

Preliminary Pharmacognostical & Phytochemical investigation of Bark & Leaves of *Lanneacoromandelica* Houtt. Merrill.

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ABSTRACT

Lanneacoromandelica Houtt. Merrill. belongs to family Anacardiaceae. The bark & leaves are known for medicinal properties, such as in ulcerative stomatitis, diarrhoea dysentery, sprains and bruises, wound, eruptions etc. The study includes macroscopy, microscopy, preliminary phytochemical analysis and physicochemical evaluation & HPTLC profile using methods given in Indian Ayurvedic Pharmacopoeia. Physicochemical parameters such as total ash value, acid insoluble ash value and water soluble ash value were determined which were 10.08% w/w, 0.77% w/w, 1.80% w/w respectively. Preliminary phytochemical analysis of extracts were carried out. The results were positive for flavonoids, tannins, terpenoids etc. These secondary metabolites are the active constituents of and it may be responsible for its pharmacological activities. Chief characters of transverse section of leaves include collenchymatous cells, Schlerenchymatous cells, endarch xylem, phloem fibers non-lignified, unicellular trichomes etc. & stem contains spherical secretory cells, stone cells etc.

Key words: *Lanneacoromandelica*, Phytochemical, pharmacognostical, flavonoids, secretory cells.

INTRODUCTION

Lanneacoromandelica Houtt. Merrill. (Anacardiaceae) also has a synonym *Jingini* (Sanskrit) is deciduous large trees, upto 15-20 m tall is located in tropical Asia. It was commonly called as Wodier or Indian ash tree. In *Ayurvedic text* *Jingini* is mentioned as a substitute for *Murva* (*Marsdeniatenecissima*)¹. Bark & leaves of *Lanneacoromandelica* is commonly used in ulcerative stomatitis, dyspepsia, general debility, gout, cholera, diarrhoea, dysentery, sore eyes, leprosy, sprains and bruises, wound, elephantiasis, eruptions, snakebite, stomachache, vaginal troubles etc.^{2,3} It was showed the presence of phenolic compounds, flavonoids, triterpenoids, tannin, alkaloids. Its Pharmacological study revealed anti-inflammatory⁴, anti-microbial^{5,6,7} hypotensive⁸, wound healing⁶, aphrodisiac, anticancerous⁹ activities. There not much more data was found on its leaf, stem and bark phytochemical analysis, microscopical study. Therefore *L. coromandelica* plants parts were investigated for its phytochemical analysis pharmacognostical study.

MATERIAL AND METHODS

The field survey was conducted to directly collect the original species of *Lannea* plant equated officially with the botanical source *Lanneacoromandelica* Houtt. Merrill. with proper record of place and time of collection, part collected and any other relevant information that is available from the field. The fresh leaves, bark of

Lanneacoromandelica Houtt. Merrill. was collected from field Rawatbhata near Kota Rajasthan The sample specimen of *Lanneacoromandelica* was identified and authenticated by Dr. P.M.Padhye, Scientist "E" & Head of office, Botanical Survey of India, Jodhpur Rajasthan. Letter no.BSI/AZC/1/120/2/2011-12/Tech.(Pl.Id.)/501 dt.08.12.2011.

The samples were subjected to organoleptic, microscopic and phytochemical study so as to generate inputs that can be considered for lying down standards in respect of this plant. The organoleptic evaluation refers to evaluation of the formulation by colour, odour, taste, texture, etc. The organoleptic characters of the fresh bark were evaluated based on the method described by Ayurvedic pharmacopoeia of India¹⁰.

Microscopical study: Microscopical study was done by sections cutting and observing the sections under suitable microscopes.

Preparation of the Specimen:

1. Take fresh sample of *Lannea* Stem. If the sample is dried soak it in water for overnight. Hold the sample between index finger & thumb. Move the blade back and forth from one end to the other, to obtain fine slices. Transfer the sections to a watch glass containing water with the help of a brush. Reject thick & oblique sections.
2. For *Lannea* leaf, cut a part of the leaf which passes through the midrib. Since the lamina is thin section

Table no. 1 Physicochemical parameters of the bark of *Lanneacoromandelica*Houtt. Merrill.

S.No.	Parameters	Average % w/w
1.	Ash values	
	Total Ash	10.08
	Acid insoluble ash	0.77
	Water soluble ash	1.80
2.	Moisture content	4.99

Table No. 2 - Inorganic profile of the drug based on qualitative tests conducted over the ash. (Micro minerals)

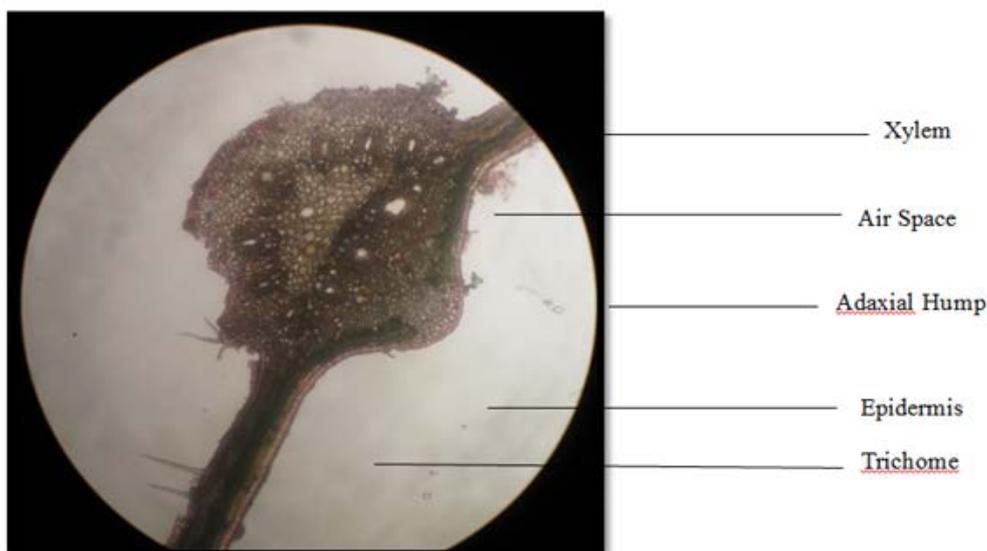
S.No.	Name of Mineral/Electrolyte	<i>Lanneacoromandelica</i> Houtt. Merrill.
1.	Calcium	-
2.	Iron	+
3.	Magnesium	-
4.	Phosphorus	+
5.	Potassium	-
6.	Sulphur	+

Table no.3 Extractive values of the bark of *Lanneacoromandelica*Houtt. Merrill. with different solvents:

S.No.	Solvents	Extractive values % w/v
1.	Ethyl acetate	6.72
2.	Acetone	8.28
3.	Ethanol	10.44
4.	Water	14.58

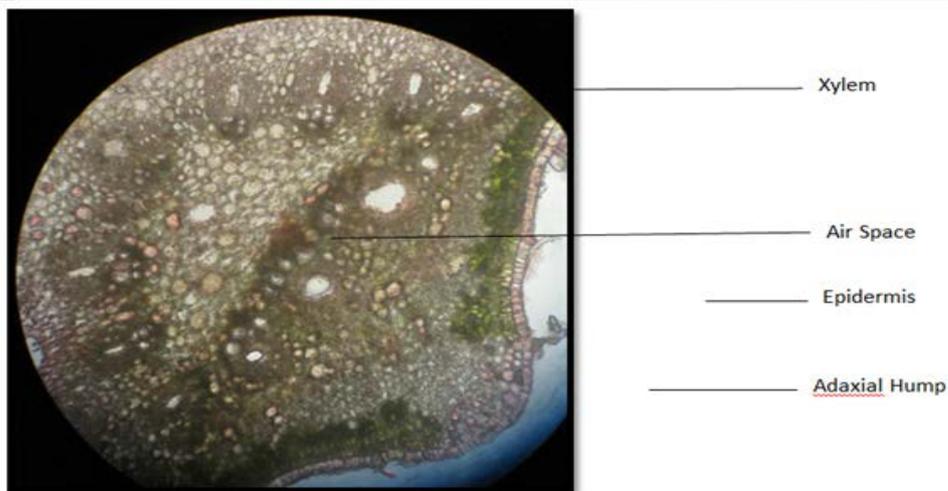
Table No.4 Observation of Qualitative analysis of Organic matter in *Lanneacoromandelica*Houtt.Merrill.

S.No.	Test	Ethyl acetate	Acetone	Ethanol	Water
1.	Carbohydrate	Molisch's test	+	+	+
2.	Alkaloid	Dragon droff's reagent test	-	+	+
3.	Protein	Ninhydrin Test	-	-	-
4.	Tannin	Vanillin HCl Alcohol reagent	+	+	-
5.	Glycoside	Killer -Killani Test	-	-	-
6.	Phenol	FeCl ₃ solution	+	+	+
7.	Terpenoids	Salkowaski test	+	+	+
8.	Flavonoids	Shinoda'test, FeCl ₃ solution	+	+	+

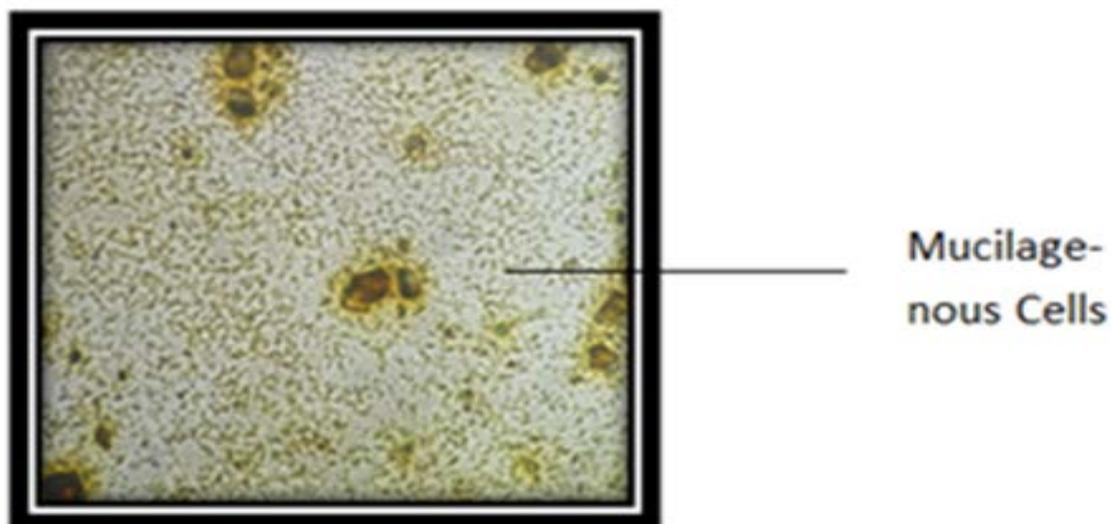


cutting is done by embedding it in a cubical block of pith or potato. Similarly cut down the sections as done in step 1.

3. Leaf sections were dehydrated with chloral hydrate before mounting.



T.S. of leaf of Lannea coromandelica Houtt. Merrill



Powered microscopy of bark

4. Then the sections were soaked in safranin stain for 5 min. & then transferred to a watch glass with plain water, so that excess stain gets washed away.

5. Transfer the section on a glass slide & add few drops of glycerine water on it. With the help of a needle place a cover slip on the section gently & observe it under the microscope.

Phytochemical Standardization and Evaluation includes: Estimation of ash value, acid insoluble ash, water soluble ash.

Qualitative analysis of ash to evaluate inorganic matter contained in drug.

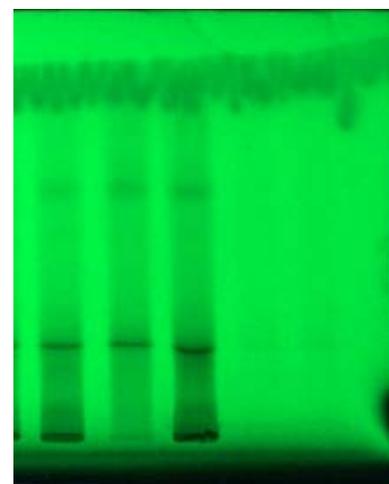
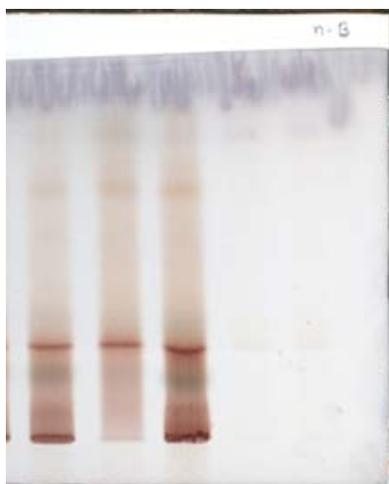
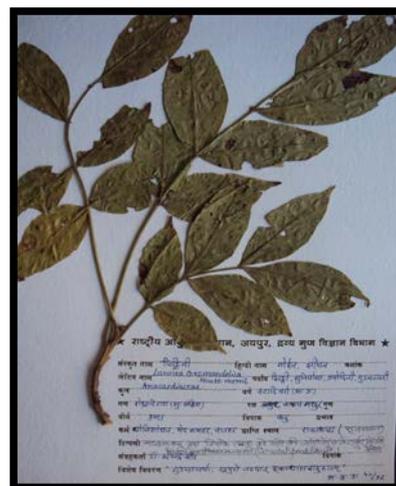
Estimation of water soluble and alcohol soluble extractive value.

Qualitative analysis of extract to evaluate general phytochemical profile.

HPTLC study on ethanolic, acetic & methanolic extract of authentic drug.

Extraction: The organic substances of the *Lannea coromandelica* Houtt. Merrill show their solubility in various solvents in different quantities. So for this purpose of determination of extractive values four solvents were selected according to their polarity. Ethyl acetate, Acetone, Ethanol, Distil water were used.

In powder microscopy oval to spherical secretory cells present, encircled by epithelium layer and filled with yellowish brown contents



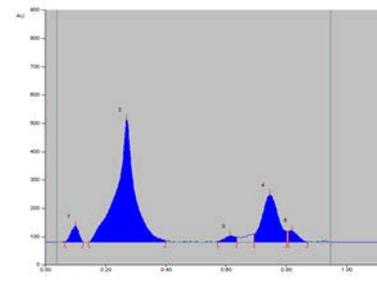
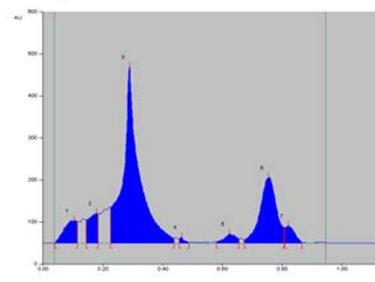
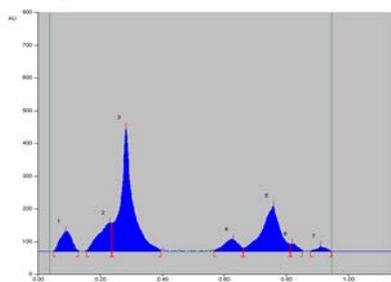
Under UV 254nm

Under UV 366nm

After derivatization

HPTLC profile of *Lanneacoromandelica*Houtt. Merrill

HPTLC graph-



ethanolic ext.

acetone ext.

methanolic ext.

Coarsely powdered air dried drug material is accurately weighed and taken in a glass stopper conical flask. Solvent is added to the flask and the flask is attached to a reflux condenser and boiled for 6hrs, on water bath. After 6hrs, the flask is allowed to cool and the content is filtered through filter paper. The filtrate is transferred to a pre-weighed flat bottomed dish and evaporated to dryness on a water bath. Then the dish is kept in oven for six hours for the contents to get dried fully. The dish is

cooled by keeping in a desiccator for 30 minutes and weighed without delay. The residual mass remained in filter paper is dried as such and is collected fully. This mass is again put into the conical flask and added with next solvent according to polarity, and fitted with reflux condenser, and extract is prepared in the same method used above. This procedure is repeated with all the seven solvents. The content of the extractable matter is calculated in mg per gms of air dried material.

Organoleptic evaluation:

Colour (ép) : Greyish white outer surface & dark reddish brown from internal surface.

Odour (g<Xa) : Without any characteristic odour.

Taste (rs) : Astringent & slightly bitter.

Texture (SpzR) : Rough

Shape (ép): Bark with longitudinal furrows, exfoliating in irregular rounded plates.

HPTLC- High performance thin layer chromatography (HPTLC) is an enhanced form of [thin layer chromatography](#) (TLC). HPTLC was performed by using a stationary phase TLC precoated plate with silica gel 60 F₂₅₄ of 0.2 mm thickness and mobile phase n-butanol : glacial acetic acid : water 4:1:5.

Volume of test solution applied - Ethanol ext.- 4.0 µl,

Acetone ext. - 7.0 µl, Methanolic ext. - 7.0 µl

Distance travelled by solvent system - 8.0 cm

Development chamber – Twin trough chamber (20 x 10 cm) with SS lid.

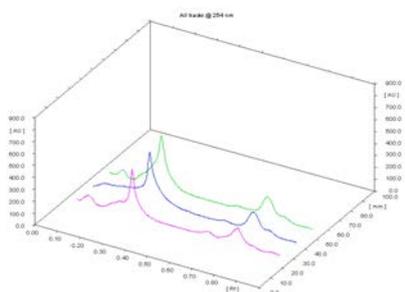
Visualization – Under 254nm; under 366nm, after derivatization with vanillin – sulphuric acid.

[254nm- Ethanol ext.- 3 major spots at Rf 0.09, 0.24, 0.66

Acetone ext. - 2 major spots at Rf 0.24, 0.66

Methanolic ext. – 2 major spots at Rf 0.24, 0.64

366nm- Ethanol ext.- 5 major spots at Rf 0.24, 0.42, 0.55, 0.60, 0.66

**All tracks at wavelength**

Acetone ext. - 4 major spots at Rf 0.24, 0.55, 0.60, 0.66

Methanolic ext. – 5 major spots at Rf 0.24, 0.40, 0.55, 0.60, 0.66

After derivatization-

Ethanol ext.- 5 major spots at Rf 0.08, 0.18, 0.24, 0.66, 0.81

Acetone ext. - 3 major spots at Rf 0.24, 0.66, 0.81

Methanolic ext. – 5 major spots at Rf 0.09, 0.16, 0.24, 0.66, 0.81

Pharmacognostical Study:

Morphology. Leaves clustered at the ends of branchlets, alternate imparipinnate, 20-40 cm long; petioles 7-13 cm long; green above, brown beneath, leaflets 3-7 pairs & odd one, elliptic-oblong or ovate-elliptic, 9-15x5-7 cm, obliquely rounded at base, acuminate at apex, stellate pubescent beneath; petiolules up to 2 mm long those of the terminal leaflets much longer; main nerves 6-8 pairs. Dried leaf powder is pale green colour and bitter in taste. It has its characteristic odour.

Microscopy Midrib. The transverse section of the leaf shows following features:

An adaxial hump at the midrib region. The leaf is 1.2 mm thick at midrib and 0.25 mm at laminar region.

Midrib has epidermal cells with a ground tissue. A well defined upper (12 µm) and lower epidermis (10 µm) are present. Ground tissue consists of an outer zone of 4-5 layered collenchymatous cells.

The vascular bundles is present in centre in the midrib are semicircular and had outer sclerenchymatous cells followed by phloem and xylem. Xylem is endarch in nature. Lignified Xylem arranged in single rows of cells. Non lignified Phloem fibres, long, non sclerenchymatous and measure about 50-70 µm in length.

Phloem tissue was intervened by airspaces. Palisade cells are present in the midrib region and a small gap was noticed at the adaxial hump. Central pith region was prominent. Stomata are present in both the surfaces.

Trichomes are unicellular. Each non-glandular trichome is attached to a central stalk in a multiserial manner and measures about 50-100 µm in length.

Lamina. The lamina is around 250 µm thick. The adaxial epidermis is comprising of a layer of thick walled cylindrical cells. The second layer consists of squarish dilated and thick walled hypodermal cells. The epidermis is covered by a cuticle. The lower epidermis has a narrow single layer of cylindrical cells and fairly wide sub epidermal cells.

RESULTS

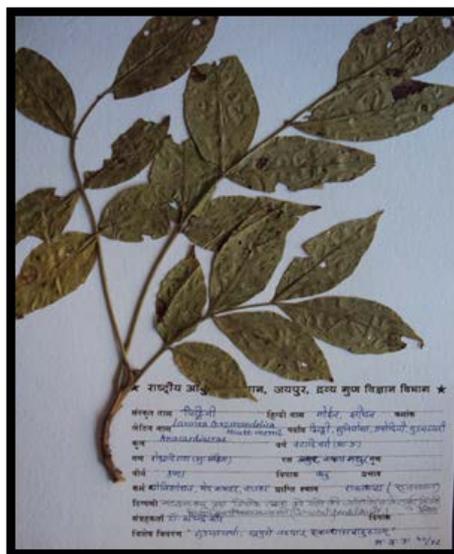
The phytochemical screening analysis of all the extracts of *Lanneacoromandelica* bark shows that the ethyl acetate, acetone, ethanol, water extracts showed the presence of phenol, triterpenoids, flavonoids, tannin, carbohydrate. Alkaloids were present in acetone, ethanol, water extract. Review on *Lanneacoromandelica* extracts also confirmed the presence of these chemical constituents¹¹. The high performance thin layer chromatography revealed that the clearly visible spots has been identified in the ethanolic, acetone and methanolic extract of the bark. The average % of total ash of bark powder of *L. coromandelica* is 10.08% w/w. The acid insoluble content was 0.77% w/w. Water soluble ash was 1.80% w/w. The plants have subjected to qualitative examination to find out the presence of micro-nutrients & heavy metal constituents. It is found that calcium, potassium, magnesium were present in bark powder of plant. However there is absence of heavy metals which is suggestive of its safety profile. Ethyl acetate extract, Acetone extract, Ethanol extract, Water extract of *Lannea* were found to be 6.72%, 8.28%, 10.44%, 14.58% respectively.

CONCLUSION

This study is in line with the quality parameters prescribed in Ayurvedic Pharmacopeia of India and also standards set by other international agencies. This work provides qualitative and quantitative standards for the identification of *Lanneacoromandelica* and from this



Leaves Branches



Herbarium of leaves branch



Main Trunk & Bark appearance



Dried Bark

study it is concluded that Pharmacognostical and phytochemical studies on *Lanneacoromandelica* will be highly useful in determining qualitative and quantitative standards which can ascertain the identity, quality and purity of this plant drug.

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