



Phytochemical profile and medicinal potentials of *Lannea Coromandelica* stem

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ABSTRACT

In the history of humanity, great ancestors of Indian sub-continent used plants that possessed unique medicinal properties. They identified the plants from jungles and derived crude drugs out of them for treating infectious diseases. This treatment process was a tradition. In the recent century, this plant-based drug extraction, processing and refining began scientifically by pharmaceutical industries. In this new era, plants with medicinal features are being used in curing broad spectrum of diseases. *Lannea Coromandelica* (L.C.) is a medicinal plant that belongs to this category. Tribals extensively used it in treating various infectious diseases and common injuries. There have been very few studies on the leaves, barks, flowers, gums and mucilage of this tree. But no preliminary phytochemical composition of L.C. stem has been studied. Therefore, the current work focusses on the screening of the phytochemical profile of the stem of L.C. by the sequential ethanolic extract. Stems of L.C. were procured from Mettur, Tamil Nadu. Thirteen different components were qualitatively analyzed using standard procedures from 100g of L.C. stem extract. In this study, seven components were identified, and their percentage was estimated. Using GC-MS, 50 components were identified of which Pentadecanoic acid, 14-methyl-methyl ester (1.0%) was the major component. The presence of these components in L.C. stem extract can be used in the treatment of different ailments through their antioxidant, anti-arthritic, anti-diabetic, anti-inflammatory and antimicrobial activities.



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INTRODUCTION

Life on earth predominantly depends upon the natural resources for its survival. Medicinal plants being

one of the essential natural resources, its purpose and utility have been well recognized by our great ancestors for many centuries. They used medicinal plants to cure infectious diseases in human beings and animals by traditional methods. Even today in this digital world, medicinal plants contribute extensively for various developments in the form of new drug discovery, drug formulas (Das, 2014) and antibiotics for treating diseases. Globally, there are many rare species of plants still unexplored for its medicinal properties. In India, one such rare medicinal plant is *Lannea Coromandelica* (L.C.) belonging to the Anacardiaceae family (Vadivel *et al.*, 2012; Juan Yun *et al.*, 2014). Traditionally, this plant was used as folk medicine by local tribes in different parts of the India Lee *et al.* (2017);

Islam *et al.* (2018) and also in other countries like China, Srilanka, Bangladesh (M *et al.*, 2016; Islam and Tahara, 2000), Africa (Premjanu and Jaynthy, 2014; Islam *et al.*, 2018), Nepal and Pakistan. It is widely known as Indian ash tree (Jain *et al.*, 2013; Venkatesham *et al.*, 2014) and by different names based on the growing location of the plant. Tribal people used various parts of this plant for treating pain (Kumar and Jain, 2015; Imam and Moniruz-zaman, 2014), wounds (Sathish *et al.*, 2010), cuts, ulcers (Tahara *et al.*, 2002; Ahmed *et al.*, 2013), gastritis, jaundice (Rahmatullah *et al.*, 2010), gout, sprains, diarrhoea (Majumder and Md, 2013), sore eyes, leprosy, impotence (Vadivel *et al.*, 2012) and dysentery. (Pavithra, 2018) in the review article illustrated medicinal importance of Odina, Wodier. She has explained that ethanol extracts of the plant showed to produce a pharmacological response which includes antioxidant activity, antimicrobial, wound healing, anti-diabetic, anti-arthritic activity. It has been found by Reddy and Joy (2011) that L.C. comprised of Flavonoids, tannins, polyphenols, Gallic acid, mucilage, gums and some sterols. The flowers and leaves of L.C. were found to constitute Ellagic acid and Quercetin-3-arabinoiside (Subramanian and Nair, 1971). No preliminary studies have been reported so far on the stem of Lannea Coromandelica. Therefore, we have delved the phytochemical compositions of L.C. stem for its pharmacological and therapeutic applications.

MATERIALS AND METHODS

Identification and Collection

Fresh samples of L.C. stems were procured in April 2019 from Siddha Medicinal Plants, Mettur, Tamil Nadu, India with the help of Dr Padma Sorna, Research officer – Botanist. The stems of the plant along with leaves, flowers and other plant parts were submitted to Siddha Medicinal Plants Garden, Mettur as herbarium. The sample specimen of L.C. was identified and authenticated by Dr P. Radha, Research officer – Botany.

Flavonoid estimation

Aluminium chloride was used to estimate flavonoid found in the L.C. stem extracts in which Quercetin was used as standard described by Sakanaka *et al.* (2005). In Ethyl acetate (10 mg/ ml) Quercetin and extracts were prepared. In the test tube, 0.9 ml of distilled water was added along with 0.1 ml of L.C. extract and mixed with 75 μ L of 5% sodium nitrate solution. This mixture was allowed to remain for about 6 minutes, and after that 10 μ L of 10%, Aluminium chloride was added. After 5 minutes, 0.5 ml of 1M Sodium hydroxide was added to the mixture.

To the mix, distilled water was added to make it up to 2.5 ml and thereby the mixture was shaken well. UV-Visible absorbance was measured at 510 nm. The estimation of flavonoid was expressed in terms of Quercetin equivalence (Q.E.) μ g/mg of L.C. extract.

Extract preparation

The stems of Lannea Coromandelica were shade dried for one month and Coarse powdered by using a mixer grinder. The powder was stored at room temperature and covered by plastic wrap to avoid moisture absorption. The finely powdered sample was subjected to sequential extraction using ethanol.

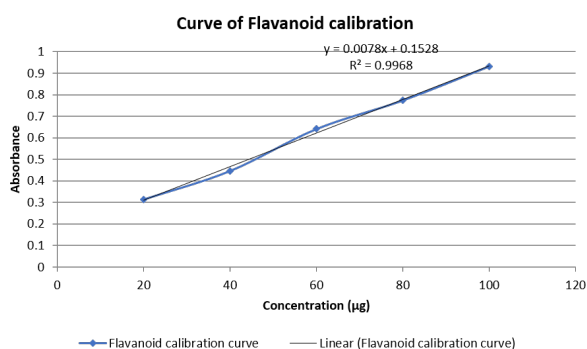


Figure 1: The standard calibration curve of Quercetin

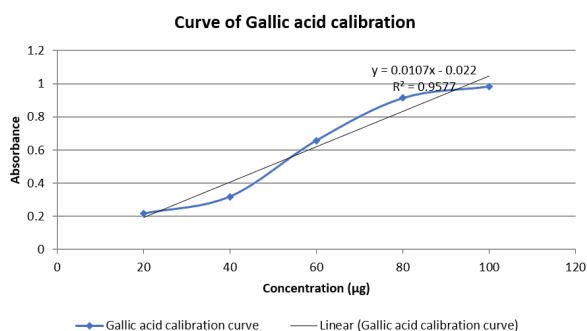


Figure 2: The standard calibration curve of Gallic acid

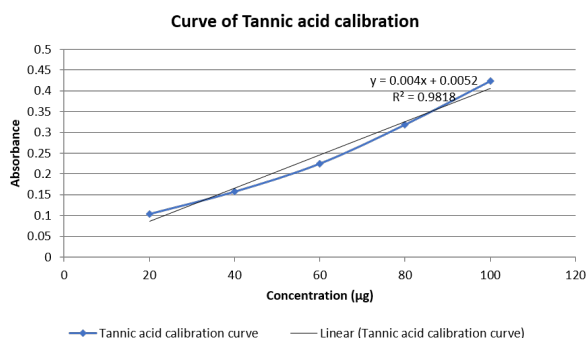


Figure 3: The standard Tannic acid calibration curve

Table 1: Phytochemical Qualitative analysis

S.No	Test	Procedure	Observation	Ethanolic extraction of L.C Stems
1.	Tannins	Extract + 5% ferric chloride	Dark blue or greenish black	+
2.	Saponins	Extract + distilled H ₂ O	Formation of 1cm layer of foam	+
3.	Flavonoids	Extract + NaOH	Yellow color	+
4.	Alkaloids	Extract + HCL, Mayer's reagent	Green color or white precipitate	+
5.	Quinones	Extract+ Conc. H ₂ SO ₄	Red color	-
6.	Glycosides	Extract + chloroform + 10% NH ₃	Pink color	-
7.	Terpenoids	Extract + chloroform + Conc. H ₂ SO ₄	Red brown color	+
8.	Phenols	Phenol Ciocalteau's reagent 15% Na ₂ CO ₃	Blue or green color	+
9.	Coumarin	Extract + 10% NaOH	Yellow color	-
10.	Steroids	Chloroform + Conc. H ₂ SO ₄	Appearance of brown ring	-
11.	Phlobatannins	Extract + 2% HCL	Red color precipitate	-
12.	Anthraquinones	Extract + 10% NH ₃	Pink color precipitate	+
13.	Cardiac Glycosides	Extract + glacial acid + 5% ferric chloride Conc.H ₂ SO ₄	Formation of brown ring interface	-

Table 2: Phytochemical Quantitative analysis

S.No	Estimation	Stem %
1.	Flavonoids (Quercetin)	0.30%
2.	Phenols (Gallic acid)	3.02%
3.	Tannins(Tannic acid)	23.7%

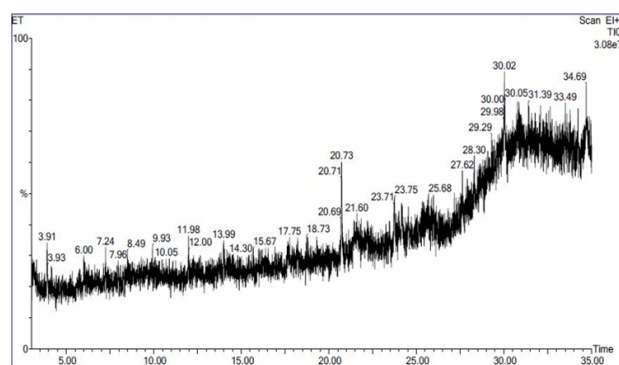


Figure 4: Gas chromatogram of L.C. stem



Figure 5: Chemical structure of compound

Table 3: Chemical compounds identified by GC-MS

S.No	RT	Area %	Compound name
1	3.013	0.369	1,4-Benzenediol, 2,6-bis(1,1-dimethylethyl)-
2	3.153	0.475	6-Amino-5-cyano-4-(5-cyano-2,4-dimethyl-1Hpyrrol-3-yl)-2-methyl-4H-pyran-3-carboxylic acid ethyl ester
3	3.194	0.257	Coumarin-6-ol, 3,4-dihydro-4,4-dimethyl-5,7-dinitro-
4	10.066	0.229	Carda-4,20(22)-dienolide, 3-[(6-deoxy-3-O-methylà-L-mannopyranosyl)oxy]-14-hydroxy-, (3à)-
5	11.98	0.343	Phenol, 3,5-bis(1,1-dimethylethyl)-
6	13.99	0.307	d-Mannitol, 1-decylsulfonyl-
7	18.20	0.340	Octadecane, 3-ethyl-5-(2-ethylbutyl)-
8	18.7	0.229	1-Benzazirene-1-carboxylic acid, 2,2,5a-trimethyl-1a-[3-oxo-1-butenyl] perhydro-, methyl ester
9	18.79	0.243	18-Norcholest-17(20),24-dien-21-oic acid, 16-acetoxy-4,8,14-trimethyl-3,11-dioxo-, methyl ester
10	20.72	1.022	Pentadecanoic acid, 14-methyl-, methyl ester
11	21.42	0.333	Urea, N-[5-(ethylsulfonyl)-1,3,4-thiadiazol-2-yl]-N, N'-dimethyl-
12	21.47	0.327	Taxa-4,11-diene
13	21.54	0.229	à-d-Xylopyranoside, methyl-2,3,4-tris-O-[9-borabicyclo[3.3.1]non-9-yl]-
14	21.626	0.302	16-Nitrobicyclo[10.4.0]hexadecan-1-ol-13-one
15	22.191	0.329	Milbemycin B, 6,28-anhydro-15-chloro-25-isopropyl-13-dehydro-5-O-demethyl-4-methyl-
16	23.72	0.243	Fumaric acid, tetradec-3-enyl tridecyl ester
17	23.74	0.354	Pregn-4-ene-3,20-dione, 17,21-dihydroxy-, bis(Omethyloxime)
18	24.02	0.354	Milbemycin B, 6,28-anhydro-15-chloro-25-isopropyl-13-dehydro-5-O-demethyl-4-methyl-
19	24.13	0.399	Aspidospermidine, 1,2-didehydro-, (5à,12à,19à)-
20	24.18	0.229	(5á)Pregnane-3,20á-diol, 14à,18à-[4-methyl-3-oxo-(1-oxa-4-azabutane-1,4-diyl)]-, diacetate
21	24.24	0.25	Echitamine
22	24.53	0.42	Strychane, 1-acetyl-20à-hydroxy-16-methylene-
23	28.68	0.283	2-(Ethyl)oxybenzylidene acetophenone
24	29.72	0.289	Carbonic acid, (1,2,3,4-tetrahydro-6-methoxycarboniloxy-3-methyl-1-naphthylidenamino)-, methyl diester
25	29.83	0.246	1-Benzazirene-1-carboxylic acid, 2,2,5a-trimethyl-1a-[3-oxo-1-butenyl] perhydro-, methyl ester
26	29.86	0.228	2-Azatricyclo[3.2.0.0(2,4)]hept-6-ene-5-carboxylic acid, 1,6,7-tri-t-butyl-4-phenyl-, t-butyl ester
27	29.92	0.283	Milbemycin B, 6,28-anhydro-15-chloro-25-isopropyl-13-dehydro-5-O-demethyl-4-methyl-
28	29.99	0.338	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-
29	30.02	0.724	Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)-
30	30.09	0.37	Acetic acid, 17-acetoxy-4,4,10,13-tetramethyl-7-oxo-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl (ester)

Continued on next page

Table 3 continued

31	30.164	0.233	Corynan-17-ol, 18,19-didehydro-10-methoxy-
32	30.504	0.234	2-Nonadecanone 2,4-dinitrophenylhydrazine
33	30.69	0.404	2-[2-(4-Chlorophenyl)-3-morpholin-4-yl-3-thioxopropenylamino] Acetamide
34	30.74	0.265	Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)-
35	30.84	0.594	2-(Ethyl)oxybenzylidene acetophenone
36	30.95	0.275	Curan-17-oic acid, 2,16-didehydro-20-hydroxy-19-oxo-, methyl ester
37	31.01	0.338	1H-Cyclopropa[3,4]benz[1,2-e]azulene-5,7b,9,9atetrol, 1a,1b,4,4a,5,7a,8,9-octahydro-3-(hydroxymethyl)-1,1,6,8-tetramethyl-, 5,9,9atriacetate, [1aR-(1aà,1bá,4aá,5á,7aà,7bà,8à,9á,9aà)]-
38	31.09	0.246	Methyl glycocholate, 3TMS derivative
39	31.18	0.253	Butanoic acid, 4-chloro-, 1,1a,1b,4,4a,5,7a,7b,8,9-decahydro-4a,7b-dihydroxy-3-(hydroxymethyl)-1,1,6,8-tetramethyl-5-oxo-9aH-cyclopropa[3,4]benz[1,2-e]azulene-9,9a-diyl ester, [1aR-(1aà,1bá,4aá,7aà,7bà,8à,9á,9aà)]-
40	31.42	0.457	Aspidofractinine-1-carboxaldehyde, 3-oxo-, (2à,5à)-
41	31.62	0.299	5-Methyl-2-N-methylaminobenzophenone Semicarbazone
42	31.73	0.553	Strychane, 1-acetyl-20à-hydroxy-16-methylene-
43	32.03	0.348	Methyl glycocholate, 3TMS derivative
44	32.21	0.353	6-Amino-5-cyano-4-(5-cyano-2,4-dimethyl-1Hpyrrol-3-yl)-2-methyl-4H-pyran-3-carboxylic acid ethyl ester
45	32.66	0.254	1,4-Benzenediol, 2,6-bis(1,1-dimethylethyl)-
46	33.49	0.364	2,4,6,8,10-Tetradecapentaenoic acid, 9a-(acetyloxy)-1a,1b,4,4a,5,7a,7b,8,9,9a-decahydro-4a,7b-dihydroxy-3-(hydroxymethyl)-1,1,6,8-tetramethyl-5-oxo-1H-cyclopropa[3,4]benz[1,2-e]azulen-9-yl ester, [1aR-(1aà,1bá,4aá,7aà,7bà,8à,9á,9aà)]-
47	33.65	0.233	5,7,9(11)-Androstatriene, 3-hydroxy-17-oxo-
48	34.61	0.228	1H-Cyclopropa[3,4]benz[1,2-e]azulene-5,7b,9,9atetrol, 1a,1b,4,4a,5,7a,8,9-octahydro-3-(hydroxymethyl)-1,1,6,8-tetramethyl-, 5,9,9atriacetate, [1aR-(1aà,1bá,4aá,5á,7aà,7bà,8à,9á,9aà)]-
49	34.69	0.894	(22S)-6á,11á,21-Trihydroxy-16à,17àpropylmethylenedioxypregna-1,4-diene-3,20-dione
50	34.77	0.269	Butanoic acid, 1a,2,5,5a,6,9,10,10a-octahydro-5ahydroxy-4-(hydroxymethyl)-1,1,7,9-tetramethyl-6,11-dioxo-1H-2,8amethanocyclopenta[a]cyclopropa[e]cyclodecen-5-yl ester, [1aR-(1aà,2à,5á,5aá,8aà,9à,10aà)]-

The extracts were filtered and concentrated to a dry mass separately, evaporated with the help of rotary evaporator at 35° C and stored in the refrigerator for the phytochemical analysis.

Phenol estimation

Folin Ciocalteu colorimetric method was used to estimate phenol compounds in the L.C. ethanolic extracts. They were with Gallic Acid as standard (10mg/10ml) by Kumar *et al.* (2009). To different test tubes, 20 to 100 μ l of standard solutions were added. 5ml of Folin-Ciocalteu (1:10 dilution) was added to a separate test tube containing extract at a concentration of 10 mg/ml, and the mixture was shaken thoroughly. For half an hour, the mixture was incubated after adding 4ml of 0.7 M sodium carbonate. UV-Visible absorbance was measured at 765nm. The estimation of phenol was expressed in terms of Gallic acid equivalence (G.E.) μ g/mg of L.C. extract.

Tannin estimation

Folin-Ciocalteu method was used to estimate the tannin found in the ethanolic extracts of L.C. by Harborne (1973). 1 ml of 35 % Na₂CO₃, 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu reagent was added to the test tube. To this mixture, 0.1 ml of the sample extracts containing 1 mg were added and by adding distilled water to this mixture, it was made up to 10ml. The mixture was kept at room temperature for 30 minutes after being thoroughly shaken. 20, 40, 60, 80 and 100 μ g/ml were used as standard reference solutions for tannic acid. UV/Visible absorbance was measured at 725 nm against the blank solution. The estimation of tannin was expressed in terms of mg of Tannic acid equivalence (T.E.) μ g/mg of L.C. extract.

GC-MS analysis of L.C. stem

Using Turbo mass software (5.2.0) and Perkin Elmer Clarus 500, the GCMS analysis was performed. A capillary column of 250 μ m column I.D. and 30m length with Elite 5MS (5% phenyl 95% dimethyl polysiloxane) was used in the Perkin Elmer Clarus 500. The temperature for the mass analyzer was 8°C/min to 200°C. The oven temperature was retained at 50°C with 1-minute holding time in the initial stage of GC. The Helium gas was used as a carrier with flow rate at 1ml/minute and 1:10 as the split ratio, Mass range was 40-600 amu, the energy of electron used was 70ev, Electron Ionization (E.I.) was used in the MS and the source temperature Transfer line was 180°C, 200°C, 1.0 μ L was injected for the analysis.

RESULTS AND DISCUSSION

The preliminary phytochemical components from the analysis showed that tannins, phenols, flavonoids, alkaloids, saponins, terpenoids and anthraquinones were present in the ethanolic extract of L.C. stems. Table 1 shows the results obtained from the qualitative analysis.

The effect of Flavonoids on the cell membrane permeability and inhibiting enzymes found on the membranes like phospholipase A2 and ATPase has been reported by Hausteen (1983). The medicinal uses of alkaloids found in the plant stems have been known for centuries, and among them, cytotoxicity is one of their biological properties (Nobori *et al.*, 1994). The presence of terpenoid serves as anti-inflammatory and anti-fungal properties. The following inferences have been identified,

1. The tannins present in the plant stem L.C. exhibits anti-cancer and anti-inflammation properties.
2. The phenols detected from the stems have anti-oxidative properties.
3. Saponins compound found in L.C. stems known to possess significant anti-cancer properties.

In this present work, different compounds showed various percentages which are mainly due to the geographical location of the plant.

The total Flavonoid content from stems of L.C. by the ethanolic extract was 3.05 μ g/mg (0.30%) expressed in Quercetin equivalence. Figure 1 shows the standard calibration curve of Quercetin. In the stems of L.C., the phenolic content was estimated to be 30.2 μ g/mg (3.02%) expressed in Gallic acid equivalence, and the calibration curve is shown in Figure 2. The tannin content in this study was found to be higher 237.5 μ g/mg (23.7%) expressed in tannic acid equivalence. The results of tannin standard calibration curve are shown in Figure 3 and given in the Table 2.

Gas Chromatography-Mass Spectroscopy (GCMS)

The GCMS analysis (Figure 4) from L.C. stem illustrates peaks showing a wide range of compounds. An inceptive inquiry of the stem revealed the identification of fifty compounds. Results of the compounds are given in Table 3. The primary component was Pentadecanoic acid, 14-methyl-methyl ester (1.0%) (Figure 5) and other compounds were present in traces. The different compounds present in the L.C. stem is mainly due to climatic and geographical location of the plant (Policegoudra *et al.*, 2012).

CONCLUSION

The presence of these chemical (Flavonoid, alkaloid, saponin, terpenoid, phenol and tannin) components from phytochemical screening in L.C. stem extract can be used in the different ailments through their anti-inflammatory, anti-cancer, anti-arthritis, antioxidant and antimicrobial properties. Further exploration of the L.C. stem could be achieved in the future by isolation and detecting its active compounds.

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Conflict of Interest

Nil

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