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Research Article

Identification of bioactive compounds from ethanolic leaf extracts of *premna serratifolia* L. using GC-MS

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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. leaves (Verbenaceae) by using Perkin-Elmer Gas Chromatography - Mass Spectrometry. The leaf sample was extracted with 99% of ethanol. Extracted sample was injected; based on the retention time and peak formation, the bioactive compounds are screened. Interpretation was done using the database of National Institute Standard and Technology (NIST). Twenty two compounds were identified. Among them,9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (36.81%) was found to be the major compound followed by phytol (13.99%), E-8-Methyl-9-tetradecen-1-ol acetate(8.00%), Vitamin E (5.29%),11,13-Dimethyl-12-tetradecen-1-ol acetate (5.13%)à-D-Glucopyranoside, O-à-D-glucopyranosyl - (1.fwdarw.3)- á- D- fructofuranosyl (4.72%), 1b, 5, 5, 6a-Tetramethyl-octahydro- 1 –oxa - cyclopropa [a] inden-6-one (3.60%) and 9, 12, 15- Octadecatrienoic acid, (Z,Z,Z)-(3.58%), n-Hexadecanoic acid (2.73%), 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol (2.58%) and Squalene (2.55 %).

INTRODUCTION

Herbs and herbal products have been used in folklore medicine throughout the world for centuries. India is endowed with an estimated 47,000 species of plant that include around 8000 plants which are known to have medicinal properties (Rekha, 2010). *Premna serratifolia* L. is one such medicinal plant belonging to the family Verbenaceae, having tremendous medicinal properties. Vernacular name of the plant is Pai Minney in Tamil and Appel and Ben-moenja in Malayalam.The plant is widespread throughout the tropical and sub tropical part of Asia. In India it is common in the plains of the coastal region. 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 thin, pale wood light brown, scented (Gamble, 1921). This plant has an important role in Ayurvedha, Siddha and Unani system of medicines.It is known as Agnimantha in Ayurvedha. The leaves have various activities likeanti-inflammatory (Rathore *et al*., 1977), anticoagulant (Gopal and Purushothaman, 1984) hypoglycemic (Ajit Kar *et al*., 2003), antiparasitic (Julie Desrivot *et al*., 2007) and cardiostimulant

activity (Rekha *et al*., 2008), Hepatoprotective and cytotoxic (Vadivu *et al*., 2009) antimicrobial (Rekha, 2010; Ravinder Singh, 2011), antioxidant (Selvam *et al*., 2012; Muthukumaran *et al*., 2013) and anti obesity (Mali *et al*., 2013). Leaves are sometimes used as 'nganga' component a *substitute for Piper betle with the seeds of Areca catechu and also used for various stomach ailments.*Roots are also aromatic. With the above background the medicinally important plant *P serratifoila* has been selected for the present study with the aim of isolating the bioactive compounds from the ethanolic extract of leaves.

MATERIALS AND METHODS

Plant material

Premna serratifolia L. leaves were collected from Coimbatore, Tamilnadu and certified by Botanical Survey of India (BSI), Coimbatore, India (No. BSI/SRC/5/23/2015/tech-1170 dt. 22.5.2015). Voucher specimen has been deposited in the PG And Research Department of Botany, Government Arts College (Autonomous) Coimbatore for future reference.

Preparation of plant extract

The fresh and healthy leaves were collected 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 (Anonymous, 1998). 25 grams of powdered whole plant sample was transferred to stoppered flask with thirty ml of ethanol and kept it for overnight soaking. The flask was shaken frequently. Then the sample was filtered using Whatmann 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 36 min.

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: 36 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 GC-MS analysis of leaf ethanol extract of ***Premna serratifolia*** revealed the identification of many phytochemicals. The GC-MS chromatogram shows the presence of 22 major peaks with the retention time range between 2.72 and 34.54 (Figure 1). The active principles with their retention time (RT), molecular formula, molecular weight (MW), the concentration (peak area percentage) and activity are presented in Table 1.

Among the identified compounds, 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-($C_{19}H_{32}O_2$) was found to be the major compound attained the largest peak (36.81%) with the retention time 14.96 min. followed by phytol ($C_{20}H_{40}O$) (13.99%) with the retention time 13.77 min.The diterpene compound phytol showed significant antimicrobial properties against many bacterial strains (Bharathy *et al*., 2012). The third largest peak is due to the presence of E-8-Methyl-9-tetradecen-1-ol acetate ($C_{17}H_{32}O_2$) having the peak area of 8.00% with the retention time 17.72 min. The compound Vitamin E ($C_{29}H_{50}O_2$) showed the peak area of 5.29% with the retention time 27.09 min. Vitamin E, the tocopherol is scavenging the free radicals (Sheela and Uthayakumari, 2013). *In vitro* antioxidant activity of *P. serratifolia* leaves were studied by Sanjay Jain *et al*. (2013). The fifth less prominent peak (5.13%) was attained by the compound 11,13-Dimethyl-12-tetradecen-1-ol acetate ($C_{19}H_{30}O_2$) with the maximum retention time of 34.54 The other compounds showing the less prominent peaks are given in Table 1.

Table 1: *Bioactive compounds identified in the ethanolic leaf extract of *P. serratifolia*

No.	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1.	3.70	Bicyclo[3.1.1]hept-2-ene-2-methanol, 6,6-dimethyl-	$C_{10}H_{16}O$	152	1.44
2.	4.54	Bornyl acetate	$C_{12}H_{20}O_2$	196	0.92
3.	5.01	(-)-Myrtenyl acetate	$C_{12}H_{18}O_2$	194	1.57
4.	5.77	Cyclohexanol, 5-methyl-2-(1-methylethenyl)-, [1R-(1à,2á,5à)]-	$C_{10}H_{18}O$	154	0.29
5.	6.16	Caryophyllene	$C_{15}H_{24}$	204	0.44
6.	6.94	Longifolene-(V4)	$C_{15}H_{24}$	204	1.04
7.	10.12	à-D-Glucopyranoside, O-à-D-glucopyranosyl-(1.fwdarw.3)-á-D-fructofuranosyl	$C_{18}H_{32}O_{16}$	504	4.72
8.	10.71	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	2.58
9.	10.95	9,12-Tetradecadien-1-ol, acetate, (Z,E)-	$C_{16}H_{28}O_2$	252	1.51
10.	11.14	10-Methyl-E-11-tridecen-1-ol propionate	$C_{17}H_{32}O_2$	268	0.79
11.	11.68	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	270	0.86
12.	12.38	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	2.73
13.	13.63	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	278	3.58
14.	13.77	Phytol	$C_{20}H_{40}O$	296	13.99
15.	14.96	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	292	36.81
16.	17.72	E-8-Methyl-9-tetradecen-1-ol acetate	$C_{17}H_{32}O_2$	268	8.00
17.	22.10	Z,Z,Z-4,6,9-Nonadecatriene	$C_{19}H_{34}$	262	1.05
18.	22.91	Squalene	$C_{30}H_{50}$	410	2.55
19.	24.58	10,13-Octadecadiynoic acid, methyl ester	$C_{19}H_{30}O_2$	290	1.10
20.	27.09	Vitamin E	$C_{29}H_{50}O_2$	430	5.29
21.	27.58	1b,5,5,6a-Tetramethyl-octahydro-1-oxa-cyclopropa[a]inden-6-one	$C_{13}H_{20}O_2$	208	3.60
22.	34.54	11,13-Dimethyl-12-tetradecen-1-ol acetate	$C_{18}H_{34}O_2$	282	5.13

*Parameters tested are not covered under the scope of NABL accreditation

Table 2.Activity of Bioactive Compounds identified in the ethanolic leaf extract of *P. serratifolia*

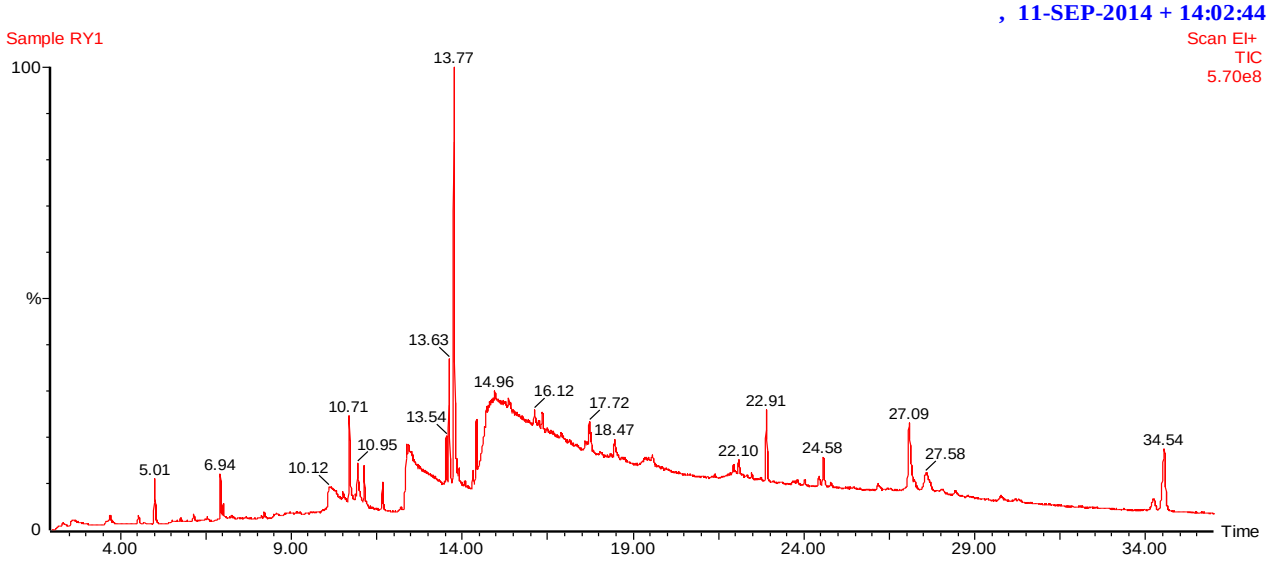
No.	Name of the compound	Molecular Formula	Compound nature	**Activity
1.	Bicyclo[3.1.1]hept-2-ene-2-methanol, 6,6-dimethyl-	$C_{10}H_{16}O$	Monoterpene oxide	Anti-tumor, Analgesic, Antibacterial, Antiinflammatory, Sedative, Fungicide, Hypocholesterolemic, Insecticide, Insectifuge, Chemo preventive, Pesticide, Antiacne,
2.	Bornyl acetate	$C_{12}H_{20}O_2$	Fragrance nature	Used in perfume manufacture Used as a plasticizer
	(-)-Myrtenyl acetate	$C_{12}H_{18}O_2$	Fragrance nature	Flavor and fragrance agent
4.	Cyclohexanol, 5-methyl-2-	$C_{10}H_{18}O$	Monoterpene alcohol	Anti-tumor, Analgesic, Antibac-

	(1-methylethenyl)-, [1R-(1à,2á,5à)]-			terial, Anti-inflammatory, Sedative, Fungicide, Hypocholesterolemic, Insecticide, Insectifuge Chemo preventive, Pesticide, Antiacne,
5.	Caryophyllene	$C_{15}H_{24}$	Sesquiterpene	Anti-tumor, Analgesic, Anti-bacterial, Antiinflammatory, Sedative, Fungicide.
6.	Longifolene-(V4)	$C_{15}H_{24}$	Sesquiterpene	Anti-tumor,Analgesic, Antibacterial, Antiinflammatory, Sedative, Fungicide.
7.	à-D-Glucopyranoside, O-à-D-glucopyranosyl-(1.fwdarw.3)-á-D-fructofuranosyl	$C_{18}H_{32}O_{16}$	Sugar moiety	Preservative
8.	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	Terpene alcohol	Antimicrobial Anti-inflammatory
9.	9,12-Tetradecadien-1-ol, acetate, (Z,E)-	$C_{16}H_{28}O_2$	Alcoholic compound	No activity reported
10.	10-Methyl-E-11-tridecen-1-ol propionate	$C_{17}H_{32}O_2$	Propionate compound	No activity reported
11.	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	Saturated fatty acid ester	No activity reported
12.	n-Hexadecanoic acid	$C_{16}H_{32}O_2$		**Antioxidant, hypocholesterolemic Nematicide, Pesticide, Lubricant AntiandrogenicFlavor, hemolytic 9.5-Alpha reductase inhibitor**
13.	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	Linolenic acid ester	Antiinflammatory, Hypocholesterolemic Cancer preventive, Hepatoprotective, NematicideInsectifuge, Antihistaminic Antieczemic, Antiacne, 5-Alpha reductase inhibitor Antiandrogenic, Antiart-hritic, Anticoronary, insectifuge
14.	Phytol	$C_{20}H_{40}O$	Diterpene	Anticancer, Anti-inflammatory, Antimicrobial, Diuretic
15.	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	Linolenic acid ester	Antiinflammatory, Hypocholeste-rolemic Cancer preventive, Hepatoprotective, NematicideInsectifuge, Antihistaminic Antieczemic, Antiacne, 5-Alpha reductase inhibitor, Antiandrogenic, Antiarthritic, Anticoronary, Insectifuge
16.	E-8-Methyl-9-tetradecen-1-ol acetate	$C_{17}H_{32}O_2$	Acetate compound	No activity reported
17.	Z,Z,Z-4,6,9-Nonadecatriene	$C_{19}H_{34}$	Alkene compound	No activity reported
18.	Squalene	$C_{30}H_{50}$	Triterpene	**Antibacterial, Antioxidant Antitumor, Cancer preventive Immunostimulant, Chemo preven- tive, Lipoxygenase-inhibitor Pesticide**
19.	10,13-Octadecadiynoic acid, methyl ester	$C_{19}H_{30}O_2$	Unsaturated fatty acid ester	No activity reported

20.	Vitamin E	$C_{29}H_{50}O_2$	Vitamin compound	Antiageing, Analgesic,Antidiabetic,Antiinflammatory, Antioxidant, Antidermatitic, Antileukemic, Antitumor, Anticancer, Hepatoprotective, Hypocholesterolemic, Antiulcerogenic, Vasodilator, Antispasmodic, Antibronchitic, Anticoronary
21.	1b,5,5,6a-Tetramethyl-octahydro-1-oxa-cyclopropa[a]inden-6-one	$C_{13}H_{20}O_2$	Ketone compound	No activity reported
22.	11,13-Dimethyl-12-tetradecen-1-ol acetate	$C_{18}H_{34}O_2$	Acetate compound	No activity reported

****Source: -**Dr.Duke's Phytochemical and Ethnobotanical Databases

Figure 1. GC-MS Chromatogram of ethanol leaf extract of *P. serratifolia*



The nature of the compounds obtained are of Monoterpene oxide, Monoterpene alcohol Sesquiterpene, Terpene alcohol, Alcoholic compound Propionate compound, Linolenic acid ester, Diterpene, Acetate compound, Alkene compound, Triterpene, Unsaturated fatty acid ester, Vitamin compound, Ketone compound and Acetate compound. Sesquiterpene is found to have the activities of Anti-tumor, Analgesic Antibacterial, Anti-inflammatory Sedative and Fungicide. Alcoholic and diterpene compounds are having antimicrobial activities. linolenic acid are having many activities such as anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic, antieczemic, antiacne, 5-alpha reductase inhibitor, antiandrogenic, antiarthritic and anticoronary. Kala *et al.* (2011) identified squalene having antioxidant property. The compound squalene with the peak area percentage of 2.55 with the retention time 22.91 min. was identified from the sample. 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 (Musa Keshavarz *et al*., 2011). Ravinder Singh *et al.* (2011) reported thirteen compounds from the ethanol extract of leaves of *P. serratifolia* where as the GC-MS analysis of the leaf ethanolic extract showed the presence of twenty two compounds in the present study.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*.

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LITERATURE CITED

AjitKar BK, Choudry and Bandyopadhyay NG, 2003. Comparative evaluation of hypoglycemic activity of some Indian medicinal plants in Alloxan dioabetic rats.*J. Ethanopharmacol*., **84**: 105-108.

Anonymous, 1998. Quality control methods for medicinal plant materials. WHO Press, World Health Organisation, Switzerland.

Bharathy V, Maria Sumathy B and Uthayakumari F, 2012. Determination of phytocomponents by GC-MS in leaves of *Jatropha gossypifolia* L. *Sci. Res. Reporter*, 2(3): 286-290.

Gamble JS, 1921. Flora of the Presidency of Madras. Published under the Authority of the Secsretary of State for India in Council, London, Vol. II, 1091 p.

Gopal RH and Purushothaman KK, 1984. Effect of plant isolates on coagulation of blood: An *in-vitro*study. *Bull. Med. Ethnobot. Res*., 5: 171-177.

Julie Desrivot , Jean Waikedre, Pierre Cabalion, Christine Herrenknecht, Christian Bories, Reynald Hocquemiller, Alain Fournet, 2007. Antiparasitic activity of some New Caledonian medicinal plants. *J. Ethnopharmacol*., **112**(1): 7-12.

Kala SMU, Balasubramanian T, Tresina Soris and Mohan VR, 2011. GC-MS determination of bioactive components of *Eugenia singampattiana* Bedd. *Int. J. Chem. Tech. Res*., **3**: 1534-1537.

Mali PY, Bigoniya P, Panchal SS and Muchhandi IS, 2013. Anti obesity activity of chloroform- methanol extract of *Premna integrifolia* in mice fed with cafeteria diet. *J. Pharm. Bioallied Sci.*, **5**(3): 229-236.

Musa Keshavarz and Abdul-Reza Dabbah, 2012. Compositions in the leaves extract, *Avicennia marina* Forssk. From Bander-e Kamair (Southern Coast of Iran). *Am. J. Scientific Res*., **45**: 85-89.

Muthukumaran P, Slomi S and Umamaheswari R, 2013. *In vitro* antioxidant activity of *Premna serratifolia* Linn. *Asian J. Res. Pharm. Sci.,* **3**(1): 15-18.

Rathore RS, Prakash A, Singh PP, 1977. *Premna intergrifolia* Lin., A preliminary study of anti-inflammatory activity. *Rheum,* **12**: 130-134.

Ravinder Singh, C, 2011. Antimicrobial effect of callus and natural plant extracts of *Premna serratifolia* L. *Int. J. on Pharmaceu. Biomedi. Res*., **2** (1): 17-20.

Ravinder Singh, C, Nelson R, Muthu Krishnan P and Pargavi B, 2011. Identification of volatile constituents from *Premna serratifolia* L. through GC-MS. *Int. J. Pharm Tech Res*., **3**(2): 1050-1058.

Rekha R, Suseela L, Meenakshi Sundaram R, and Saleem Basha N, 2008. Cardiac stimulant activity of bark and wood of *Premna serratifolia* L. *A J. of the Bangladesh Pharmcol. Soc.,* **3**: 107-113.

Rekha, R, 2010. Antimicrobial activity of different bark and wood of *Premna serratifolia* Lin. 2010.*Int. J. Pharm. Bio Sci*., **6** (1): 1-9.

Sanjay Jain, Mamta Singh, Rakesh Barik and Neelesh Malviya, 2013. *In-vitro* antioxidant activity of *Premna integrifolia* Linn. Roots. *Res. J. Pharmacol. Pharmacodynamics*, **5**(5): 293-296.

SelvamTN, Venkatakrishnan V, Damodar KS and Elumalai P, 2012. Antioxidant and tumor cell suppression potential of *Premna serratifolia* Linn. leaf, *Toxicol. Int*., **19**(1): 31-34.

Sheela D and Uthayakumari F, 2013. GC-MS anaylsis of bioactive constituents from coastal sand dune taxon- *Sesuvium portulacastrum* (L). *Bioscience Discovery*, **4**(1): 47-53.

Usha R and Maria Victorial Rani S, 2015. Gas chromatography and mass spectrometric analysis of *Padina pavonia* (L.) Lamour. *Bioscience Discovery*, **6**(1): 1-5.

Vadivu R, Jerad Suresh A, Girinath K, Boopathi Kannan P, Vimala R and Sathish Kumar NM., 2009. Evaluation of hepatoprotective and *in-vitro* cytotoxic activity of leaves of *Premna serratifolia* Linn. *J. Sci. Res*., **1**(1): 145-152.

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