Pomegranate is indigenous to the Middle East, which was the Center of Origin IV by Vavilov, encompassing the interior of Asia Minor, all of Transcaucasia, Iran, and the mountainous regions of Turkmenistan.

Bioactive compounds are plant derived substances and they are considered as secondary metabolites which as various biological activities such as antioxidant, antimicrobial, anti viral etc. The bioactive compounds are inherently present in all the plant parts which besides bark, leaves, stems, roots, flowers, fruits, and even in seeds. The quantity of phytochemicals found may be able to vary in different parts of the plant. Additionally, the plant's secondary metabolites possess chemical and pharmaceutical attributes that are advantageous for human life. (Raskin *et al.*, 2002; Reddy *et al.*, 2003).

The extraction technique described is straightforward, quick, and cost-effective, with minimized solvent usage. The efficient tool for analyzing the compounds extracted from the plant materials is GC-MS which assessing the levels of certain active compounds in herbs utilized in cosmetics, pharmaceuticals, drug formulation, the food sector, as well as in environmental and forensic contexts (Uma *et al*. 2009). This method integrates the analytical approaches for examining mixtures of chemical substances. Gas chromatography helps in separating the components within the mixture based on retention time and fragmentation pattern of compounds. (Deng et al. 1964; Hedeji)

2. MATERIALS AND METHODS

Punica granatum L. root was chosed for the research obtained from Kothagiri District, Tamilnadu, India. The plant was authenticated by Botanical Survey of India, Tamil Nadu Agricultural University (TNAU) Campus, Coimbatore. The sample was washed under running tap water, air dried and powdered in electric blender.

3. EXTRACTION PROCESS

A 100 g sample of dried plant powder was subjected to extraction using 500 ml of methanol in an orbital shaker for duration of 72 hours. The extraction process was repeated with the same solvent until a clear, colorless solution was achieved. The resulting extract was then evaporated to dryness and stored in an airtight container at 4 °C for future use.

4. GC-MS ANALYSIS:

GC-MS analyes of methanolic extract of Punica granatum L. root were carried out using the Perkin-Elmer Clarus 680 system (Perkin-Elmer, Inc. U.S.A) equipped with a fused silica column, packed with Elite-5MS) capillary column (30 m in length × 250 µm in diameter × 0.25 µm in thickness). Helium gas was used as carrier gas at a constant flow of 1 ml/min. 1 µL of extract was injected over the instrument and followed to temperature 60 °C (2 min); followed by 300 °C at the rate of 10 °C min−1; and 300 °C, where it was held for 6 min. The compounds present in the test samples were identified based on their retention time (min), peak area, peak height and mass spectral patterns with those spectral databases of authentic compounds stored in the National Institute of Standards and Technology (NIST) library.

5. RESULTS AND DISCUSSION

The findings from the GC-MS analysis of the methanolic extract of *Punica granatum L.* root extract resulted in the identification of several compounds. These compounds were discerned using mass spectrometry coupled with gas chromatography. The diverse constituents identified as detected by the GC-MS technique, are presented in the accompanying figure.

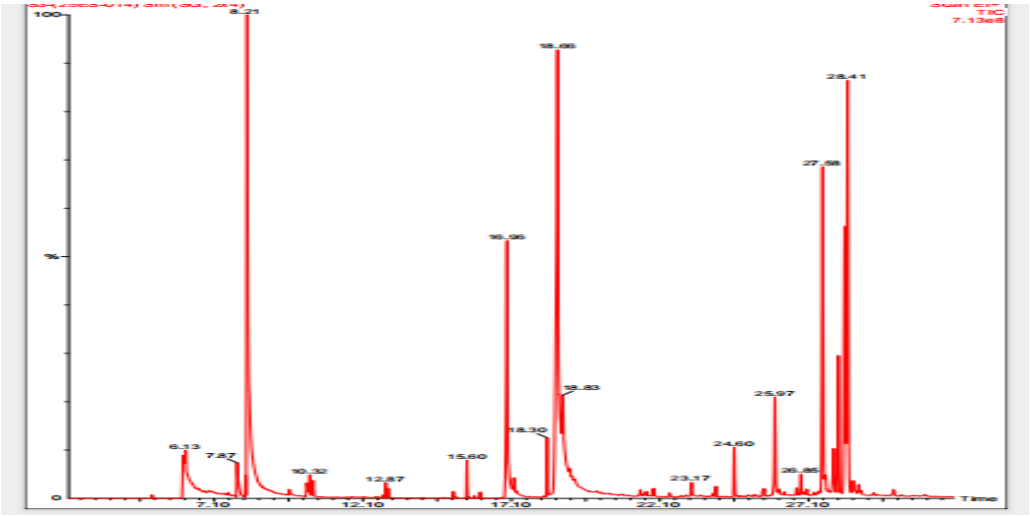

Fig: 1 GC-MS chromatogram of methanolic extract of *Punica granatum* Root

Table 2: Compounds identified in the methanolic extract of *Punica granatum* Root In GC-MS

RT	Compounds	Molecular Formula	Molecular weight
6.135	PROPANOIC ACID, 3-(ACETYLTHIO)-2-METHYL-, (S)-	$C_6H_{10}O_3S$	162
7.37	GERMACYCLOHEXANE, 1,1-DICHLORO-	$C_5H_{10}C_{l2}Ge$	214
8.21	2-FURANACETAMIDE, .ALPHA.-METHYL-N-PHENYL-	$C_{13}H_{13}O_2N$	215
10.32	1-PROPANAMINE, N-METHYL-N-NITROSO-	$C_4H_{10}ON_2$	102
12.67	OXONANE	$C_8H_{16}O$	128

16.60	2-CARBAMYL-9-[.BETA.-D-RIBOFURANOSYL]HYPOXANTHINE	$C_{11}H_{13}O_5N_5$	295
18.30	PHYTOL	$C_{20}H_{40}O$	296
18.660	10-UNDECYN-1-OL	$C_{11}H_{20}O$	168
18.830	13-TETRADECE-11-YN-1-OL	$C_{14}H_{24}O$	208
19.070	13-TETRADECE-11-YN-1-OL	$C_{14}H_{24}O$	208
23.17	HYDROXYLAMINE, O-DECYL-	$C_{10}H_{23}ON$	173
24.60	HYDROXYLAMINE, O-DECYL-	$C_{10}H_{23}ON$	173
25.87	1-HEXACOSANOL	$C_{26}H_{54}O$	382
26.85	.BETA.-SITOSTEROL	$C_{29}H_{50}O$	414
27.68	URS-12-EN-28-OL	$C_{30}H_{50}O$	426
28.109	2,6,10,14-HEXADECATETRAEN-1-OL, 3,7,11,15-TETRAMETHYL-, ACETATE, (E,E,E)-	$C_{22}H_{36}O_2$	332
28.319	1-METHYLENE-2B-HYDROXYMETHYL-3,3-DIMETHYL-4B-(3-METHYLBUT-2-ENYL)-C	$C_{15}H_{26}O$	222
28.41	2R-ACETOXYMETHYL-1,3,3-TRIMETHYL-4T-(3-METHYL-2-BUTEN-1-YL)-1T-CYCLOH	$C_{17}H_{30}O_3$	282

The GC-MS spectrum confirm the presence of multiple compounds, each indicate characteristic retention times with peak area, as depicted in [Figure 1]. The mass spectrometer assesses the compounds that are eluted at varying intervals to find out their nature and structural characteristics. Larger compounds undergo fragmentation into smaller entities, resulting in the disclosure of peaks corresponding to different m/z ratios.

This study contributes to the forecasting of the formula and structure of 19 biomolecules. Subsequent research may facilitate the isolation of bioactive compounds, along with their structural elucidation, while screening for pharmacological activity will be advantageous for future drug development.

6. CONCLUSION

The identification of multiple bioactive compounds through GC-MS analysis of the methanolic extract of *Punica granatum L.* root supports the traditional used for various applications. Nonetheless, the isolation of specific phytochemical constituents and their subsequent evaluation for biological activity is likely to yield significant insights and pave the way for further research into the individual components and their pharmacological efficacy. The findings indicate that *Punica granatum L.* Root is rich in bioactive compounds. Ongoing assessments of its pharmacological properties are in progress. Consequently, this plant is recommended for its potential significance in phyto-pharmaceutical applications.

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