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Gas Chromatography - Mass Spectrometry (GC-MS) analysis of bioactive compounds present in methanolic extract of the flowers of *Punica granatum* Linn.

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Abstract

The chemical components were analysed from the methanolic flowers extract of *Punica granatum* by using Perkin Elmer Gas chromatography and Mass spectroscopy (GC-MS). The results of GC-MS compounds in the extract were relevant to the National Institute of Standards and Technology (NIST) library. GC-MS analysis of the methanolic extract of *Punica granatum* flowers revealed the presence of 24 compounds including isoquerecitrin, 1,2,3 benzenetriol, N-hexadecanoicacid, *5,6,7,8,22 ,52 - hexahydroxy-42 -methoxy flavanone-7-â-D xylopyra noside* (granatumflavanylxyloside), punicanyl benzoate, uroslic acid, 3,7,8,4'-tetrahydroxy-3'-myrt-8-enylflavone, squalene, and masilinic acid. The highest peak area (%) of 48.16 was obtained by 3,7,8,4'-tetrahydroxy-3'-myrt-8-enylflavone with retention-time 10.90 and the lowest peak area of 0.76% was obtained in Beta sitosterollaurate with the retention time of 34.98. Thus this type of GC-MS analysis is the first step towards understanding the nature of active principles present in this medicinal plant. The phytochemical analysis proved that *Punica granatum* could be a new potential source of raw materials for the pharmaceutical industry.

Key words: Gas chromatography; Mass spectrum; *Punica Granatum; Methanolic extract*

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INTRODUCTION

Medicinal plants and human beings have unique relationship since time immemorial. Plants are nature's "Chemical factories" providing the richest source of organic chemicals on earth. The world is blessed with a great variety of natural vegetations, some of which are used as traditional medicine to cure various illnesses and diseases (Okeniyi *et al*., 2007). Modern drugs have saved many lives and have prolonged life span of many patients during the last few decades. But the use of chemical form of drugs is like using of double edged weapon resulting in a variety of physical and drug-induced diseases. The synthetic drugs being pure synthetic chemicals could induce cellular changes, act as foreign substance to the body system and cause several side or toxic effects resulting in ulcer, allergy and hemorrhage and also could leads to death. In the context of present day, prevalent use of chemical drugs and their side effects, the traditional medicinal practices and folk medicines have been put into scientific validation, and it has been widely accepted as viable alternative to chemical drugs. India is repository of rich biodiversity and traditional medicinal practices such as folklore medicines, Siddha and Ayurveda. Nevertheless lack of scientific validation and technological application did not permit to exploit the available resources. However, research on this aspect in gaining momentum in the recent years.

Punica granatum L. (Punicaceae), known as pomegranate, is a deciduous small tree grow up to a height of 8m with attractive reddish scarlet edible fruits. The species originated in Iran, Afghanistan and Baluchistan, found wild in the warm valleys of the Himalayas and is cultivated throughout India (Satyavati *et al.,* 1990). The dried flowers known as Gulnar, are efficacious to treat haematuria, haemoptysis, diarrhoea, dysentery, nasal hemorrhage (Nadkarni, 2002) and in Unani it is used as a remedy for diabetes (Jurjani *et al*., 1878 and Majoosi *et al*., 1889). Flower juice is recommended as a gargle for sore throat and to treat leucorrhoea, hemorrhages and ulcers of the uterus and rectum. The root bark and stem bark of the plant are astringent and used as anthelmintic, especially tapeworms. Fruit rind is valued as an astringent in diarrhoea and dysentery. The powdered flower buds are useful in bronchitis. The seeds are

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reputed as stomachic and the pulp as cardiac and stomachic. The green leaf paste is applied to relieve conjunctivitis (Anonymous,1969) .In addition *Punica granatum* is considered as "a pharmacy unto itself" in ayurvedic medicine and is used as an antiparasitic agent, a blood tonic, and to ulcers (Naqvi, 1991). *Punica granatum* exhibited many pharmacological activities such as anti cancer, antioxidant anti inflammatory, neuroprotective, reproductive function, anti antherogenic, hypoglycemic and antiglycemic and hepatoprotective function.

The present article deals with qualitative and quantitative determination of phytochemical compound present in the methanolic flower extract of *Punica granatum* by using Gas Chromatography – Mass Spectrum (GC-MS) analysis.

MATERIALS AND METHODS

Collection and authentication of plant material

The flowers of *Punica granatum* L. were collected from in and around Mannargudi, Thiruvarur District, Tamil Nadu, India. They were identified and authenticated by Dr. S. John Britto, Department of Botany, RAPINAT Herbarium and Center for Modular Systematics, St. Joseph's College, Tirchirappalli, Tamil Nadu, India.

Fig.1. Flowers of *Punica granatum* Linn.

Preparation of plant material

The collected flowers were thoroughly washed with distilled water and then dried under shade at room temperature for few days. The dried flowers were ground well into a fine powder using blender. The powdered samples were then stored in airtight containers for further use at room temperature. The powdered materials were used for GC-MS analysis.

Sample preparation

5 g of the powdered sample was soaked in 100 ml of methanol overnight and keep in shaker for one hour. The sample was filtered with Whatman No.1 filter paper and evaporated to 1ml at 40°C. The concentrated extract was diluted to 5ml and injects 1ml of the extract was injected in GC- MS.

GC-MS analysis of plant extract

The methanolic extract of powdered flower sample was analyzed for different phytochemicals present in the methanolic extract of the sample using GC-MS. It was performed by using a Perkin Elmer GC Claurus 500 system and gas chromatograph interfaced to a Mass spectrometer equipped with elite-1 fused silica capillary column (30m×1µl was Mdf .composed of 100% Dimethyl polysiloxane. For GC-MS detection electron ionization energy of 70ev was used. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1ml / min and an injection volume of 2µl was employed, injector temperature was 250°C. The oven temperature was programmed from 110°C (isothermal for 2min), with an increase of 10°C / min to 200°C then 5°C / min to 280°C ending with a 90 min isothermal at 280°C. Mass spectra were taken at 70eV a scan interval of 0.5 second and fragment from 45 to 450 Da. The relative percentage of each component was calculated by comparing its average peak area to the total area. Software adopted to handle mass spectra and chromatogram was a Turbo mass Ver. 5.2.0.

RESULTS AND DISCUSSION

The phytochemical compounds such as isoquerecitrin, 1,2,3-benzenetriol,N-hexadecanoicacid,*5,6,7,8,22 ,52 - hexahydroxy-42-methoxyflavanone-7-â-D-xylopyranoside* (granatum flavanylxyloside), punicanyl benzoate, uroslic acid, 3,7,8,4'-tetrahydroxy-3'-myrt-8-enylflavone, squalene, and masilinic acid, ethyl brevifolincarboxylate, beta sitosterolaurate, (Beta-Sitosterol) 17- (5-Ethyl-6-methylheptan-2-yl) -10,13-dimethyl 2,3,4,7,8,9,11,12, 14, 15, 16,17-dodecahydro-1H-cyclopenta [a] phenanthren-3-ol were identified. The highest peak area (%) of 48.16 was obtained by 1, 2, 3 benzenetriol with retention-time 10.90 and the lowest peak area of 0.76% was obtained in Beta sitosterollaurate with the retention time of 34.98. The results of the GC-MS profile can be used as pharmacognostical tool for the identification of the compounds with different chemical structures. The presence of various bioactive compounds confirms the application of *Punica granatum* for various ailments by traditional practitioners. The active compounds with their retention time (RT), molecular formula (MF) and molecular weight (MW) in the methanolic extract of P*unica granatum* were presented in Table 1 and Figure 1.

Table 1: Components identified in methanol extract of *Punica granatum* Linn by GC-MS

S. No.	RT	Name of the compound	Molecular Formula	Molecular Weight	Peak area %
1	1.78	Catechin((2R,3S)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-	$C_{15}H_{14}O_6$	290	0.89
2	3.62	Gallic acid,	$C_7H_6O_5$	170	1.47
3	6.1	(Gallic acid -4-glycoside) 3,5-dihydroxy-4-[[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy]benzoic acid	$C_{13}H_{16}O_{10}$	332	1.59
4	7.6	Ethyl brevifolincarboxylate	$C_{15}H_{12}O_8$	320	2.08
5	8.55	5,6,7,8,2?,3?,5?-heptahydroxy-4?-methoxyflavanone	$C_{19}H_{18}O_{10}$	406	0.87
6	9.6	Isoquerecitrin	$C_{21}H_{20}O_{12}$	464	1.85
7	10.9	1,2,3 benzenetriol	$C_6H_6O_3$	126	11.33
8	12.89	3,7,8,4'-tetrahydroxy-3'-myrt-8-enyl-flavone	$C_{25}H_{24}O_6$	420	48.16
9	14.1	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)	$C_{19}H_{32}O_2$	292	1.78
10	15.08	N-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	9.5
11	17.8	Grantumoside	$C_{18}H_{28}O_9$	388	1.84
12	18.45	Daucosterol	$C_{35}H_{60}O_6$	576	3.45.
13	19.17	Beta sitosterol aurate	$C_{41}H_{73}O_2$	598	0.84
14	20.85	5, 6, 7, 8, 2?,5?-hexahydroxy-4?-methoxyflavanone-7-β- D-xylopyranoside (granatumflavanylxyloside)	$C_{21}H_{22}O_{13}$	482	4.05
15	21.94	Squalene	$C_{30}H_{50}$	410	4.16
16	25.04	Methyl galate	$C_8H_8O_5$	184	11.16
17	26.56	Ursolic acid	$C_{30}H_{48}O_3$	456	1.72
18	27.25	Maslinic acids	$C_{30}H_{48}O_4$	472	4.89
19	27.97	Punicanyl benzoate	$C_{16}H_{22}O_3$	262	5.42
20	29.05	9-Octadecenoic acid (Z)-phenyl methyl ester	$C_{25}H_{32}O_2$	296	3.55
21	32.14	(Beta-Sitosterol) 17- (5-Ethyl-6-methylheptan-2-yl) -10,13-dimethyl 2,3,4,7,8,9,11,12, 14, 15, 16,17-dodecahydro-1H-	$C_{29}H_{50}O$	414	13.56
22	33.96	Granatumol *(13- (15,19,19-* trimethylcyclohex-16-en)-yl-6,10-dimethyl-tridec-10-en-3beta,	$C_{24}H_{44}O_4$	396	4.65
23	34.98	Beta sitosterol laurate	$C_{41}H_{73}O_2$	598	0.76
24	36.89	3,3?,4?-tri-O-methylellagic acid	$C_{17}H_{12}O_8$	344	3.49

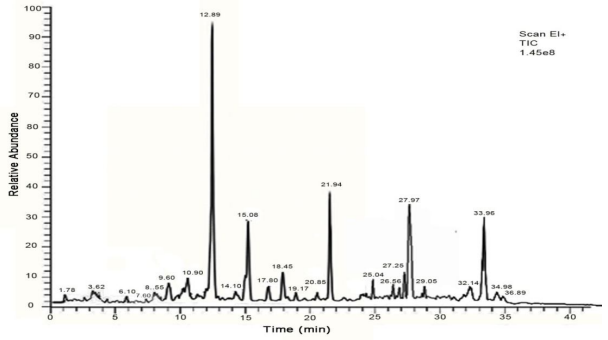

Fig.1. GC-MS spectrum of methanolic extract of *Punica granatum*

The GC-MS spectra of flower extract showed that the identified compounds were mostly the derivatives of terpenoids and flavonoids. GC-MS analysis on methanolic flowers extract revealed the presence of 24 compounds, where 3,7,8,4'-tetrahydroxy-3'-myrt-8-enyl-flavone, constituted the major part. The constituents were identified by comparing GC-MS data with those given in library and reported in literature. The plant has shown both similar and dissimilar biological active compounds.

Gas Chromatography is an advanced technique that cannot be compared with other modern analytical equipment but can be complemented by mass spectrophotometer to achieve GC-MS. It has broad range of applications that caters to academic research, quality control as well as industrial applications. Its short, efficient, automated system gives fast, reproducible and effective results that serve a key role in encroachment of Science and Technology. This versatile analytical technique could be explored for better prospects in future. These biological activities of compounds present in the methanolic extract of the flowers of *Punica granatum* L. support the medicinal application of the plant. The study revealed major bioactive compounds present in the methanol extracts of flowers. Identification of these compounds in the plant serves as the source of determination for potential health benefits of the plant prominent to further biological and pharmacological studies. In addition, the squalene was also reported to have anticancer, anti-oxidant, analgesic, antibacterial and antihelmenthic properties and n-Hexadeconic acid (Palmitic acid) has anti-oxidant, hypocholesterolemic, nematicide and pesticide activities. Further isolation, characterization and structural elucidation of active compounds and clinical studies on the isolated compounds would through more light on their therapeutic usefulness and application, to pave the way for development of new therapeutic biological compounds.

CONCLUSION

GC-MS is a direct and fast analysis approach for identification of secondary metabolites and only few grams of plant material is required. The present study reveals the presence of phytochemicals compounds in *Punica granatum* Linn suggests that the flowers of *Punica granatum* could potentially used in the pharmacological industries for the formulation and development of new drugs.

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