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DETECTION OF ACTIVE CONSTITUENTS FROM THE LEAF EXTRACT OF *LANNEA COROMANDELICA* BY GC-MS TESTING AND ASSESSMENT OF ITS PHARMACOLOGICAL ACTIVITY

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ABSTRACT: *Lannea coromandelica* (Anacardiaceae) is a tropical tree commonly called as Indian ash tree. Its various parts is said to possess medicinal property and used in Ayurveda and other ancient system of medicine for the treatment of various ailments including treatment of inflammation, gout, cholera, leprosy, vaginal troubles etc. Its Pharmacological study revealed anti-inflammatory, antimicrobial, hypotensive, anticancerous activities. Leaves of *Lannea coromandelica* were collected from Azhagar malai, Madurai. The sample specimen of *Lannea coromandelica* was identified and authenticated by Dr. Jaya Raman, Taxonomist, Chennai. The present work was carried out to identify some of the phytocomponents present in the ethanolic leaf extract of the *L. coromandelica* by GC-MS technique, to ascertain the medicinal properties of the plant and also to assess its anti-oxidant and anti-arthritic potential of ethanolic leaf extract of *Lannea coromandelica*. The results revealed that ethanolic extract is mainly composed of oxygenated hydrocarbons and predominantly phenolic hydrocarbons. These different active phytochemicals may be responsible for wide range of activities, which may help in the protection against incurable diseases. The future *in vivo* investigation should be carried out to confirm the activity in animal models.

INTRODUCTION: Since times immemorial nature was being a font of medicinal agents. India is one of the richest countries in the world in stare to possessions of medicinal plants. As they are short of adequate scientific documentation, the conventional Indian system of medicine chiefly in scientific knowledge. *Lannea coromandelica* prevalently known as Indian Ash tree was found in isolated places and in jungles all over India principally southern part of India. *Lannea coromandelica* belongs to Anacardiaceae family which has diversity of medicinal uses.

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Linguistic variations show the way in a choice of names to the plant. It is used as folk medicine to treat fever, dyspepsia, general debility, gout, dysentery, sore eyes, leprosy, sprains, ulcers, inflammations, Impotency, wounds and much more disorders. Generally leaves, bark, stem and gum of *Lannea coromandelica* are used to treat an assortment of diseases. Some say it is as important as Neem as it has amity number of uses. It was expansively analysed for its chemical components and pharmacological actions [1]. *Lannea coromandelica* was found to be precious anti-inflammatory agent and wound healing agent [2] and anti-bacterial [3] as it is assumed in ayurveda.

Since there is no further systematic, scientific, research work has been executed on *in vitro* anti-arthritic activity of *Lannea coromandelica* leaves, this study has been engaged for the existing research work.

MATERIALS AND METHODS:

Collection of Plant:

The plant leaves were collected from Madurai, Tamilnadu, India and it was examined , recognized and autentified by Professor P. Jayaraman , Plant Anatomy Research Centre, Chennai. The Leaf was dried in shade for 15 days and pulverised in plant mill and accumulated in air tight box for future use.

Preparation of Extract:

The Leaf powder was subjected to successive extraction by cold maceration for 72 hours, 48 hours and then for 24 hours at room temperature. The leaf powder was consecutively treated with 200 ml of solvents (petroleum ether, benzene, chloroform, ethyl acetate and Ethanol). Solvent was distilled from the extract under reduced pressure.

Phytochemical screening of the leaf extract:

The primary and secondary metabolites were recognized by performing a variety of biochemical tests. The leaf extract was assessed for the existence of phenols, glycosides, quinones, terpenoids, alkaloids, saponins, flavanoids, triterpenoids, steroids and tannins.

Anti-oxidant activity:

Protocol for Nitric oxide scavenging assay:

Sodium nitroprusside (5M) In phosphate – buffered saline(1×PBS pH 7.4) was assorted in the midst of 3 ml of different plant extracts (different concentrations that is 0.25, 0.50, 1.0, 1.5, 2.0, 2.5 mg /ml) and incubated at 25°c for 150 min. The samples from them were reacted with 1.5 ml of Griess reagent. The absorbance was interpreted at 546nm. Ascorbic acid was taken as optimistic control. The scavenging percentage of nitric oxide of plant extracts was calculated via the following formula:

NO scavenging (%) = [(absorbance of control-absorbance of test sample)/ (absorbance of the control) ×100

Protocol for reducing power:

Distinct amount of the extract (62.5, 125, 250, 500, 1000, 2000 µg/ml))and standard drug Ascorbic acid at equivalent concentration , 2.5ml phosphate buffer pH 6.6 together with 2.5ml of 1% potassium ferricyanide were incubated at 50°c for 20 minutes, 2.5ml of 10% Trichloroacetic acid (TCA) were supplemented to the mixture. Centrifuge for 10 minutes at 3000 r.p.m. Following the centrifugation, supernatant liquid of 2.5ml were diluted with 2.5ml of water and traumatized with 0.5ml freshly prepared 0.1% ferric chloride. The absorbance was measured at 700nm.

Augmented absorbance of the response of mixture is a sign of amplify in reducing power, which is calculated using the formula,

$$\% \text{ increase in Reducing Power} = [A_{test} / A_{blank} - 1] \times 100$$

Anti-arthritic activity:

Inhibition of protein denaturation method:

0.5ml of Test solution, Test control solution, Product control solution, Standard solution was prepared. Various concentrations (50, 100, 250, 500, 1000, 2000µ/ml) of test dugs and standard drug diclofenac sodium (50, 100, 250, 500, 1000, 2000µ/ml) were prepared. 1N HCl was used to adjust the pH to 6.3 for all the above solutions. The samples were incubated at 37°c for 20 minutes and the temperature was increased to keep the samples at 57°c for 3 minutes. After cooling, 2.5 ml of phosphate buffer was added to the above solutions. The absorbance was measured at 416nm [4]. The control represents 100% protein denaturation. The percentage inhibition of protein denaturation can be calculated as

Percentage Inhibition =

100 – [{(optical density of test control – optical Density of product control)/optical density of test solution} × 100

The control represents 100% protein denaturation

Gas chromatography-mass spectroscopy (GC-MS) analysis:

The additional defined information in qualitative examination can be attained by Gas-chromatography together with mass spectrometry (GC-MC) [5]. The GC-MS analysis of the *Lannea coromandelica* leaf extract within absolute alcohol was achieved using a Clarus 500 Perkin Elmer gas chromatography along with a Elite-5 capillary coloumn (5%phenyl 95% dimethyl polysiloxane) (30nm × 0.25mm ID × 0.25 µmdf). Mass detector of Turbo mass gold company was functioning in EI mode. Carrier gas used was Helium at a run time of 1 ml/min. The injector was controlled at 250°c and

the oven temperature was automated as follows;50°c at 8°c/min to 200°c(5min) at 7°c/min to 290°c(10min) at 110°c(2min)to 200°c at 10°c/min to 280°c(9min) at 5°c/min. Total operation time is 36 minutes. The effective volatile constituents present in the extract was achieved using GC-MS and the interpretation on mass spectrum of GC-MS was concluded using the Database of Indian Institute of Crop Processing Technology (IICPT) having more than 75,000 patterns. The plant extract was liquefied in ethanol and pass through a filter with polymeric solid phase extraction (SPE) column and investigated in GC-MS for different components.

RESULTS AND DISCUSSION:

Preliminary phytochemical studies:

First round of phytochemical learning of the successive extracts of the leaves of *Lannea coromandelica* was made. The extracts exposed the occurrence of metabolites like flavonoids, steroids, saponins, terpenoids, triterpenoids, glycosides, alkaloids, phenols and tannins, which is specified in **Table 1**.

TABLE 1: PRELIMINARY PHYTOCHEMICAL ANALYSIS OF *LANNEA COROMANDELICA*

Name of test	Pet ether	Benzene	Chloroform	Ethyl acetate	Ethanol
Quinones	-	-	+	-	-
Glycosides	-	-	-	-	+
Terpenoids	-	+	-	+	+
Alkaloids	-	-	+	+	-
Saponins	-	-	-	-	+
Flavonoids	-	-	-	-	+
Triterpenoids	-	+	-	+	-
Steroids	+	+	-	+	-
Tannins	-	-	-	-	+
Phenols	-	-	-	-	+

Anti-oxidant activity:

Nitric Oxide Radical Scavenging Activity:

Nitric Oxide (NO) is a forceful pleiotropic negotiator in nomerous physiological process like smooth muscle relaxation, inhibition of platelet aggregation, neuronal signalling and cell mediated toxicity parameter. This free radical is a diffusible one, which plays a lot of roles as an effect or molecule in many parts biological systems together with neuronal messenger, anti-microbial, anti-inflammatory and anti-tumor activities [6]. Furthermore Nitric Oxide plays a key position in repairs of wound by influence angiogenesis and inflammation [7]. Hence this study will assist us to considerate the role of our extract in various physiological scheme such as diminution of inflammation, smooth muscle relaxation and platelet aggregation.

The scavenging activity of the extract in opposition of nitric oxide released by sodium nitroprusside was inspected. Nitric oxide (NO) scavenging assay is based on the scavenging capability of the extracts as well as Ascorbic acid, which is used as standard. The Scavenging of NO was found to amplify in dose dependent manner. Maximum inhibition of NO was observed in the extracts of uppermost concentration (25 mg/ml) for ethanol extract. At this highest concentration, inhibition was found to be 83.43 for extract and 82.12 for Ascorbic acid which serve up as standard. The results are presented in **Table 2**.

TABLE 2: NITRIC OXIDE SCAVENGING EFFECT OF SUCCESSIVE LEAF EXTRACT OF *LANNEA COROMANDELICA*

S.no	Concentration(µg/ml)	% inhibition					
		Ascorbic acid	Pet ether	Ethyl acetate	Benzene	Chloroform	Ethanol
1	0.25	49.12	47.80	52.27	49.85	52.27	52.48
2	0.50	58.28	50.41	59.38	50.83	53.22	59.12
3	1.0	68.34	57.18	63.37	57.92	64.15	69.34
4	1.5	73.76	66.20	68.30	61.56	71.30	72.34
5	2.0	80.81	71.39	72.32	68.05	74.97	79.16
6	2.5	82.12	77.77	76.94	78.16	79.87	83.43

Reducing power activity:

Reducing power is connected with antioxidant activity which might serve as a considerable reflection of the antioxidant activity [8]. Reducing power effect of the components was considered as a sign of that, they are electron donors and can trim down the oxidized intermediates of lipid peroxidation processes, as a result , they can act as primary and secondary antioxidants [9]. In this assay, the colour of the test solution was yellow and it vary to a variety of shades of blue and green depending on theability orthe reducing power of each compound. Existence of reducers causes the translation of the Fe3+/ferri cyanide complex used in this method. Among each and every one of the extracts ethanolic leaf extract of *Lannea coromandelica* exhibited respectable activity with utmost inhibition of about 79.3 % at a concentration of 2000 µg/ml when paralled to standard Ascorbic acid at the same concentration for which the percentage inhibition is 86.6%. The results are presented in **Table 3**.

TABLE 3: REDUCING POWER ACTIVITY OF ETHANOLIC LEAF ECTRACT OF *LANNEA COROMANDELICA*

S. no	Concentration (µg/ml)	%inhibition					
		Ascorbic acid	*Lannea coromandelica*				
			Pet ether	Benzene	Chloroform	Ethyl acetate	Ethanol
1	62.5	47.3	36.2	45.6	29.7	44.6	43.1
2	125	58.6	45.8	56.8	34.9	48.3	50.6
3	250	69.1	51.3	59.4	45.9	56.7	59.2
4	500	75.9	59.8	60.5	49.4	59.9	68.8
5	1000	80.4	62.8	56.0	53.9	63.3	70.4
6	2000	86.6	68.5	66.5	62.7	69.1	79.3

Anti-arthritic activity:

Inhibition of Protein Denaturation Method:

Protease inhibitory activity of ethanolic leaf extract of *Lannea coromandelica* was estimated as described in the literature [10]. Diclofenac sodium was included as standard. *In vitro* Anti-Arthritic assay was done by inhibition of protein denaturation method. The highest percentage inhibition of ethanolic extract of *Lannea coromandelica* was about 81.08% at concentration of 1000µg/ml in contrast to standard which was about 89.36% at same concentration. The results were exposed in the **Table 4**.

TABLE 4: ANTI-ARTHRITIC ACTIVITY OF ETHANOLIC LEAF EXTRACT OF *LANNEA COROMANDELICA* BY PROTEINASE INHIBITION.

S.no	Concentration(µg/ml)	% inhibition	
		Diclofenac sodium	*Lannea coromandelica*
1	50	44.1 ± 0.34	34.26 ± 0.14
2	100	54.3 ± 0.20	40.28 ± 0.27
3	250	58.10 ± 0.12	57.23 ± 0.18
4	500	68.64 ± 0.40	61.66 ± 0.16
5	1000	73.27 ± 0.25	70.14 ± 0.32
6	2000	89.36 ± 0.31	81.08 ± 0.64

Gas Chromatography-Mass Spectroscoy (GC-MS) analysis:

GC-MS chromatogram of the ethanolic leaf extract of *Lannea coromandelica* (**Fig. 1**) illustrate peaks indicating the presence of a wide range of compounds. The compound recognized in GC-MS analysis was benzyl alcohol (8.3%) , DL-Arabinitol (5.69%), phenol, 2-methoxy-4-(1-propenyl) (2.84%), phenol, 2, 4-bis(1,1-dimethylethyl) (1.28%), 4H-1-benzpyran, 4, 4, 5, 8 –tetramethyl (1.42%), 2, 6, 10 – dodecatriene - 1- imine - 3, 7, 11 - trimethyl - 1 (1%), 1, 6-Anhydro – β – D - glucopyranose (11.38%), 5 – Isopropenyl – 2 - methylcyclopent-1-enecarboxaldehyde (1.28%), Amyl nitrite (2.84%) , 1, 1'-bicyclohexyl, 2-methyl-trans - (7.11%), 3 - methyl - 5 - (2, 6 - dimethylheptyl) - 1, 5 - pent - 2 - enolide (2.84%), cyclohexanecarboxylic acid, 4 – propyl – 4 - methoxyphenyl ester (15.65%), 3, 7, 11, 15-tetramethyl - 2 - hexadecen - 1 - ol (1.42%), n-Hexadecanoic acid (21.34%), Hexadecanoic acid, ethyl ester (2.84%), Phytol (11.38%), 1, 2-benzenedicarboxalic acid, bis (4-methylpentyl)ester (1.14%) .

***Components identified in the Sample DE-1 - 420**
[GC MS study]

No.	RT	Name of the compound	Molecular formula	MW	Peak Area %
1	2.60	Benzyl Alcohol	C_7H_8O	108	8.53
2	3.29	DL-Arabinitol	$C_5H_{12}O_5$	152	5.69
3	5.93	Phenol, 2-methoxy-4-(1-propenyl)- [Synonyms: Isoeugenol]	$C_{10}H_{12}O_2$	164	2.84
4	7.71	Phenol, 2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	206	1.28
5	7.76	4H-1-Benzopyran, 4,4,5,8-tetramethyl-	$C_{13}H_{16}O$	188	1.42
6	7.82	2,6,10-Dodecatriene-1-imine, 3,7,11-trimethyl-1-(dimethylamino)-	$C_{17}H_{30}N_2$	262	1.00
7	8.49	1,6-Anhydro-β-D-glucopyranose [levoglucosan]	$C_6H_{10}O_5$	162	11.38
8	8.97	5-Isopropenyl-2-methylcyclopent-1-enecarboxaldehyde	$C_{10}H_{14}O$	150	1.28
9	9.83	Amyl nitrite	$C_5H_{11}NO_2$	117	2.84
10	10.70	1,1'-Bicyclohexyl, 2-methyl-, trans-	$C_{13}H_{24}$	180	7.11
11	10.95	3-methyl-5-(2,6-dimethylheptyl)-1,5-Pent-2-enolide	$C_{15}H_{26}O_2$	238	2.84
12	11.07	Cyclohexanecarboxylic acid, 4-propyl-, 4-methoxyphenyl ester	$C_{17}H_{24}O_3$	276	15.65
13	11.26	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	1.42
14	12.81	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	21.34
15	13.04	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	2.84
16	14.52	Phytol	$C_{20}H_{40}O$	296	11.38
17	20.35	1,2-Benzenedicarboxylic acid, bis(4-methylpentyl) ester	$C_{20}H_{30}O_4$	334	1.14

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

GC-MS Chromatogram of the Sample DE-1 - 420

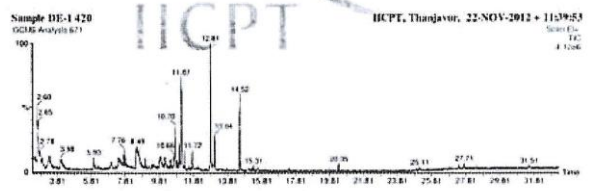

FIGURE 1: GC/MS SPECTROGRAM FOR THE *LANNEA COROMANDELICA* ETHANOLIC LEAF EXTRACT

Most of them are oxygenated hydrocarbons and primarily phenolic hydrocarbons, these phytochemicals may be accountable for various pharmacological behaviour like anti-oxidant and anti-arthritic activity, etc.

In view of preliminary phytochemical studies, *in vitro* anti-oxidant and anti-arthritic study ethanolic extract showed more appreciable results, GC-MS examination was carried out to recognize the components responsible for its potency. The present study in *Lannea coromandelica* recommends that, because of the presence of these components anti-arthritic potency was enhanced, though it should be further evaluated in future by appropriate *in vivo* studies.

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REFERENCES:

1. Vadivel K, Thangabalan B, Narayana KV, Grac BCJ, Kumar DP, Babu SM: Preliminary phytochemical evaluation of leaf extracts of Lannea *coromandelica*. Int J pharmacol. Res 2012; 2(2): 64-68.
2. Alam B, Horsain S, Flabib R, Rea J, Islam A: Anti-oxidant, Analgesic activity of *Lannea coromendelica bark linn* . Int.J.pharmacol 2012; 8(4): 224-233.
3. Reddy AK, Joy JM, Kumar CKA: Wound healing, Hypotensive activity and Antimicrobial activity of *Lannea coromandelica* . J pharm.Res 2011; 4(3): 577-579.
4. Singh AP: Distribution of steroid like compound in plant flora, Phcog. Mag. 2006; 2(6): 87-89.
5. Cong Z, Meiing Q, Qinglong S, Shan Z and Ruinong FJ: GC-MS analysis of *Cassia italica* leaf methanol extract. Pharm.Biomed. Anal 2007; 44: 464.
6. Hagerman AE, Reidl KM, Jones GA, Sovik KN, Richard NT, Hartzfeld PW, Reich TL: High molecular weight plant phenolics (tannins) as biological anti-oxidants. J.Agric.food Chem 1998; 46:1887-1892.
7. Luo JD, Chen AF: Nitric oxide: a newly discovered functions on wound healing. Acta Pharmacol. Sin 2005; 26: 259-264.
8. Oktay M, Gulcin I, Kufrevioglu OI: Determination of invitro antioxidant activity of fennel (Foeniculum vulgare) seed extracts. Lebensum.-Wiss.U.-Technol 2003; 36: 263-271.
9. Chanda S, Dave R: In vitro models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: An overview. African Journal of Microbiology Research 2009; 3(13): 981-996.
10. Chippada SC, Vangalapati M: Antioxidant, an antiinflammatory and anti-arthritic activity of *Centella asiatica* extracts. Journal of Chemical, Biological and Physical Sciences. 2011; 1(2) B: 260– 269.

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