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Identification of Volatile Constituents from *Premna serratifolia* L.through GC-MS

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Abstract : Medicinal plants are source of important therapeutic aids for alleviating human ailments. Thus natural products have been a major source of drugs for centuries. In tune with this effort, the objective set for the present investigation is to identify the chemical constituents of the leaves and roots of *Premna serratifolia* L. In order to determine the nature of the principle component responsible for its medicinal property. All parts of the plant have medicinal properties. Taking into consideration the medicinal importance of the plant, the volatile organic constituents were analyzed using GC-MS (gas chromatography-mas spectrometry) and the structures were conformed by genesis. A total 29 compounds from both leaves and roots, where the major compounds are 1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1aà,4aà,7á,7aá,7bà)]- (2.98 %), 2-Furancarboxaldehyde, 5-(hydroxymethyl)- (2.44 %), 2-Hydroxy-3-methylbenzaldehyde (6.39 %), : 2s,6s-2,6,8,8-Tetramethyltricyclo[5.2.2.0(1,6)]undecan-2-ol (6.35 %), Benzofuran, 2,3-dihydro- (29.94 %), Glycerin (1.14 %), n-Hexadecanoic acid (13.94%), 2-Propenoic acid, 3-(4-methoxyphenyl)- (13.84 %) have been identified.
Key words: *Premna serratifolia* L, Volatile Constituents,GC-MS.

Introduction

Premna serratifolia L. (Verbenaceae) is an important woody, medicinal plant, it is locally known as munnai and has prominent place in Ayurvedha, Siddha, Unani system of medicines [1]. The leaves and roots are astringent, anti-inflammatory, antibacterial properties and are used in cardiac disorder, cough, leprosy, skin disease, constipation, fever, diabetes, obesity, stomach-ache and tumour [2]. And it has cardiotonic [3], anti- hypoglyceamic properties [4], anti-coagulant [5], anti- arthritics [6] and cardio protective effect [7]. The main objective of this study is to identify the chemical constituents from the leaf and root of *Prema srratifolia* L. This might be responsible for the reported biological activity of this plant. In the present study, Volatile organic matter of the leaves and roots of *Premna serratifolia* L. was analyzed for the first time. This work will help to identify the new compounds, which may helps to produce important therapeutic products.

Material and Methods

The plant *Premna serratifolia* L. was collected from Keelathaniam, Pudukottai District, Tamil Nadu, India and scientific authenticated in Rhbinath herbarium, Trichy, Tamil Nadu, India.

Plant Materials Preparation

The leaf and root were collected from 8 years old *Premna serratifolia* L. and washed in tap water

and then chopped into small fragments. Then materials were dried under shade conditions for 30 days and the drying operation was carried out under controlled conditions to avoid chemical changes. The dried samples were powdered roughly with hands. The powdered samples were stored in polythene containers at room temperature.

Extraction of Samples

The organic constituents from dried plant tissue (leaf and root) prepared by continuous extracting the powdered materials in Soxhlet apparatus with ethanol as solvent. The extracts were concentrated to one third of their original volume and used for testing the chemical constituents. After completion of extraction, the extract was filtered and concentrated to dryness under hot air oven at 55° C. The residue appeared as a dark brown powder.

GC-MS Programme:
Column: Elite-1
(100% Dimethyl poly siloxane), 30m x 0.25mmID x 1µm df
Equipment: GC Clarus 500 Perkin Elmer
Carrier gas: Helium 1ml/min
Detector: Mass detector- Turbo mass gold- Perkin Elmer, Software- Turbomass 5.1.

Sample injected: 1µl
Split: 10:1

Oven Temperature programme:
110deg-2min hold
Up to 280 deg at the rate of 5 deg/ min-9 hold
Injector temperature: 250 deg c
Total GC time: 45 min

MS Programme
Library used: NIST Ver.2.1
In let Line temperature: 200 deg c
Source temperature: 200deg c
Mass scan: (m/z) 45- 450
MS Time: 46 min

Phytochemical studies

The preliminary phytochemical screening test has been attempted in difference parts of *P. serratifolia* to find out the presence or absence of certain bioactive compounds Table-1. All the extracts were used to test for the presence of alkaloids, sugar, reducing sugar, catachins, anthroquilnones, amino acids, flavonoids, steroids, terpenoids, tannins, phenolics and saponins. The methods of preliminary phytochemical analysis were based on the methods of Brindha *et al.*.[8]

1. Preliminary Phytochemical Test (Brindha *et al*., 1981).

Sl.No	Test	Observation	Inference
1.	Test solution + minimum quantity of chloroform, 3-4 drops of acetic anhydride and one drop of conc. H_2SO_4	Purple colour changes to blue or green	Presence of steroids
2.	Test solution + piece of tin +3 drops of thionyl chloride	Violet or purple colour developed	Presence of triterpenoids
3.	Test solution + 2ml of Fehlings reagent + 3ml of H_2O	Red – orange colour formed	Presence of reducing sugars
4.	Test solution + very small quantity of anthrone + few drops of conc. $H2SO_4$ and heat	Green to purple colour developed	Presence of sugar
5.	Test solution taken with 2 NHCL. The aqueous layer formed was decanted and to this one or few drops of Mayers reagent was added	White precipitate or turbidity formed	Presence of alkaloids
6.	Test solution in alcohol + one drop of natural ferric chloride (5%) solution	Intense colour developed	Presecne of phenolic compounds
7.	Test solution in alcohol + Ehrilich reaget and few drops of conc. HCl	Pink colour formed	Presence of catachins
8	Test solution in alcohol + a bit of magnesium and one or two drops of conc. HCl and heat	Red or orange red colour formed	Presence of flavanoids
9.	Test solution + H_2O and shake	Foamy lather formed	Presence of saponins
10	Test solution + H_2O + lead acetate	White precipitate formed	Presence of Tannins
11.	Test solution + magnesium acetate solution	Pink colour formed	Presence of anthroquiones
12.	Test solution + 1 % ninhydrin in alcohol	Blue or violet colour developed	Presence of amino acids

Table-2 Phytochemicals identified in the alcoholic extract of the leaf *Premna serratifolia*

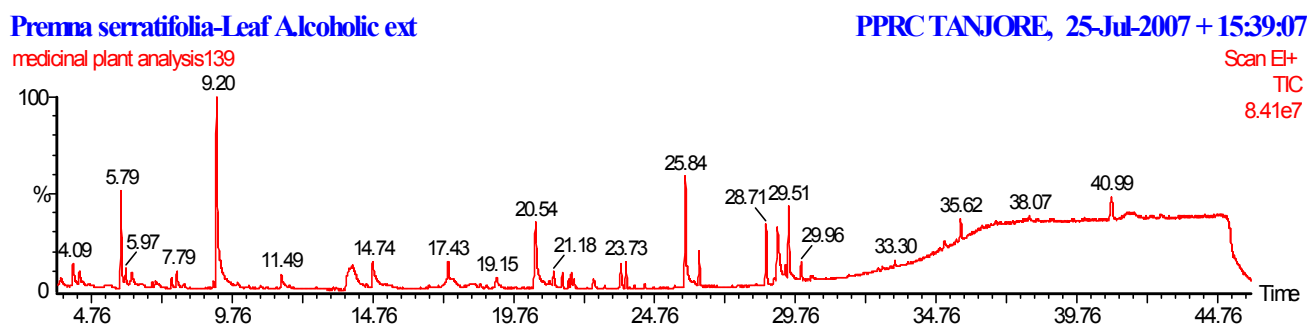| Sl. No	RT	Name of the compound	Formula	MW	Peak area %
1	4.09	: Glycerin	$C_3H_8O_3$	92	2.79
2	5.80	2,5-Furandione, 3-methyl-	$C_5H_4O_3$	112	9.27
3	9.20	Benzofuran, 2,3-dihydro-	C_8H_8O	120	29.94
4	14.74	2-Hydroxy-3-methylbenzaldehyde	$C_8H_8O_2$	136	6.39
5	17.43	Dodecanoic acid	$C_{12}H_{24}O_2$	200	7.88
6	20.54	2-Propenoic acid, 3-(4-methoxyphenyl)-	$C_{10}H_{10}O_3$	178	13.84
7	21.18	Phenol, 4-(3-hydroxy-1-propenyl)-2-methoxy-	$C_{10}H_{12}O_3$	180	1.54
8	21.49	2-Propenoic acid, 3-(4-methoxyphenyl)-, ethyl ester	$C_{12}H_{14}O_3$	206	1.35
9	23.73	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{22}O_4$	278	2.50
10	25.84	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	13.94
11	28.71	Phytol	$C_{20}H_{40}O$	296	6.78
12	29.96	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312	1.68
13	35.62	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	$C_{16}H_{50}O_7Si_8$	578	2.11

Fig-1 Phytochemicals identified in the alcoholic extract of the leaf *Premna serratifolia*

Table-3 Phytochemicals identified in the alcoholic extract of the root *Premna serratifolia*

Sl. No	RT	Name of the compound	Molecular Formula	MW	Peak Area%
1	4.08	: Glycerin	$C_3H_8O_3$	92	1.14
2	5.79	2,5-Furandione, 3-methyl-	$C_5H_4O_3$	112	2.89
3	9.06	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	$C_6H_6O_3$	126	2.44
4	9.20	Benzofuran, 2,3-dihydro-	C_8H_8O	120	9.86
5	14.74	2-Hydroxy-3-methylbenzaldehyde	$C_8H_8O_2$	136	34.58
6	16.24	Seychellene	$C_{15}H_{24}$	204	2.30
7	17.43	Dodecanoic acid	$C_{12}H_{24}O_2$	200	0.71
8	18.30	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1aà,4aà,7á,7aá,7bà)]-	$C_{15}H_{24}O$	: 220	2.98
9	20.65	2-Propenoic acid, 3-(4-methoxyphenyl)-	$C_{10}H_{10}O_3$	178	13.99
10	22.71	2s,6s-2,6,8,8-Tetramethyltricyclo[5.2.2.0(1,6)]undecan-2-ol	$C_{15}H_{26}O$	222	6.35
11	23.56	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	1.34
12	25.85	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	4.87
13	28.71	Phytol	$C_{20}H_{40}O$	296	1.90
14	29.96	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312	0.59
15	32.69	2-Phenanthrenol, 4b,5,6,7,8,8a,9,10-octahydro-4b,8,8-trimethyl-1-(1-methylethyl)-, (4bS-trans)-	$C_{20}H_{30}O$	286	4.77
16	37.01	unknown	***	***	9.29

Fig-2 Phytochemicals identified in the alcoholic extract of the root *Premna serratifolia*
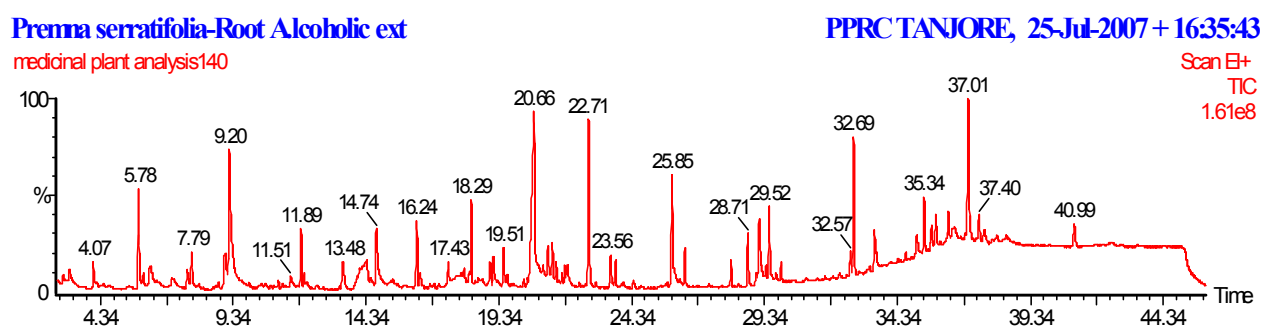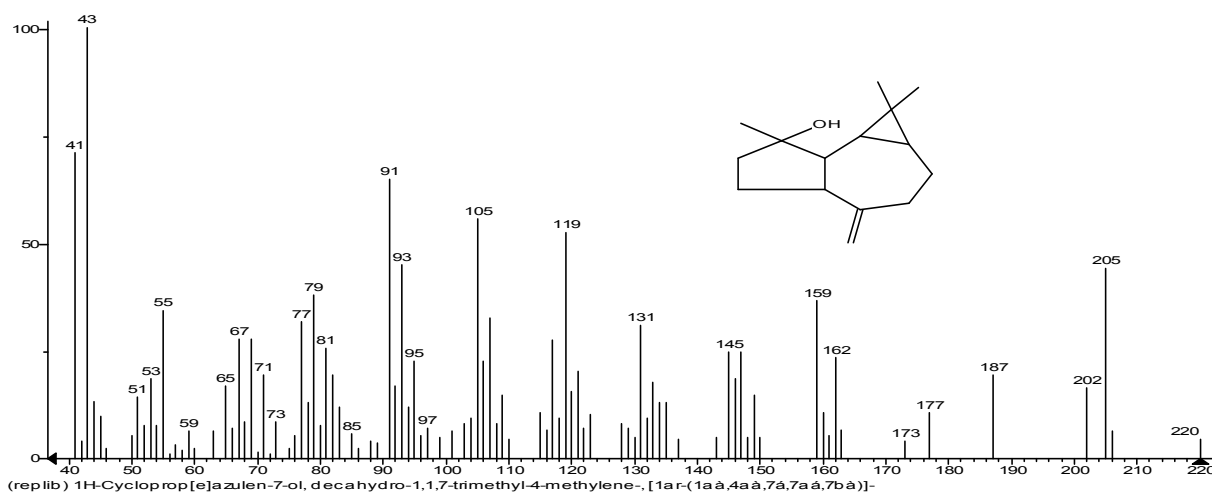
Fig-3
Name: 1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1aà,4aà,7á,7aá,7bà)]-
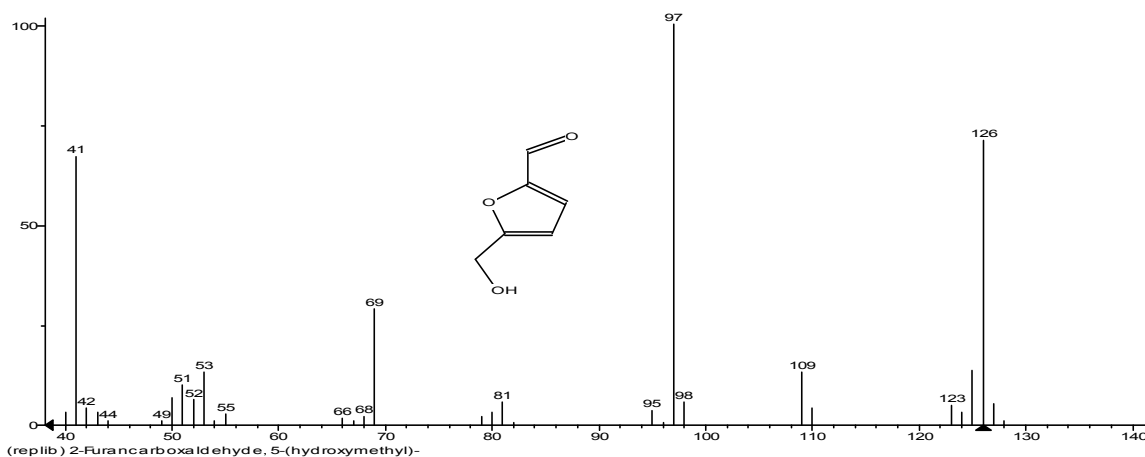Formula: $C_{15}H_{24}O$
MW: 220 CAS#: 6750-60-3 NIST#: 107043 ID#: 1780 DB: replib
Other DBs: None
Contributor: N.W. Davies, Centr. Sci. Lab., Univ. Tasmania, Hobart, Australia
10 largest peaks:

         43 999 | 41 709 | 91 647 | 105 555 | 119 524 |
         93 449 | 205 440 | 79 379 | 159 365 | 55 343 |

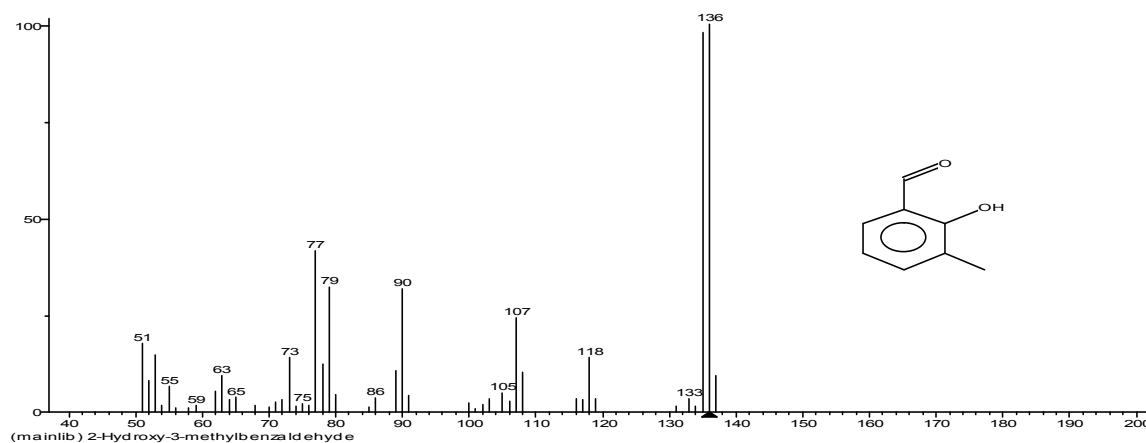**Fig-4**
Name: 2-Furancarboxaldehyde, 5-(hydroxymethyl)-
Formula: $C_6H_6O_3$
MW: 126 CAS#: 67-47-0 NIST#: 60544 ID#: 12832 DB: replib
Other DBs: None
Contributor: D.HENNEBERG, MAX-PLANCK INSTITUTE, MULHEIM, WEST GERMANY
10 largest peaks: 97 999 | 126 710 | 41 668 | 39 336 | 69 289 |
                        29 173 | 125 136 | 109 131 | 53 131 | 51 100 |

(mainlib) 2-Hydroxy-3-methylbenzaldehyde

Fig-5
Name: 2-Hydroxy-3-methylbenzaldehyde
Formula: $C_8H_8O_2$
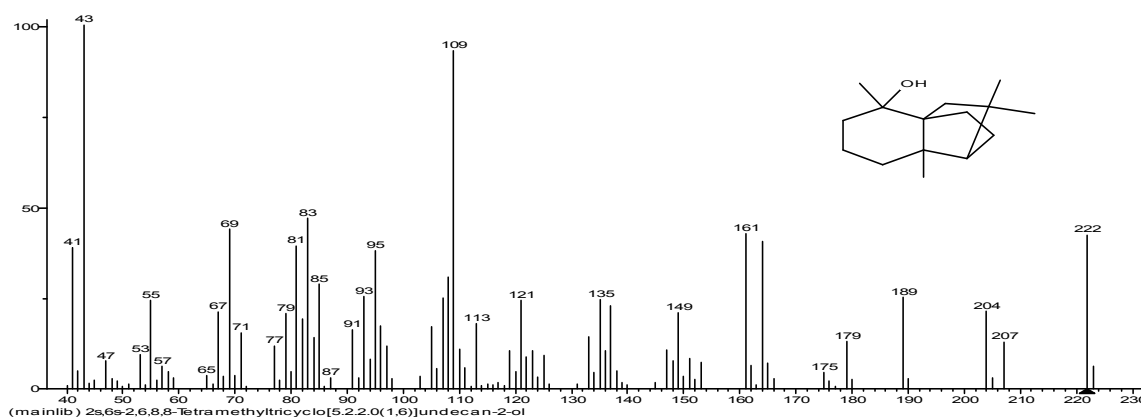MW: 136 CAS#: 824-42-0 NIST#: 72528 ID#: 80172 DB: mainlib
Other DBs: None
Contributor: R.SELF, AGRIC. RES. COUNC., FOOD RES. INST., NORWICH, U.
10 largest peaks:
          136 999 | 135 977 | 77 414 | 79 321 | 90 316 |
          107 242 | 51 175 | 53 147 | 73 140 | 118 139 |

(mainlib) 2s,6s-2,6,8,8-Tetramethyltricyclo[5.2.2.0(1,6)]undecan-2-ol

Fig-6
Name: 2s,6s-2,6,8,8-Tetramethyltricyclo[5.2.2.0(1,6)]undecan-2-ol
Formula: $C_{15}H_{26}O$
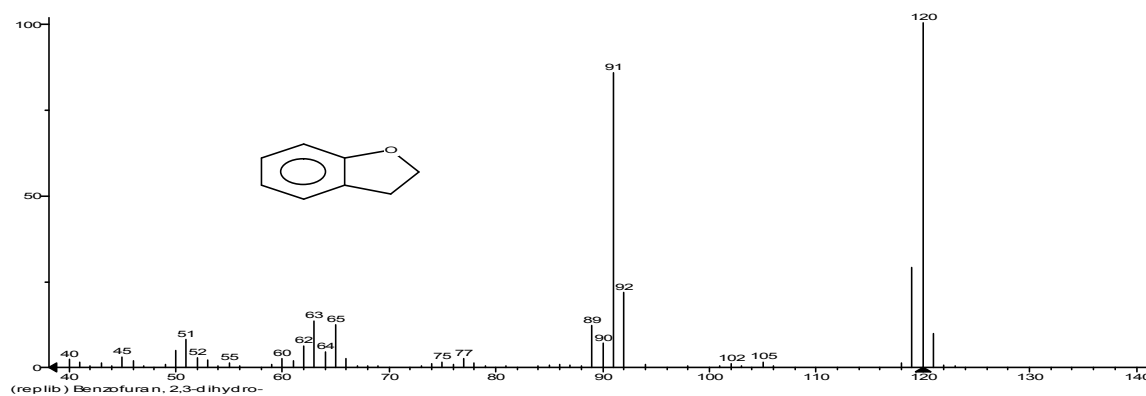MW: 222 CAS#: N/A NIST#: 140230 ID#: 9434 DB: mainlib
Other DBs: None
Contributor: B. Derendyaev, Novosibirsk Institute of Organic Chemistry
10 largest peaks:
                    43 999 | 109 928 | 83 469 | 69 438 | 161 425 |
                    222 421 | 164 404 | 81 392 | 41 386 | 95 379 |

Fig-7
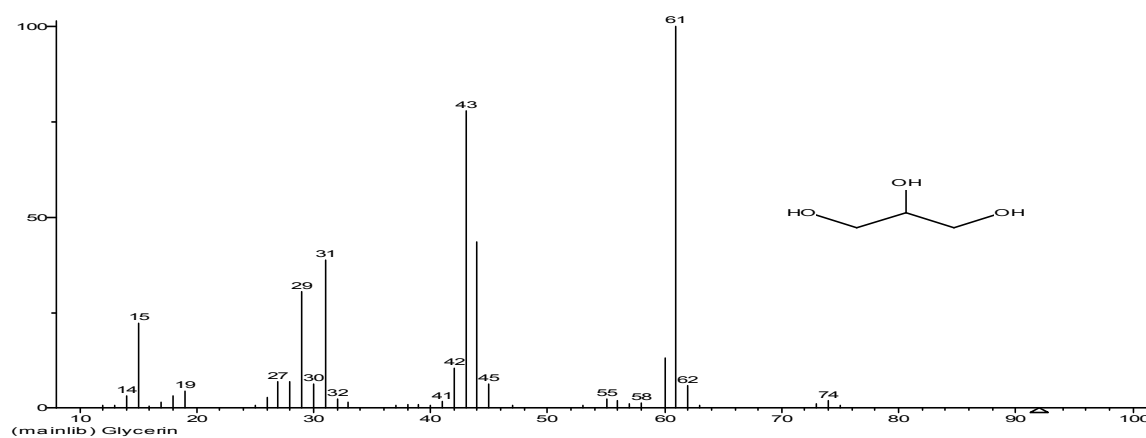Name: Benzofuran, 2,3-dihydro-
Formula: C_8H_8O
MW: 120 CAS#: 496-16-2 NIST#: 109771 ID#: 16183 DB: replib
Other DBs: None
Contributor: Philip Morris R&D
10 largest peaks:
         120 999 | 91 855 | 119 288 | 92 216 | 39 159 |
          63 134 | 65 122 | 89 121 | 12196 | 5180 |

Fig-8
Name: Glycerin
Formula: $C_3H_8O_3$
MW: 92 CAS#: 56-81-5 NIST#: 229308 ID#: 6829 DB: replib
Other DBs: None
Contributor: Japan AIST/NIMC Database- Spectrum MS-NW-5532
10 largest peaks:
         61 999 | 43 740 | 44 462 | 31 289 | 15 273 |
         29 151 | 60 90 | 18 68 | 27 61 | 28 58 |

Fig-9
Name: n-Hexadecanoic acid
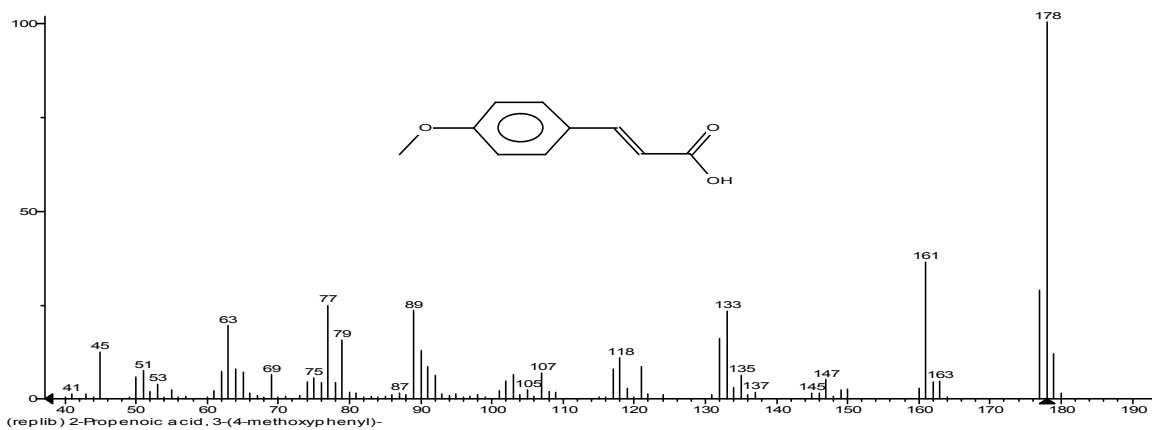Formula: $C_{16}H_{32}O_2$
MW: 256 CAS#: 57-10-3 NIST#: 251929 ID#: 1725 DB: replib
Other DBs: None
Contributor: Div. of Experiment Therapeutics WRAIR, WRAMC, Washington DC 20307
10 largest peaks:
   43 999 | 41 753 | 60 580 | 55 526 | 73 523 |
   57 476 | 69 236 | 71 193 | 45 132 | 42 119 |

Fig-10
Name: 2-Propenoic acid, 3-(4-methoxyphenyl)-
Formula: $C_{10}H_{10}O_3$
MW: 178 CAS#: 830-09-1 NIST#: 75919 ID#: 22426 DB: replib
Other DBs: None
Contributor: RADIAN CORP
10 largest peaks:
         178 999 | 161 361 | 177 286 | 77 246 | 89 233 |
         133 232   63 194 | 132 159 | 79 154 | 90 127 |

Result and Discussion

Volatile organic compounds are products of plant secondary metabolites, consisting of complex mixture of mono-, di-, tri- terpene hydrocarbons and oxygenated biological materials. In the present investigation, 13 compounds (Fig-1) from leaves and 16 compounds (Fig-2) from root of *Premna serratifolia* L. were identified through GC-MS analysis (Table-2,3). Among the total compounds, 8 major compounds A total 29 compounds from both leaves and roots, where the major compounds are 1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1aà,4aà,7á,7aá,7bà)]- (2.98 %) Fig-3, 2-Furancarboxaldehyde, 5-(hydroxymethyl)- (2.44 %) Fig-4, 2-Hydroxy-3-methylbenzaldehyde (6.39 %) Fig-5, : 2s,6s-2,6,8,8-Tetramethyltricyclo [5.2.2.0 (1,6)]undecan-2-ol (6.35 %) Fig-6, Benzofuran, 2,3-dihydro- (29.94 %) Fig-7, Glycerin (1.14 %) Fig-8, n-Hexadecanoic acid (13.94%) Fig-9, 2-Propenoic acid,

3-(4-methoxyphenyl)- (13.84 %) Fig-10 have been identified. from both leaves and roots have been identified with comparisons of mass spectrum (Fig-3). The similar findings (Volatile organic compounds) reported from Mimusops elengi [9], *Acorus calamus*, [10], *Morinda morindoides* [11]. Our study suggests that *Premna serratifolia* L. may be a potential source of the reported important disease use in medicine.

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