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Physicochemical and Phytochemical Investigation of the roots of *Lannea coromandelica* (Houtt.) Merr.

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ABSTRACT

The present study was undertaken for the development of physicochemical and phytochemical parameters of the roots of *Lannea coromandelica* (Houtt.) Merr. belonging to the family Anacardiaceae. The plant is known in Hindi as Jhingan, in Kannada as Manjistha, in English as Wodier, in Sanskrit as Jhingini and in Konkani as Moi. The physicochemical and phytochemical investigation confirms the purity and authenticity of *Lannea coromandelica* roots by using standard methods. The physicochemical studies revealed the presence of moisture content as13.7 % w/w, total ash as 10.725 % w/w, acid insoluble ash as 0.975 % w/w, water soluble ash as 1.825 % w/w, alcohol soluble extractive as 10 % w/w, water soluble extractive as 10.8 % w/w, ether soluble extractive as 2.3 % w/w, foaming index as less than 100 and swelling index as 0.733 cm. The fluorescence analysis in short wavelength, long wavelength and day light is also reported, which is a tool to determine the chemical nature of crude drug. Preliminary phytochemical screening of the ethanolic extract of the roots revealed the presence of alkaloids, carbohydrates, glycosides, proteins, flavonoids, triterpenoids, steroids, tannins and saponins. All these methods will help in setting down pharmacopoeial standards in determining the quality and purity of the *Lannea coromandelica* roots.

Keywords: *Lannea coromandelica*, Anacardiaceae, Fluorescence, Physicochemical, Phytochemical.

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INTRODUCTION

Lannea coromandelica (Synonym- L.grandis (Dennst.) Engl.; Odina wodier Roxb.) belonging to the family Anacardiaceae[1]. Is a medium sized to large tree 12-28m in height with grayish bark rough, exfoliating in thin irregular flakes, leaves imparipinnately compound, crowded at the ends of branches, leaflets membranous, 7-9, oblong-ovate, green above, brown beneath, glabrous, base acute or rounded, often oblique, main nerves 6-8 pairs; flowers small, yellowish or purplish, the male racemes compound, the female simple; fruits reniform, compressed, 1-seeded red drupes[2]. It is distributed throughout India in deciduous forests, ascends to an altitude of 1,500m in the Himalayas[3].

Figure 1: *Lannea coromandelica*

The bark is sweet, hot, acrid; stomachic; useful in vaginal troubles, allays thirst, dispels foul breath; cures skin eruptions, heart disease[4]. It is also considered to be useful as an astringent and used as a lotion for bruises, wounds, ulcers and sore eyes. It is also used in gout, and as a decoction in tooth ache. Boiled leaves are applied for sprains and bruises, local swellings, elephantiasis and in bodyache. The juice of the green branches with tamarind, is employed as an emetic in Bengal. The gum is used in asthma and also by lactating women as a cordial[3].The plant is considered to be useful by the tribals (Santals) in sores and carbuncles, haematuria, diarrhoea, dysentry, cholera and trinder pest[5]. A paste made from the stem bark is applied to wounds in cattle[6]. In Ayurveda bark and resin is used cardiac diseases, diarrhea and diseases of the nervous system[7]. The pharmacological activities such as antioxidant and free radical scavenging property are reported from the bark of the plant[8]. *L. coromandelica* fruits were found to contain terpenes and alkaloids and devoid of sterols, saponins, tannins and mucilage[9]. The flowers on methanolic extraction yielded ellagic acid, quercetin, isoquercetin and morin[10], while on alcoholic extraction gave allegic acid and quercetin-3-arabinoside[11]. Leaves were found to contain leucocyanidin and leucodelphidin in traces[10]; β-sitosterol, ellagic acid, quercetin and quercetin-3-α-arabinoside[11]. The jeol gum from the plant was found to contain L-arabinose, D-galactose and D-galactouronic

acid as the constituent units. An aldobiuronic acid, shown to be 3-O-(D-galactopyranosyl uronic acid)-D-galactose was also reported[12]. A partial structure of degraded gum was also proposed[13,14]. The roots contain cluytyl ferulate; heartwood gave lanosterol; bark, dl-epi-catechin and (+)-leucocyanidin[1]. From the extensive literature survey, it was revealed that no substantial work has been carried out on the roots of the plant. Hence, an effort was made to investigate the physicochemical parameters, fluorescence analysis and preliminary phytochemical screening of *L. coromandelica* roots.

MATERIALS AND METHOD

Plant Collection

Fresh roots were collected from fully grown plants of *L. coromandelica* from Sancoale-Goa in the month of September 2013. It was authenticated by Dr. K. Gopalkrishna Bhat, Dept. of Botany, Poornaprajna College, Udupi, Karnataka and by Mr. Dinesh Nayak (sasyashymala) advisor green belt Mangalore –India[15, 16].

Preparation of Ethanolic Extract

The roots were then washed thoroughly to remove the soil and adhering materials and dried in shade. The dried roots were then powdered and used for physicochemical evaluation, fluorescence analysis and preparation of ethanolic extract. The dried roots powder (500g) was exhaustively extracted by maceration with ethanol (95%) for three days. After three days ethanolic layer was decanted off. The process was repeated thrice. The solvent from the total extract was distilled off using Rotary vaccum evaporator (superfit) and the concentrate was evaporated to dryness (20g) and then used for the preliminary phytochemical investigation[17].

PHYSICOCHEMICAL EVALUATIONS

Determination of swelling index

The specified quantity (1cm) of dried root powder of *L. coromandelica* was taken which was previously reduced to the required fineness. It was introduced in a 25 ml glass stoppered measuring cylinder. The internal diameter of the cylinder should be about 16 mm, the length of the graduated cylinder should be 125 mm and it was subdivided in 0.2 ml and marked from 0 to 25 ml in an upward direction. 25 ml of water was added to the powdered roots and mixture was shaken thoroughly at an interval of every 10 min for 1 hour. It was allowed to stand for 3 hour at room temperature. The volume was measured in ml occupied by the plant material, including any sticky mucilage. The mean value of the individual determinations was calculated[18].

Determination of foaming index

1 g of the plant material was taken and was reduced to a coarse powder (sieve no. 1250). Then

weighed and transferred to a 500 ml of conical flask containing 100 ml of boiling water. It was maintained at moderate boiling for 30 min, cooled and filtered into 100 ml volumetric flask. Sufficient amount of water was added to make up the volume to 100 ml. The above decoction was poured in 10 stoppered test tubes (height 16 cm, diameter 16 mm) in series of successive portions of 1 ml, 2 ml, 3 ml, etc. up to 10 ml and volume of the liquid in each test tube was adjusted to 10 ml with water. The tubes were stoppered and shaken in lengthwise motion for 15 seconds (2 shake per second). Then it was allowed to stand for 15 min and the height of the foam was measured.

If the height of foam in every test tube is less than 1 cm, the foaming index is less than 100. If in any test tube a height of foam of 1 cm is measured, the dilution of the plant material in this test tube is the index sought. If this tube is the first or second tube in the series, it is necessary to have an intermediate dilution prepared in the similar manner to obtain a more precise result. If the height of the foam is more than 1 cm in every test tube, the foaming index is over 1000. In this case the determination needs to be made on a new series of dilution of the decoction in order to obtain the result[18].

Determination of extractive value

Method: cold maceration

4.0 g of coarsely powdered air dried material was weighed accurately in a glass stoppered conical flask. This was macerated with 100 ml of 90 % alcohol for 6 hours, shaking frequently and then allowed to stand for 18 hours. It was filtered rapidly and 25 ml of the filtrate was transferred to tarred flat bottom dish and evaporated to dryness on water bath. Later it was dried at 105^0C for 6 hours, cooled in a desiccator for 30 minutes and weight was recorded. Percentage of alcohol soluble extractive was calculated with reference to the air dried sample.

For water soluble extractive value the same procedure was followed and % was calculated by using water as solvent. For ether soluble extractive value the same procedure was followed and % was calculated by using ether as solvent[18].

Determination of moisture content

2 g of dried root powder of *L. coromandelica* was taken into glass dish previously weighed. This glass dish was kept into the hot air oven at 100-105^0C. The weight was noted every hour till two successive readings comes same. At last the weight of drug was calculated and percent yield was determined[19].

Determination of ash values

Total ash:

2 g of the powdered drug was accurately weighed and was taken in a tarred platinum or silica dish previously ignited and weighed. The powdered drug was spread in a fine even layer at the bottom of the dish and then ignited by gradually increasing the heat to 500-600^{0}C until free from carbon. It was cooled in a desiccator and weighed. The % of ash (total ash) was calculated with reference to the air dried drug.

Acid insoluble ash:

To the crucible containing total ash, 25 ml of HCl (approx.70g/l) test solution was added, covered with a watch glass and boiled gently for 5 min. The watch glass was rinsed with 5 ml of hot water which was then added to the crucible. The insoluble matter was collected on an ash less filter paper and washed with hot water until the filtrate is neutral. The filter paper containing the insoluble matter was then transferred to original crucible, dried on hot plate and ignited to constant weight. The residue was allowed to cool in desiccator for 30 min and weighed without delay. The % of acid insoluble ash was calculated with reference to the air dried drug.

Water soluble ash:

To the crucible containing the total ash 25 ml of water was added and boiled for 5 min. The insoluble matter was collected on an ash less filter paper. It was washed with hot water and ignited for 15 min, at a temperature not exceeding 450^{0}C. The weight of this residue obtained was subtracted from the weight of total ash. The % of water soluble ash was calculated with reference to the air dried drug[18].

Determination of fluorescence analysis

Powdered roots of the plant *L. coromandelica* was subjected to analysis under ultra violet light (254 and 366 nm) and visible light after treatment with various chemical and organic reagents as shown in the table 2 [20-22].

PRELIMINARY PHYTOCHEMICAL SCREENING [20-22]

The preliminary phytoconstituents studies were performed for testing the different phyto-constituents present in the ethanolic extract of the roots of *L .coromandelica*. The phytochemical tests were performed as per the standard procedure.

ALKALOIDS [20-22]

Dragendroff's reagent

To 2 mg of the ethanolic extract, 5 ml of distilled water was added; 2 M Hydrochloric acid was added until an acid reaction occurs. To this 1 ml of Dragendroff's Reagent was added. Formation of orange – red precipitate indicated the presence of alkaloids.

Mayer's test

To 2 mg of ethanolic extract, a few drops of Mayer's Reagent was added. Formation of white or yellow precipitate indicated the presence of alkaloids.

Wagner's test

To 2 mg of ethanolic extract, 1 ml of dilute Hydrochloric acid was added along with few drops of Wagner's reagent. A yellow or brown precipitate indicated the presence of alkaloids.

Hager's test

To 2 mg of the ethanolic extract taken in a test tube, a few of Hager's reagent was added. Formation of yellow precipitate confirmed the presence of alkaloids.

CARBOHYDRATES [20-22]

Molisch's test

In a test tube containing 2 ml of extract, 2 drops of freshly prepared 20% alcoholic solution of α-naphthol was added. 2 ml of Conc. Sulphuric acid was added so as to form a layer below the mixture. Red-violet ring appeared, indicating the presence of carbohydrates, which disappeared on the addition of excess of alkali.

Benedict's test

To 0.5 ml of extract, 5 ml of benedict's solution was added and boiled for 5 min. Formation of brick red coloured precipitate indicated the presence of carbohydrates.

Fehling's Test

To 2 ml of extract, 1ml mixture of equal parts of Fehling's solution A and B were added and boiled for few minutes. Formation of red or brick colored precipitate indicated the presence of reducing sugar.

FLAVONOIDS [20-22]

Shinoda test

In a test tube containing 0.5 ml of extract, 10 drops of dilute hydrochloric acid followed by a small piece of magnesium were added. Formation of pink, reddish or brown colour indicated the presence of flavonoids.

Lead acetate test

To 2 mg of plant extract, 1ml of lead acetate solution was added. Formation of yellow precipitate indicated the presence of flavonoids.

Vanillin- hydrochloric acid test

Vanillin HCl was added to the alcoholic solution of drug, formation of pink colour indicated the presence of flavonoids.

TRITERPENOIDS AND STEROIDS [20-22]

Libermann- Burchard's test

2 mg of dry extract was dissolved in acetic anhydride, heated to boiling, cooled and then 1 ml of concentrated sulphuric acid was added along the sides of the test tube. Brown ring was formed at junction of 2 layers and formation of a deep red colour in the upper layer indicated the presence of triterpenoids.

Salkowski test

2 mg of dry extract was shaken with chloroform, to the chloroform layer; sulphuric acid was added slowly by the side of test tube. Formation of red colour indicated the presence of steroids.

TANNINS AND PHENOLIC COMPOUNDS [20-22]

To 1-2 ml of the extract, few drops of 5% w/v $FeCl_3$ solution were added. A green colour indicated the presence of gallotannins; blue colour indicated the presence of hydrolysable tannins while brown colour indicated the presence of pseudotaninns

To 1-2 ml of extract add lead acetate solution. White precipitate indicated presence of tannins and phenolic compounds.

RESINS [20-22]

1 ml of extract was dissolved in acetone and the solution was poured in distilled water. Turbidity indicated the presence of resins.

PROTEINS [20-22]

Biuret's test

To 1 ml of hot extract, 5-8 drops of 10% w/v sodium hydroxide solution, followed by 1 or 2 drops of 3% w/v copper sulphate solution were added. Formation of violet red colour indicated the presence of proteins.

Millon's test

1 ml of extract was dissolved in 1ml distilled water & 5-6 drops of Millon's reagent were added. Formation of white precipitate, which turns red on heating, indicated the presence of proteins.

GLYCOSIDES [20-22]

Free sugar content of the extract was determined and hydrolysed with mineral acids (dil. HCl/dil.H_2SO_4). The total sugar content of hydrolysed extract was again determined. Increase in the sugar content indicated the presence of glycosides in the extract.

Test for cardiac glycosides

Baljet's test

A thick section showed yellow to orange colour with sodium picrate.

Legal's test

To the aqueous or alcoholic extract, 1 ml pyridine and 1 ml of sodium nitroprusside were added. Pink to red colour indicated the presence of cardiac glycosides.

Test for deoxysugars (keller-kiliani test)

To 2 ml extract, glacial acetic acid, one drop 5% $FeCl_3$ and conc.H_2SO_4 were added. Reddish brown colour appeared at the junction of the two liquid layers and upper layer appeared bluish green indicated the presence of deoxysugars.

Liebermann's test (test for bufadienoloids)

3 ml extract with 3 ml of acetic anhydride were mixed. It was heated and cooled. Few drops of conc. H_2SO_4 were added. Blue colour appeared indicated the presence of bufadienoloids.

Test for Anthraquinone glycosides

Borntrager's test for anthraquinone glycosides

To 3 ml extract, 5% dil. H_2SO_4 was added. It was boiled and filtered. To the cold filtrate, equal volume of benzene or chloroform was added and shaken well. The organic solvent was separated and then ammonia was. Ammoniacal layer turned pink or red indicated the presence of anthraquinone glycosides.

Modified Borntrager's test for C-glycosides

To 3 ml extract, 5 ml 5% dil. HCl, few drops of $FeCl_3$ were added. It was then heated for five minutes in boiling water bath and cooled. To the cold filtrate equal volume of benzene or organic solvent was added. It was shaken well. The organic solvent was separated and ammonia was added. Ammoniacal layer turned pink or red indicated the presence of C-glycosides.

Test for saponins

Foam test

The drug extract or dry powder was shaken vigorously with water. Persistent foam observed indicated the presence of saponins.

Test for coumarin glycosides

Powder was moistened and taken in a test tube. The tube was covered with filter paper soaked in dilute NaOH and kept in water bath. Later the filter paper was exposed to UV light. Yellowish – green fluorescence indicated the presence of coumarin glycosides.

$FeCl_3$ test

To the conc. Alcoholic extract of the drug few drops of alcoholic $FeCl_3$ solution was added. Formation of deep green colour, which turned yellow on addition of conc. HNO_3 indicated the presence of coumarins.

Starch [20-22]

0.01 g of Iodine and 0.075 g of potassium iodide were dissolved in 5 ml of distilled water and 2-3 ml of extract was added. Formation of blue colour indicated the presence of starch.

RESULTS AND DISCUSSION

The root of *L. coromandelica* was subjected to systemic physicochemical, fluorescence and preliminary phytochemical analysis. The data generated is helpful in determining the quality and the purity of the crude drug, especially in the powdered form. In this study the parameters included for the evaluation of root of *L. coromandelica* were moisture content, ash values (total ash, water soluble ash and acid insoluble ash), extractive values using alcohol, water and ether as solvents, swelling index, foaming index (Table 1).

Table 1: results of all above physicochemical test on the powdered roots of *L. coromandelica*

Sr. No.	Physicochemical Test	Results
1	Determination of Swelling Index	0.733
2	Determination of Foaming Index	Less than 100
3	Determination of extractive value	
	• Alcohol soluble extractive of sample	10 %w/w
	• Water soluble extractive of sample	10.8 %w/w
	• Ether soluble extractive of sample	2.3 %w/w
		Since no physicochemical work has been carried out on the roots of Lannea Coromandelica there are no standard limits.
4	Determination of moisture content	13.7 %w/w
5	Determination of ash values	
	• Total ash	10.725 %w/w
	• Acid insoluble ash	0.975 %w/w
	• Water soluble ash	1.825 %w/w

The extractive values are however, moderate but will be useful for the further extraction of phytoconstituents from the plant. The alcohol soluble extractive indicated the presence of polar constituents like phenols, flavonoids etc. The total ash is particularly important in the evaluation of purity of drugs i.e. the presence or absence of foreign matter such as metallic salts or silica. The fluorescence analysis performed showed a wide range of fluorescent colours for the crude drug with different reagents (Table 2).

Fluorescence study of root powder helps in qualitative evaluation which can be used for its identification. The preliminary phytochemical screening of the ethanolic extract of root was performed and it was found to contain alkaloids, carbohydrates, glycosides, proteins, flavonoids, triterpenoids, steroids, tannins and saponins (Table 3).

Table 2: Determination of Fluorescence analysis of powdered roots of *L. coromandelica* using various solvents.

Sr. No	Drug +reagent	Visible light	Short wavelength 254nm	Long wavelength 366nm
1	Powder	Brown	Brown	Light Brown
2	Powder +50% NaOH(aqueous)	Dark Brown	Dark Brown	Dark Green
3	Powder +50% NaOH (alcoholic)	Light Brown	Light Brown	Grey
4	Powder + Ammonia	Brown	Brown	Light Green
5	Powder +Picric acid	Light Brown	Light Brown	Light Brown
6	Powder +10% HCl	Brown	Light Green	Light Green
7	Powder +10% H_2SO_4	Light Brown	Light Brown	Light Brown
8	Powder + Conc. HCl	Brown	Brown	Light Green
9	Powder +Conc.H_2SO_4	Red	Black	Dark Green
10	Powder +Conc. HNO_3	Brown	Dark Brown	Dark Brown
11	Powder +10% NaOH	Dark Brown	Black	Dark Blue
12	Powder + dist. H_2O	Brown	Brown	Light Brown
13	Powder+ Methanol	Brown	Brown	Light Blue
14	Powder +Pet. Ether	Brown	Purple	Purple
15	Powder +$CHCl_3$	Brown	Dark Brown	Blue
16	Powder+Ethyl Acetate	Light Brown	Light Brown	Light Brown
17	Powder +Acetone	Light Brown	Light Brown	Blue
18	Powder+Solvent Ether	Light Brown	Light Brown	Aqua Blue
19	Powder+Ethanol	Light Brown	Light Brown	Light Purple
20	Powder+Toluene	Light Brown	Light Brown	Light Purple

Table 3: Phytochemical analysis of ethanolic extract of *L. coromandelica* roots.

Sr. No.	Chemical test	Result
1.	Alkaloids	+
2.	Carbohydrates	+
3.	Flavonoids	+
4.	Triterpenoids	+
5.	Steroids	+
6.	Tannins	+
7.	Resins	-
8.	Glycosides	+
9.	Cardiac glycosides	-
10.	Anthraquinone glycosides	-
11.	Saponins	+
12.	Coumarins	-
13.	Proteins	+
14.	Starch	-

+ = Present, - = Absent, *L. coromandelica* =*Lannea cormandelica*

CONCLUSION

Early mankind depended on nature for both health and illness. Today nearly 70% of the human population is reported to be dependent on plant based medicines. The global market for herbal drug is yet to be exploited fully because of inadequacy or non availability of quality standards for

herbal medicine. Since the material traded mostly as roots, bark, twigs, flowers, leaves and fruits visible authentification of the material is difficult so there is need to set standards for such material in order to avoid adulteration of crude drugs. Despite the modern techniques, identification of plant drugs by Pharmacognostic method is more reliable. The physicochemical parameters, fluorescence analysis and chemical test performed in the study will further guide in pharmacological and therapeutic evaluation of the species and will assist in standardization for quality, purity, and identification of drugs. In conclusion the parameters reported in the study will be useful in the development of further studies on the plant.

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REFERENCES

1. Khare CP. Indian Medicinal Plants: An Illustrated Dictionary. Berlin: Springer; 2007: 361.
2. Warriers PK, Nambiar VPK, Ramankutty C. Indian Medicinal Plants: A compendium of 500 species. Hyderabad Universities press (India) Pvt Limited; 2010: 297. (Vol. 3)
3. Satyavati GV. Medicinal Plants of India. New Delhi: Indian Council of medical Research; 1976: 130-131. (Vol.2)
4. Kirtikar KR, Basu BD. Indian Medicinal plants. Dehradun: International book distributers; 2005: 665. (Vol.1)
5. Jain SK, Tarafder CR. Medicinal plant-lore of the Santals (A revival of P.O. Boddings work). Econ Bot 1970; 24: 241.
6. Hemadri K, Raj PV, Rao SS, Sharma CRR. Folklore claims from Andhra Pradesh-I. J Sci Res PI Med 1980; 1(2): 37.
7. Shetty BV, Kaveriappa KM, Bhat KG. Plant Resources of Western Ghats and Lowlands of Dakshina Kannada and Udupi Districts. Manglore: Pilikula Nisarga Dhama Society Moodushedde; 2002: 185.
8. Stalin JD, Babu T, Kumar SS. A Study on the Antioxidant and Free Radical Scavenging Property of *Lannea Coromandelica* Bark Extract. Int J Univers Pharm Life Sci . 2013; 3(5): 43-49.
9. Bhattacharjee AK, Das AK. Phytochemical screening of some Indian plants. Quart J

Crude Drug Res.1969; 9: 1408.

10. Nair AGR, Subramanian SS, Sridharan K. Chemical examination of the flowers of *Lannea coromandelica*. Curr Sci. 196; 32: 115.
11. Subramanian SS, Nair AGR. Angiospermae Dicotyledonae (Anacardiaceae): Polyphenols of *Lannea coromandelica*. Phytochemistry 1197; 10: 1939.
12. Dhar PK, Mukherjee S. Structure of the jeol (*Odina wodier*) gum: Nature of sugars and aldobiouronic acid in jeol gum. J Sci Ind Res 1959; 18B, 219.
13. Bhattacharya AK, Rao CVN. Gum jeol: The structure of the degraded gum derived from it. Can J Chem 1964; 42: 107.
14. Gupta AK, Mukherjee S. Structure of degraded gum from Odina wodier Roxb. (jeol gum). Indian J Chem 1973; 11: 648.
15. Cooke T. The flora of the presidency of Bombay. Calcutta: Botanical Survey of India; 1967: 223.
16. Nadkarni KM. Indian Materia Medica. Bombay: Bombay Popular Prakashan; 2009: 1089.
17. Hegde K, Thakker SP, Joshi AB. Asian J Chem. 2009; 21(7): 5399-5402.
18. World Health Organisation. Quality Control Methods for Medicinal Plants Materials. WHO/PHARMA/92.559; 1998: 4-46.
19. Khandelwal KR. Practical Pharmacognosy Techniques and Experiments. 20th ed., Pune: Nirali Prakashan; 2010: 23.7.
20. Evans WC, Evans D, Trease and Evan's Pharmacognosy.15th ed., London: W.B. Saunders Company Ltd.; 2002: 193.
21. Shah B, Seth AK. Textbook of Pharmacognosy and Phytochemistry. 1st ed., New Delhi: Elsevier Pvt. Ltd.; 2010: 233-234.
22. Khandelwal KR. Practical Pharmacognosy Techniques and Experiments. 20th ed., Pune: Nirali Prakashan; 2010: 25.1-25.6.