

RESEARCH ARTICLE

PHYTOCHEMICAL SCREENING OF ETHANOLIC EXTRACT OF *PREMNA CORIACEA* THROUGH AN INTEGRATIVE GC-MS AND LC-MS

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Abstract

Premna coriacea var. *villosa* is wood climber belongs to the family Lamiaceae. *Premna* genus plants have high medicinal importance in indian system of medicines to treat various diseases. The main objective of the present study is to analyse and find the phytoconstituents present in the leaves ethanolic extract of *Premna coriacea*. The Phytochemical analysis was done by standard procedure to find out the secondary metabolites, GCMS is carried out to find the volatile constituents and LCMS for non-volatile constituents. The preliminary phytochemical analysis confirms the presents of presence of Alkaloids, Carbohydrates, Glycosides, Steroids and Flavonoids. The GCMS analysis shows the presents of 68 compounds in which 23 have medicinal properties. The LCMS analysis shows the presents of 28 compounds in which most of them have the medicinal properties. The present phytochemical study on *Premna coriacea* ethanolic extract reveals the presence of phytoconstituents like Methyl Salicylate, hexadecanoic acid, Caryophyllene, Eugenol, Ledol, cis-Vaccenic acid, n-Hexadecanoic acid, Piperine, Phytol, Diethyl Phthalate etc. *Premna coriacea* could be a potential source for anti-inflammatory, analgesic, antimicrobial, antifungal and anticancer agents.

Keywords: *Premna coriacea*, Phytochemical analysis, LCMS, GCMS, Phytol, Ledol, Piperine.

INTRODUCTION

Natural products have been still remains a major source for drug discovery in development of synthetic molecules. Chemical diversity in nature is based on biological and geographical diversity. The use of traditional plant extract in the treatment of various diseases has been flourished. In the early 19th century, when chemical analysis first became available, scientists began to extract and modify the active ingredients from plants. Plants have been a rich source for drug discovery. The World Health Organization estimated that about 80 % of the world population relays on herbal medicines. Nowadays medicinal plants receive more attention to researchers because of their safety and curative property which is due to the complex mixtures secondary metabolites.¹

Premna is widely distributed in the tropical and subtropical regions of Australia, Africa and Asia. The genus accommodated earlier in the family Verbenaceae has been recently transferred to the family Lamiaceae based on the molecular data. The generic name is derived from the Greek word "Premnon" the stump of a tree; dwarf type species. The genus comprises of 200 species distributed worldwide and among 31 districts in India. There are 8 species and one variety in Kerala. In that one the variety is *Premna coriacea* var. *villosa* C.B. Clarke A Rajedran & P. Daniel.

Plant Morphology:

Scandent shrubs, 4 - 5 m high. Stems with pale ashy brown bark. Leaves ovate, cordate at base, entire, slightly undulate along the margins, acuminate at apex, 7 - 24 x 3 - 14 cm, puberulous and dark green above, paler and densely villous beneath; lateral nerves 4 - 6, raised beneath. Inflorescence a corymb, c. 10 cm in diam., manyflowered; rachis purple. Calyx cupular, slightly wavy, 2-lipped, faintly 5-toothed. Corolla infundibuliform, hairy at neck, 2-lipped, 4-lobed; lobes ovate, c. 1.5 x 4 mm, white. Stamens 4; didynamous; filaments filiform, c. 3 mm long. Ovary obovoid, 1.8 - 2 x 1.4 - 1.6 mm, glabrous; style slender; stigma 2-lobed. Drupes obovoid, c. 2.5 x 1 mm; fruiting calyx saucer-shaped. Flowering & Fruiting: March - October. Distribution: India, Bangladesh, Thailand and Vietnam. In India, distribution of this species is restricted to the Kerala parts of Western Ghats. Wayanad district in Kerala is the type locality of this species. Habitat: Which is usually seen in the moist deciduous and semi-evergreen forests and the entire area is along the Western Ghats with altitudes ranging from 200 m to 2100 m msl. Sugandagiri, Pookkodu Lake and Chandanathode areas are coming under the forest areas of Wayanad district.²

Based on the literature review there is no scientific reports on phytochemical compounds of *Premna coriacea*. The present study has made an attempt to identify the chemical constituents from the leaves of *Premna coriacea* through GC-MS and LC-MS.

MATERIALS AND METHODS

Plant material

The plant *Premna coriacea* was collected from Kesavanpara, Nelliampathy, Palakkad District, Kerala, India and it has been identified and authenticated by Dr. Udayan P.S., Professor, Sreekrishna College, Guruvayur, Thrissur, Kerala, India. The leaves of the *Premna coriacea* were collected during November-December month and washed with water. Then the plant leaves material was shade dried for 10 days. The dried plant materials have been powdered using mechanical grinder to get uniform coarse particles. The powdered plant material was stored in polythene air tight containers at room temperature for further use.

Preparation of plant extract

The shade dried coarse powdered bark of *Premna coriacea* (100 g) was packed in the soxhlet extraction apparatus and extracted with 1 L of 95% ethanol at a temperature of 40-50°C for 72 hr. The extract was filtered and the filtered extract was then concentrated to dryness in a rotary evaporator under reduced pressure at temperature of 40°C. The resultant green color residue was stored in a desiccator for use in subsequent experiments and considered as the crude ethanol extract. The yield of the ethanolic extract was 12% w/w.

Phytochemical analysis

The preliminary phytochemical screening test was carried out in ethanolic extract of *Premna coriacea* to find out the nature of chemical compounds as per the standard procedures³⁻⁶ and the phytoconstituents were identified through GCMS and LCMS.

GC-MS and LC-MS Specifications

GCMS Make – Agilent ;Model – GC – 7890A, MS – 5975C ;Column – DB – 5MS – 30 m x 0.25mm x 0.25um;Carrier Gas – He;Flow Rate – 1.0 ml/min;Column Oven Temp. – 400 C for 5 min. – 5 0 C/min to 280 °C- hold for 10 min;Injector Temperature –250 0 C;Injection Mode – Split – 50:1;Source – 230° C;Quad – 150 0 C;Sample Preparation – 100.0 mg sample + 1.0 ml solvent.HPLC- Agilent Technologies 1260 Infinity;MS- 6120 Quadrupole;Coloumn- Agilent- Eclipse plus C-18 4.6 x 250 mm;Mobile phase;10Mm Ammonium acetate in water and methanol (15:85);Injection Volume –10µL;Flow rate - 0.4ml/ mint; Sample Preparation;5mg sample prepared in 5 ml methanol and filter.

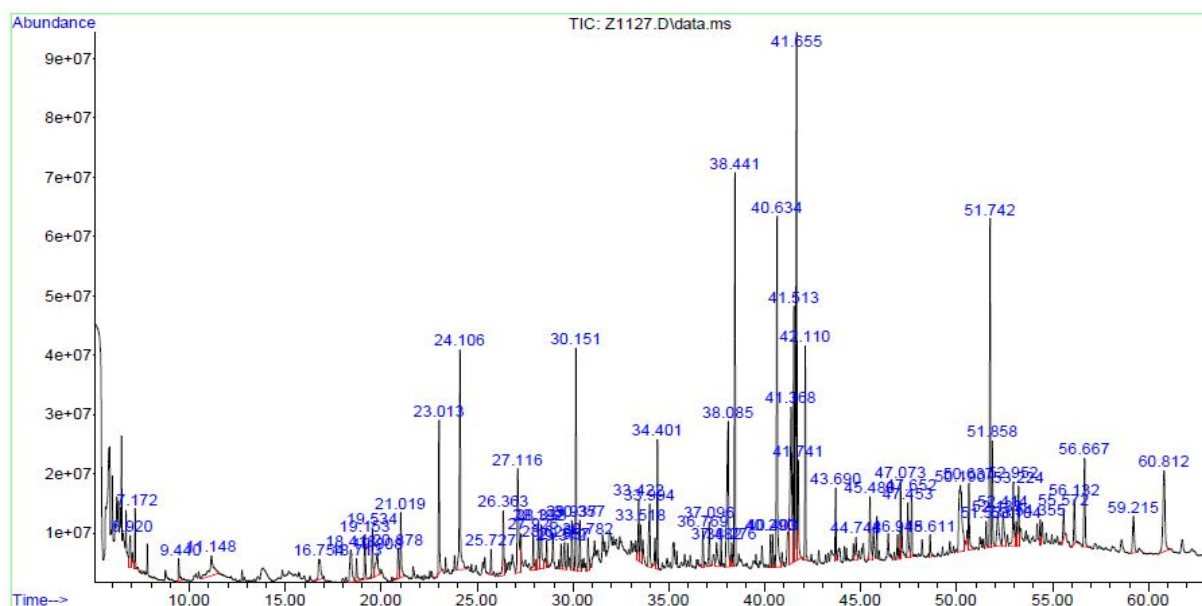


Figure 1 : GC/MS chromatogram of ethanolic extarct of *Premna coriacea*

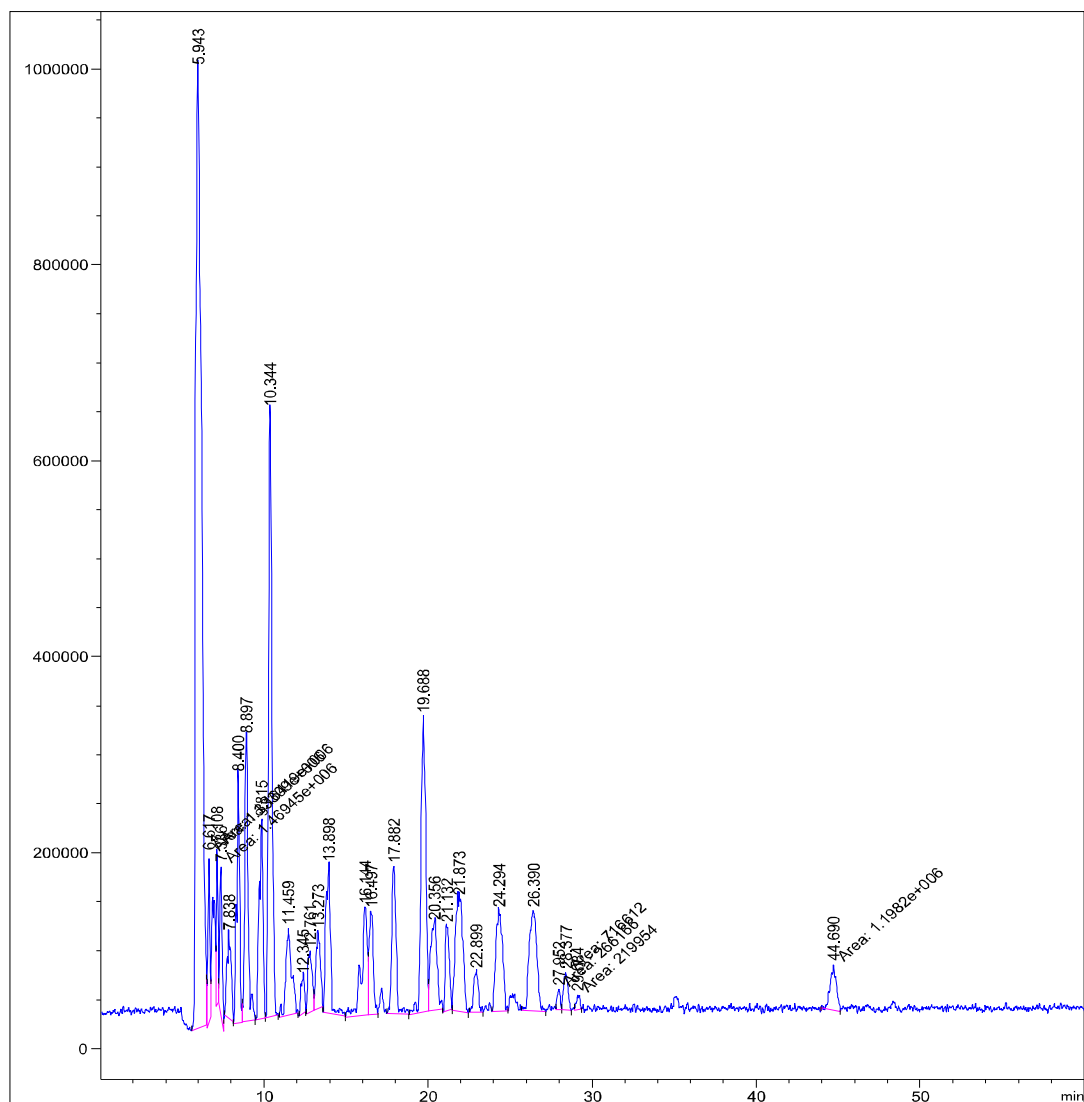


Figure 2 : LC/MS chromatogram of ethanolic extarct of *Premna coriacea*

Table 1 :

S.No.	Test performed	Premna Coriacea
1.	Alkaloids	+
2.	Carbohydrates	+
3.	Glycosides	+
	Test for cardiac glycosides	
i)	Keller kiliani test	-
ii)	Legal test	-
iii)	Baljet test	-
	Test for cyanogenetic glycosides	
i)	Sodium carbonate test	-
	Test for coumarin glycosides	
i)	Ferric chloride test	+
ii)	Flouresence test	+
	Test for anthraquinone glycosides	
i)	Borntragers test	-
4.	Terpenoids	-
	1. Liberman burchard test	-
	2. Salkowski test	-
	3. Antimony trichloride test	-
5.	Proteins	-
6.	Amino acids	-
7.	Steroids	+
8.	Flavonoids	+
	1. Shinoda test	+
	2. Sodium hydroxide test	+
	3. Lead acetate test	+
9.	Phenols	+
	1. Ferric chloride test	+
	2. Bromine water test	+
10.	Tannins	-
11.	Quinones	-
12.	Anthraquinones	-
13.	Saponins	+

Preliminary Phytochemical test carried out in ethanolic extract of *Premna coriacea*.

Table 2 : GC/MS Analysis of ethanolic extarct of *Premna coriacea* library search results

Karthikeyan *et al.* Phytochemical screening of ethanolic extract of *Premna coriacea* through an integrative GC-MS and LC-MS

Sl.no	Compounds	Retens ion time	First Scan	Max Scan	Last Scan	Peak height	Area %
3.	2,2'-Bioxirane	6.920	163	171	177	5447047	0.700%
4.	Propanoic acid, 2-oxo-, methyl ester	7.172	188	194	201	9622368	0.490%
5.	2-Cyclopentene-1,4-dione	9.440	399	406	423	3754264	0.398%
6.	1,2-Cyclopentanedione	11.148	515	565	601	3343841	1.365%
7.	Cyclopentanol	16.754	1067	1087	1107	3426722	0.767%
8.	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	18.418	1229	1242	1261	5448744	1.112%
9.	Isoborneol	18.713	1261	1269	1286	3706998	0.411%
10.	Cyclohexanol, 1-methyl-4-(1-methylethyl)-	19.153	1286	1310	1322	7494622	0.862%
11.	Methyl salicylate	19.534	1338	1346	1354	8360678	0.798%
12.	Ethyl hydrogen succinate	19.808	1354	1371	1385	3850456	0.946%
13.	Benzofuran, 2,3-dihydro-	20.878	1447	1471	1479	5021009	0.642%
14.	Benzaldehyde,4-(1-methylethyl)-	21.019	1479	1484	1506	1097830 5	1.103%
15.	2-Methoxy-4-vinylphenol	23.013	1651	1670	1683	2609968 9	2.128%
16.	Eugenol	24.106	1753	1772	1803	3698396 4	3.877%
17.	Caryophyllene	25.727	1912	1923	1930	4212804	0.362%
18.	Benzofuran-2-carboxaldehyde	26.363	1955	1982	1987	1098869 3	1.217%
19.	Benzaldehyde, 2-hydroxy-6-methyl	27.116	2041	2052	2064	1747292 7	2.610%
20.	Cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl)	27.926	2114	2127	2136	5904624	0.551%
21.	Naphthalene, 1,2,4a,5,8,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, [1S-(1 α ,4a β ,8a α)]	28.192	2144	2152	2157	7354072	0.719%
22.	1,3-Benzodioxole, 4-methoxy-6-(2-propenyl)	28.338	2157	2166	2179	7416027	1.169%
23.	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl	28.626	2188	2193	2204	4447377	0.367%
24.	3',5'-Dimethoxyacetophenone	29.372	2256	2262	2269	4113951	0.366%
25.	Dodecanoic acid	29.557	2269	2279	2289	4519110	0.559%
26.	Diethyl Phthalate	29.937	2309	2315	2325	8335120	0.803%
27.	Ledol	30.151	2325	2335	2342	3723354 3	2.885%
28.	1H-Cycloprop[e]azulen-4-ol, decahydro-1,1,4,7-tetramethyl-, [1ar-(1 α ,4 β ,4a β ,7 α ,7a β ,7b α)]-	30.357	2342	2354	2360	8656757	0.758%
29.	Megastigmatrienone	30.782	2386	2394	2404	5451491	0.645%
30.	7-Oxabicyclo[4.1.0]heptane, 1-(2,3-dimethyl-1,3-butadienyl)-2,2,6-trimethyl	33.422	2632	2639	2644	1014997 9	0.899%
31.	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	33.518	2644	2648	2655	6267037	0.742%
32.	Tetradecanoic acid	33.994	2681	2693	2703	1085593 1	1.381%
33.	Tetradecanoic acid, ethyl ester	34.401	2724	2731	2738	2144706 6	1.503%
34.	Dibutyl phthalate	36.769	2945	2951	2960	6160310	0.551%
35.	Hexadecanoic acid, methyl ester	37.096	2971	2982	2989	7356521	0.657%
36.	5-Isopropyl-2,8-dimethyl-9-oxatricyclo[4.4.0.0(2,8)]decan-7-one	37.482	3012	3018	3034	3951588	0.610%
37.	n-Hexadecanoic acid	38.085	3058	3074	3081	2436697 1	3.862%
38.	Ethyl 9-hexadecenoate	38.276	3088	3092	3098	3915958	0.331%

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39.	Hexadecanoic acid, ethyl ester	38.441	3098	3107	3113	6559236 8	5.086%
40.	Heptadecanoic acid, ethyl ester	40.290	3274	3279	3284	5391672	0.378%
41.	9-Octadecenoic acid (Z)-, methyl ester	40.403	3284	3290	3295	5426539	0.403%
42.	Phytol					5706573	
		40.634	3303	3311	3331	9	5.620%
43.	cis-Vaccenic acid					2606704	
		41.368	3367	3380	3387	5	4.673%
44.	9,12-Octadecadienoic acid, ethyl ester					4265476	
		41.513	3387	3393	3398	9	3.603%
45.	Ethyl Oleate					8823589	
		41.655	3398	3406	3410	5	8.193%
46.	Octadecanoic acid					1631999	
		41.741	3410	3414	3429	9	1.456%
47.	Octadecanoic acid, ethyl ester					3582291	
		42.110	3438	3449	3468	2	2.746%
48.	4a,7,7,10a-Tetramethyldodecahydrobenzo[f]chromen-3-ol	43.690	3589	3596	3603	1216059	0.973%
						4	
49.	4,8,12,16-Tetramethylheptadecan-4-olide	44.748	3686	3694	3705	3791219	0.493%
50.	Methyl 19-methyl-eicosanoate					1041088	
		45.480	3757	3763	3774	8	1.074%
51.	Phenol, 2,4-bis(1-phenylethyl)	46.915	3888	3896	3903	4063181	0.368%
52.	Phthalic acid, dodecyl pentyl ester					1298167	
		47.073	3903	3911	3915	4	0.971%
53.	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	47.453	3937	3946	3957	8942303	0.989%
54.	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	47.652	3957	3965	3972	1064538	0.877%
						9	
55.	Docosanoic acid, ethyl ester	48.611	4049	4054	4065	3638523	0.302%
56.	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	50.190	4188	4201	4221	1107552	3.500%
						5	
57.	Phthalic acid, bis(7-methyloctyl) ester					1118999	
		50.637	4238	4243	4264	2	1.109%
58.	Ethyl tetracosanoate	51.533	4318	4326	4337	4344471	0.375%
59.	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)	51.742	4337	4346	4351	5601838	4.319%
						5	
60.	Benzoic acid, 4-methoxy-, 2-methylpropyl ester	51.858	4351	4357	4371	1767170	1.349%
						8	
61.	1-Pyrrolidinebutanoic acid, 2-[(1,1-dimethylethoxy)carbonyl]- α -nitro-, 2,6-bis(1,1-dimethylethyl)-4-methoxyphenyl	52.133	4371	4382	4400	5203519	1.058%
62.	Octacosane					1045096	
		52.952	4436	4459	4468	9	1.197%
63.	Pyridine-3-carbonitrile, 2-[2-(3,4-dihydroxyphenyl)-2-oxoethylthio]-4-methoxymethyl-6-methyl	53.104	4468	4473	4478	4044009	0.487%
64.	Piperine					1007498	
		53.224	4478	4484	4490	8	1.079%
65.	1-Heptatriacotanol	54.355	4584	4589	4595	4266130	0.474%
66.	2(3H)-Furanone, 3,4-bis(1,3-benzodioxol-5-ylmethyl)dihydro-, (3R-trans)	55.572	4693	4703	4709	5044536	0.484%
67.	Tetratetracontane	56.132	4739	4755	4763	7520536	0.769%
68.	Vitamin E					1482943	
		56.667	4792	4805	4821	5	1.784%
69.	Stigmasterol	59.215	5026	5042	5060	6198109	0.987%
70.	γ -Sitosterol					1358086	
		60.812	5160	5191	5220	8	2.725%

Table 3 : LC/MS Analysis of ethanolic extarct of *Premna coriacea*

Sl.no	Time	Area	Height	Width	Area%	Symmetry
1	5.943	24694746	979473.8	0.3746	27.732	0.577
2	6.617	1333089	171848.6	0.1293	1.497	1.13
3	7.108	1164194	161690.2	0.12	1.307	0.859
4	7.336	1469449	150524.5	0.1627	1.65	0.62
5	7.838	1530701	86145.1	0.3079	1.719	0.93
6	8.4	3308741	252576.3	0.2031	3.716	1.213
7	8.897	4151053	290424.2	0.2271	4.662	0.801
8	9.815	3212708	202663.7	0.2855	3.608	1.351
9	10.344	8937027	618960.1	0.2288	10.036	0.799
10	11.459	2135424	88319.2	0.3626	2.398	0.692
11	12.345	606240.7	36971.3	0.2718	0.681	1.285
12	12.761	1131985	61918.6	0.2938	1.271	0.797
13	13.273	1486337	78450.8	0.3216	1.669	0.979
14	13.898	3093775	150581.7	0.3403	3.474	1.102
15	16.144	2770188	109523.3	0.3554	3.111	2.159
16	16.497	1890419	105466.2	0.2695	2.123	0.86
17	17.882	2730416	150166.7	0.2926	3.066	0.817
18	19.688	6087380	301017.8	0.3365	6.836	0.957
19	20.356	2493642	88005.2	0.4412	2.8	1.208
20	21.132	1507261	86624.9	0.2935	1.693	0.787
21	21.873	3753224	114664.5	0.5428	4.215	1.021
22	22.899	846144.8	40980.9	0.3415	0.95	1.037
23	24.294	2966601	102285.8	0.4993	3.332	0.856
24	26.39	3344730	102197.7	0.5529	3.756	1.092
25	27.952	266165.8	20679.8	0.2145	0.299	1.233
26	28.377	716612.1	37991.3	0.3144	0.805	1.166
27	29.084	219953.6	15253.8	0.2403	0.247	0.564
28	44.69	1198196	46643.3	0.4281	1.346	1.08

Discussion

In this study, the preliminary phytochemical test revealed the presence of Alkaloids, Carbohydrates, Glycosides, Steroids and Flavonoids. Through GCMS and LCMS analysis sixty-eight components was identified, in which twenty-three compounds have biological activity. The GC-MS analysis of *Premna coriacea* leaves revealed the presence of twenty-three phytoconstituents that contribute the medicinal property of the plant. The results of preliminary phytochemical analysis (Table 1) and GCMS and LCMS analysis results were shown in Table 2 & 3. The Chromatogram of GCMS and LCMS were shown in Figure 1 and 2. Constituents identified are listed in the table 1&2. The phytochemicals with pharmacological property identified are such as hexadecanoic acid, ethyl ester possess anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematocidal, insectifuge, anti-

histaminic, anti-eczemic, anti-acne, alpha reductase inhibitor, anti-androgenic, antiarthritic, anticoronary. Cosmetics/antipsychotic, medication/Antioxidant, hypocholesterolemic/nematicide, pesticide, anti-androgenic flavour, haemolytic, 5-Alpha reductase inhibitor.⁷⁻⁹ Propanoic acid, 2-oxo-, methyl ester has been used as flavor, fungicide, irritant, perfumery, pesticide.¹⁰ Methyl Salicylate possess analgesic and anti-inflammatory activities.^{11,12} Diethyl Phthalate possess antimicrobial, Antifouling.¹³ Phytol found to be effective indifferent stages of arthritis, antimicrobial, antiinflammatory, antioxidant, diuretic, antimicrobial, anticancer, anti-inflammatory, anti-diuretic, immunostimulatory and anti-diabetic, anti-mycobacterial activity against mycobacterium tuberculosis.¹⁴ Octadecanoic acid, ethyl ester possess antiinflammatory. ¹⁵ 2(4H)Benzofuranone,5,6,7,7atetrahydro4,4,7 atrimethyl have pharmacological activities like anti-inflammatory, analgesic Pesticide, Ant-Repellent, nematicide and antimicrobial property.^{16,17} Dodecanoic acid possess antifungal activity, antimicrobial/increasing HDL / fatty acid and Antieczemeic.¹⁸ Stigmasterol possess anti-inflammatory, inhibit tumor promotion and anti-HIV reverse transcriptase.^{19,20} Gamma.-Sitosterol have anti-diabetic, anti-angeogenic, anticancer, antimicrobial, anti-inflammatory, antidiarrhoeal, Pain killer used in Jaundice and antiviral. Cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4 hexenyl) possess antimicrobial agents. ^{20,22} Vitamin Epossess antidermatitic, Antileukemic, Antitumor, Anticancer, Hepatoprotective and Antispasmodic.²³ Benzofuran, 2,3-dihydro have antiangiogenic activity. ²⁴ Isoborneol, 2-Methoxy-4-vinylphenol, and 1,2-Cyclopentanedione are used as Flavour. Eugenol is used as an antiseptic, an anesthetic, antibacterial, antiviral effects and antiinflammatory action.²⁵ Caryophyllene is used as an anaesthetic, antifungal, antiseptic and antibacterial.²⁶ Ledol is an antifungal, toxic sesquiterpenoid, which exhibits expectorant and antitussive effects, and has been reported to increase blood pressure.²⁷ Tetradecanoic acid is used as larvicidal and repellent activity [28]. n-Hexadecanoic acid possess antibacterial and antifungal.²⁹ cis-Vaccenic acid up-regulates immunoglobulin synthesis.³⁰ Piperine possess sedative-hypnotic, tranquilizing, muscle-relaxing actions and used as an antiepileptic drug in treating different types of epilepsy. ³¹

Conclusion

The present study has revealed the presence of various phytochemical constituents of ethanolic leaves extract of *Premna coriacea*, which have potent pharmacological and biological property. This will help the researchers to carry out the research based on the active principles present and to conform the pharmacological activity with mechanism, this may support the use the plant in folk medicine. Based on the preliminary study we suggest that the *Premna coriacea* could be a potential source for anti-inflammatory, analgesic, antimicrobial, antifungal and anticancer agents.

Acknowledgments

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Conflicts of Interest

The author declares no conflict of interest

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