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Research Article

GC-MS analysis of ethanolic extract of *Premna serratifolia* L. fruits

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Abstract

The aim of the present study was to isolate the bioactive compounds from the ethanolic extract of *Premna serratifolia* L. (Verbenaceae) fruits by using Perkin-Elmer Gas Chromatography - Mass Spectrometry. The fruit samples were extracted with 99% ethanol. The extracted sample was injected and the bioactive volatile compounds were screened based on their retention time and peak formation. Interpretation was done using the database of National Institute Standard and Technology (NIST). Fifty compounds were isolated from the fruit extract. Among them, sitosterol (22.35%), o-tolualdehyde (8.26%), stigmasterol (5.62%), anisole, p-vinyl (5.19%), carbolic acid (4.79%), alpha glyceryllinolenate (4.75%), di-iso-octyl phthalate (4.09%), phenol, 4-ethyl (3.74%), 2-Pyrrolidinecarboxylic acid-5-oxo-, ethyl ester (2.50%), α-monopalmitin (2.38%) ,5-Methyl furfural (2.35%), a-glyceryllinoleate (2.21%), campesterol (2.03%), Phenol, 2-methoxy (1.92%), linolenic acid ethyl ester (1.84 %), squalene (1.66%), phytol (1.64%) and linoleic acid ethyl ester (1.64%) were found to be the major compounds.

INTRODUCTION

Premna serratifolia (Verbenaceae) is a popular shrub used in traditional medicines for a number of health problems. It is growing in the tropical and subtropical regions of Asia, Africa and Australia. It is also known as Munnai in Tamil, Munja in Malayalam and Agnimantha in Sanskrit. It is traditionally used as cardiotonic, antibiotic, stomachic, anticoagulant, laxative, hepato protective etc. It is a small tree with the trunk and older branches with opposite spines, leaves opposite toothed, the greenish yellow flowers in corymbose usually unpleasantly scented. Bark is thin, pale wood is light brown and scented. This plant has an important role in Ayurvedha, Siddha and Unani system of medicines. With the above background the medicinally important plant *Premna serratifoila* has been selected for the present study with the aim to isolate the bioactive compounds from the ethanolic extract of fruits using GC-MS analysis. As per the literature survey, the volatile bioactive compounds of fruits of *Premna serratifolia* is analysed for the first time in the present investigation.

MATERIALS AND METHODS

Plant Material

Premna serratifolia L. was collected from Coimbatore, Tamilnadu and certified by BSI, Coimbatore No.BSISRC5232015tech-1170 dt.22.05.2015. Voucher specimen has been deposited in the PG and Research Department of Botany, Govt. Arts and Science College, Coimbatore for future reference.

Preparation of extract

Fruits were collected from the fresh and healthy plants and washed thoroughly in tap water followed by distilled water to remove the dirt and dust particles, then shade dried and pulverized to fine powder using a mechanical grinder. 25 grams of powdered sample was transferred to stoppered flask with 30 ml of ethanol and kept it for overnight soaking. The flask was shaken frequently. Then the samples were filtered using Whatman filter paper with sodium sulphate to remove the sediments and traces of water in the filtrate. The filtrate was concentrated with the help of nitrogen flushing. 2 µl of purely prepared sample was injected into the programme GC-MS instrument.

GC Programme

Column: Elite-5MS (5% Diphenyl / 95% Dimethyl poly siloxane), 30 x 0.25mm x 0.25µm df

Equipment: GC Clarus 500 Perkin Elmer; Carrier gas: 1ml per min, Split: 10:1; Detector: Mass detector Turbo mass gold-Perkin Elmer; Software: Turbomass 5.2; Sample injected: 2µl.

Oven temperature Programme

110°C -2 min hold; Up to 200°C at the rate of 10°C/min-No hold; Up to 280°C at the rate of 5°C / min-9 min hold; Injector temperature 250°C; Total GC running time 63 minutes.

MS Programme

Library used NIST Version-Year 2005; Inlet line temperature 200°C; Source temperature 200°C, Electron energy:70 eV; Mass scan (m/z): 45-450; Solvent Delay: 0-2 min; Total MS running time: 63 min.

Characterization of Compounds

Interpretation on GC mass spectrum was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

RESULTS AND DISCUSSION

The results obtained from the GC-MS analysis of fruit ethanolic extract of *P. serratifolia* revealed the identification of many phytoconstituents. The GC-MS chromatogram shows the presence of 50 major peaks with the retention time range between 3.91 and 62.03 (Figure 1). The biologically active compounds with their retention time (RT), molecular formula, molecular weight (MW), the concentration (peak area percentage) are presented in Table 1.

The major volatile compounds identified from among the ethanolic extract of fruits were γ-sitosterol (22.35%), o-tolualdehyde (8.26%), stigmasterol (5.62%), anisole, p-vinyl (5.19%), carbolic acid (4.79%), alpha glyceryllinolenate (4.75%), di-iso-octyl phthalate (4.09%), phenol,4-ethyl (3.74%), 2-pyrrolidinecarboxylic acid-5-oxo-, ethyl ester (2.50%), α-monopalmitin (2.38%), 5-methyl furfural (2.35%), alpha-glyceryllinoleate (2.21%), campesterol (2.03%), phenol, 2-methoxy (1.92%), linolenic acid ethyl ester (1.84 %), squalene (1.66%), phytol (1.64%), linoleic acid, ethyl ester (1.64%), palmitic acid, ethyl ester (1.49%) and cinnamic acid, p-methoxy-, methyl ester (1.30 %) .

The data revealed a highest percentage of steroid alcohols namely γ-sitosterol and stigmasterol along with campesterol in medium concentration. These compounds are known to have antimicrobial activities (Hemlal and Subban Ravi, 2012) and were isolated and characterized (Luhata Lokadi Pierre and Munkombwe Namboole Moses, 2015). Phenolic compounds are also found to be in higher number which strongly suggests its antioxidant property. The nature of the other compounds obtained from the essential volatile oil of fruit extract of *P. serratifolia* are of monoterpene oxide, monoterpene alcohol, terpene alcohol, alcoholic compound, propionate compound, linolenic acid ester and diterpenes. Linolenic acid is having many activities such as anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicidal, insectifuge, antihistaminic, antieczemic, antiacne, 5-alpha reductase inhibitor, antiandrogenic, antiarthritic and anticoronary. Longifolene, linoleic acid and palmitic acids were reported from black cumin acetone extract through GC-MS (Singh *et al*., 2005). In the present study on *P. serratifolia* also these compounds were identified from different parts of the plant in different proportion. Phytol is a natural diterpene compound and has pharmaceutical applications as anxiolytic, antidepressant and anticonvulsant in nervous system diseases in humans (Pereira Costa *et al*., 2014). The compound phytol with the peak area percentage of 1.64 with the retention time 41.18 min. was identified from the fruit sample.

Table 1: Bioactive compounds identified from the ethanolic fruit extract of *P. serratifolia*

SL NO	R.T	COMPOUND NAME	MOLECULAR FORMULA	MW	Peak area %
1	3.91	Acetal	$C_6H_{14}O_2$	118	0.26
2	4.1	1-Pentanol	$C_5H_{12}O$	88	0.41
3	4.2	1-Butanol, 2-methyl	$C_5H_{12}O$	88	0.74
4	4.37	Pyridine	C_5H_5N	79	0.98
5	7.28	3-Cyclopentene-1-acetaldehyde, 2-oxo-	$C_7H_8O_2$	124	0.87
6	8.24	Furfuryl alcohol	$C_5H_6O_2$	98	0.71
7	12.19	5-Methyl furfural	$C_6H_6O_2$	110	2.35
8	12.97	Carbolic acid	C_6H_6O	94	4.79
9	16.10	α-Methyl-α-[4-methyl-3-pentenyl]oxiranemethanol	$C_{10}H_{18}O_2$	170	0.58
10	16.54	Phenol, 2-methoxy-	$C_7H_8O_2$	124	1.92
11	16.63	cis-Linaloloxide	$C_{10}H_{18}O_2$	170	0.37
12	17.00	Sorbic acid, ethyl ester	$C_8H_{12}O_2$	140	0.38
13	17.47	Phenol, 2-ethyl-6-methyl-	$C_9H_{12}O$	136	1.11
14	18.73	Anisole, p-vinyl-	$C_9H_{10}O$	134	5.19
15	19.12	Phenol, 4-ethyl-	$C_8H_{10}O$	122	3.74
16	19.54	Succinic acid, diethyl ester	$C_8H_{14}O_4$	174	0.52
17	19.74	Fumaric acid, diethyl ester	$C_8H_{12}O_4$	172	0.26
18	20.72	o-Tolualdehyde	C_8H_8O	120	8.26
19	22.02	Diethyl dl-malate	$C_8H_{14}O_5$	190	0.72
20	22.29	p-Ethylguaiacol	$C_9H_{12}O_2$	152	0.32
21	23.32	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150	0.63
22	24.00	4-Cyano-3,5-dimethylphenol	C_9H_9NO	147	0.44
23	24.31	Syringol	$C_8H_{10}O_3$	154	0.80
24	24.47	o-Allylguaiacol	$C_{10}H_{12}O_2$	164	0.48
25	26.76	2-Pyrrolidinecarboxylic acid-5-oxo-, ethyl ester	$C_7H_{11}NO_3$	157	2.50
26	32.36	Cinnamic acid, p-methoxy-, methyl ester	$C_{11}H_{12}O_3$	192	1.30
27	34.14	2-Propenoic acid, 3-(4-methoxyphenyl)-, ethyl ester	$C_{12}H_{14}O_3$	206	0.85
28	35.90	Ethanol, 2-(9-octadecenyloxy)-, (Z)-	$C_{20}H_{40}O_2$	312	0.40
29	36.31	Dibutyl phthalate	$C_{16}H_{22}O_4$	278	0.50
30	36.77	Ethanol, 2-(9-octadecenyloxy)-, (Z)-	$C_{20}H_{40}O_2$	312	0.28
31	37.68	Palmitic acid, methyl ester	$C_{17}H_{34}O_2$	270	0.34
32	38.35	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	0.79
33	39.03	Palmitic acid, ethyl ester	$C_{18}H_{36}O_2$	284	1.49
34	40.88	8,11-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294	0.40
35	41.02	10-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	0.68
36	41.18	Phytol	$C_{20}H_{40}O$	296	1.64
37	42.10	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	302	1.647
38	42.21	Linolenic acid, ethyl ester	$C_{20}H_{34}O_2$	306	1.84

39	42.72	Stearic acid, ethyl ester	$C_{20}H_{40}O_2$	312	0.95
40	42.86	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	$C_{26}H_{54}$	366	0.11
41	47.92	α-Monopalmitin	$C_{19}H_{38}O_4$	330	2.38
42	48.23	Di-iso-octyl phthalate	$C_{24}H_{38}O_4$	390	4.09
43	50.589	Alpha-glyceryllinoleate	$C_{21}H_{38}O_4$	354	2.21
44	50.689	Alpha-glyceryllinolenate	$C_{21}H_{36}O_4$	352	4.75
45	51.04	Glycerin 1,3-distearate	$C_{39}H_{76}O_5$	624	0.95
46	52.42	Squalene	$C_{30}H_{50}$	410	1.66
47	56.68	Sigmastan-3,5-diene	$C_{47}H_{82}O_2$	678	1.14
48	59.66	Campesterol	$C_{28}H_{48}O$	400	2.03
49	60.32	Stigmasterol	$C_{29}H_{48}O$	412	5.62
50	62.03	γ-Sitosterol	$C_{29}H_{50}O$	414	22.35

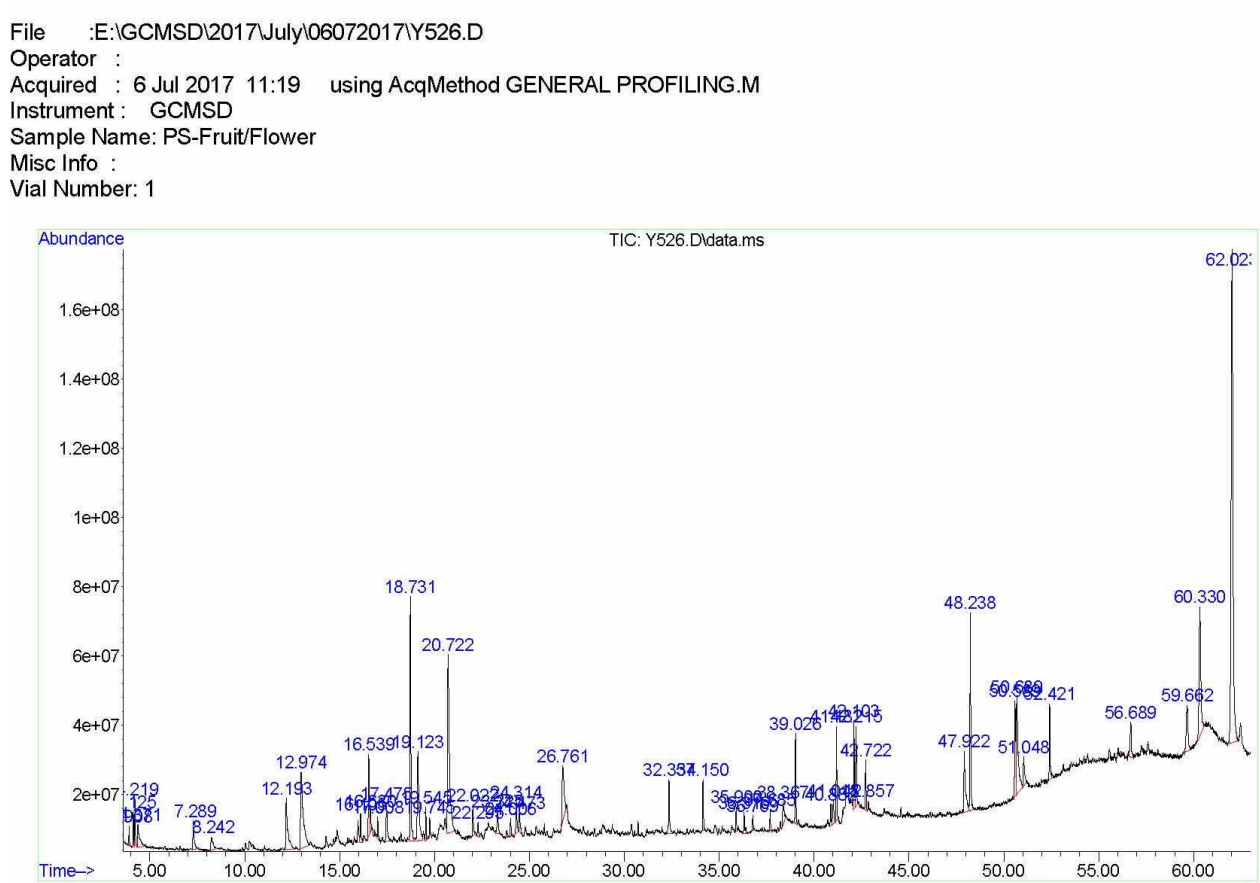

Fig. 1. GC-MS chromatogram of ethnanilc extract of *P.serratifolia* Fruit

Meenakshi Ammal and Viji Stella Bai (2013) also reported phytol as a prevailing compound with peak area percentage 23.53 with the retention time 14. 38. The compound squalene with the peak area percentage of 1.66 with the retention time 52.42 min. was identified from the fruit sample. Kala *et al*. (2011) and Wolosik *et al*. (2013) identified squalene having antioxidant property and can be used to solve the skin physiological problems. The compound squalene holds antibacterial, antioxidant, cancer preventive, antitumor, immunostimulants, pesticide, haemopreventive, lipoxygenase inhibitor, nematicide, antiarthritic and hepatoprotective properties and also used in perfumeries (Revathi and Rajeswari, 2015).

The hydrocarbon and triterpene compound squalene involved in the synthesis of cholesterol, steroid hormones and vitamin D in human body and it is also able to protect human against cancer (Keshavarz *et al*., 2012). Phytol and squalene were also found to be reported from the leaves of *P. serratifolia* (Rency *et al*., 2015). Phytol compound is also reported from the leaf oil of *Premna latifolia* by Renjana and Thoppil (2013). The steroid compound stigmasterol has antimicrobial, antioxidant, anti-inflammatory, antiarthritic, antiasthma and diuretic properties (Revathi and Rajeswari, 2015). Usha and Maria Victorial Rani (2015) reported the compounds n-hexadecanoic acid, 3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol and squalene from *Padina pavonia*. Singh *et al*. (2011) reported compounds such as n-hexadecanoic acid, 2-propionic acid, 3-(4-methoxy phenyl) and phytol from the seeds of *Nigella sativa* which are identified in the present study.

CONCLUSION

The result obtained from the ethanolic fruit extract of *P. serratifolia* in the present study suggests that the identified phytochemicals are pharmacologically active compounds used for treating various ailments.

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