

Protective effect of *Lannea coromandelica* Houtt. Merrill. against three common pathogens

Rupinder Kaur, Mohan Lal Jaiswal, Vivek Jain¹

Department of Dravyaguna, National Institute of Ayurveda, Jaipur, ¹Department of Pharmacy, Faculty of Science and Technology, Banasthali Vidhyapith, Tonk, Rajasthan, India

ABSTRACT

Background: Ayurvedic text reports suggested *Lannea coromandelica* is used in various microbial origin disorders like dysentery, sore eyes and leprosy, genital wounds. **Objective:** The present study was designed to investigate the antimicrobial effect of *L. coromandelica* Houtt. Merrill. (Anacardiaceae) on microbes which cause female reproductive tract infection. **Materials and Methods:** Ethanolic and aqueous bark extract (Ext.) of *L. coromandelica* were screened against strains of *Streptococcus pyogenes*, *Staphylococcus aureus*, and *Candida albicans*. Antimicrobial assay had been done with agar well diffusion method. **Results:** Ethanolic extracts [100% (16 mg), 75% (12 mg) and 50% (8 mg)] of *L. coromandelica* exhibited zone of inhibition (ZI) 19.21 mm, 18.45 mm, 16.41 mm and 18.12 mm, 17.35 mm, 16.35 mm against *S. aureus* and *S. pyogenes*, respectively. However, only 100% and 75% ethanolic extract showed (ZI-19.18 mm, 16.29 mm) activity against *C. albicans*. Nevertheless, aqueous extract (100%) showed higher antifungal activity (ZI-16.97 mm). Ciprofloxacin and amphotericin B were used as a standard drugs in the present study. **Conclusion:** The results demonstrated that *L. coromandelica* Houtt. Merrill. have antibacterial activity against *S. pyogenes*, *S. aureus* and antifungal property against *C. albicans*. Our findings corroborate the ethnobotanical use of *L. coromandelica* in traditional medicine system (Ayurveda) of India.

Key words: Agar well diffusion method, antibacterial, antifungal, ayurveda, *Candida albicans*, female reproductive tract infection, *Lannea coromandelica*, *Staphylococcus aureus*, *Streptococcus pyogenes*

INTRODUCTION

Reproductive tract infections (RTIs) are recognized as a serious global health problem with impact on women and men, their families, and communities. These infections have second rank after maternal morbidity and mortality as the cause of healthy life loss among women of reproductive age in developing countries.^[1] The estimate indicates that about 40% of women have RTI/STI at any given point of time in India.^[2] RTIs include endogenous

infections, iatrogenic infections, and sexually transmitted infections (STIs).^[3] If left untreated, RTIs can have severe consequences, including infertility, ectopic pregnancy, cervical cancer, menstrual disturbances, pregnancy loss, chronic pelvic pain, miscarriage, low birth weight babies, and increased risk of HIV transmission.^[4] Pathogenic organisms that are responsible for RTI's are *Gardnerella vaginalis*, *Mobiluncus* spp., *Mycoplasma* spp., *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Chlamydia trachomatis*, *Bacteroides*, *Neisseria gonorrhoeae*, *Klebsiella pneumoniae*, *Treponema pallidum*, *Mycoplasma hominis*, *Actinomyces israelii*, *Pseudomonas*, *Mobiluncus* spp. *Streptococcus* both hemolytic and anerobic pathogens, HPV and HSV viruses as a minimum. Besides bacteria, fungi *Candida albicans* and protozoa *Trichomonas vaginalis* are also responsible for RTI.

Present medical therapy for RTI comprises the use of systemic or topical antibiotic, antifungal, and antiprotozoal preparations. RTI being a disorder of multifactorial etiology, a single-line therapy is often inadequate and recurrence is a common complication. Nevertheless, these medications may temporarily reduce infection; they often disrupt the balance of genital bacterial flora (pathogenic vs. nonpathogenic) and often lead to recurrent infection. Therefore, as complementary to these medications (there

Address for correspondence:

Mr. Vivek Jain, Department of Pharmacy, Faculty of Science and Technology, Banasthali Vidhyapith, Tonk - 304 022, Rajasthan, India. E-mail: vivek19j@gmail.com

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are no large scale clinical studies to prove this point); herbal therapy is gaining popularity in women on account of its lesser side effects and restoration of the normal vaginal Flora.^[5-7]

L. coromandelica Hoult. Merrill. belongs to family Anacardiaceae known as *Jhingini* in Sanskrit and woder or Indian ash tree in English is located in tropical Asia. It is large deciduous tree, up to 15-20 m tall, thick trunk, leaves imparipinnate, 25-45-cm long, crowded at the end of branches, leaflets 3-7 pairs, elliptic oblong or ovate-elliptic, bark grey or whitish, exfoliating in irregular rounded plates, appearing when the tree is bare of leaves, yellowish green, crowded in cymose fascicles, fruit drupe, reniform, and red when ripe. Seed solitary, compressed, a mucilagenous gum, known as Jhingan gum, exudes from wounds and cracks in the bark, yellowish white when fresh, turning brown, and ultimately black on drying.

Furthermore, various part of plant contains polyphenols and flavonoids. Polyphenols include tannins like ellagic acid and Gallic acid. Some flavonoids like quercetin, kaempferol, isoquercetin, and gums and mucilage which on hydrolysis gives Arabino-3, 6-galactan containing D-galactose, L-arabinose, 4-O-methyl uronic acids, L-rhamnose and Proteins. Additionally, some sterols have been isolated including β -Sitosterol. In addition to that some other flavonols like physicion, leucocyanidine, and leucodelphidin have been isolated.^[8]

L. coromandelica have been documented for its potential as anti-inflammatory^[9] anti-microbial,^[10,11] hypotensive,^[12] wound healing,^[11] and aphrodisiac activities. The plant also illustrated its beneficial effect on ulcerative stomatitis, dyspepsia, general debility, gout, cholera, diarrhoea, dysentery,^[13] sore eyes, leprosy, sprains and bruises,^[14] elephantiasis,^[15] eruptions, snakebite, stomach ache, and vaginal trouble.^[16-18] Moreover, the plant gum is given in sprains,^[19] asthma and as a cordial to women during lactation.^[20,21]

However, its potential in RTI is not explore yet. Therefore, the present study was design to investigate the role of ehtanolic and aqueous bark extract of *L. coromandelica* in RTI.

MATERIALS AND METHODS

Plant material

Bark of *L. coromandelica* Hoult. Merrill. was collected from forest of Rawatbhata near Kota, Rajasthan. The sample specimen of *L. coromandelica* was identified and authenticated by Dr. P. M. Padhye, Scientist "E" and Head of office, Botanical Survey of India, Jodhpur Rajasthan, letter no. BSI/AZC/I/120/2/2011-12/Tech. (Pl.Id.)/501 dt. 08.12.2011.

The bark was shades dried under room temperature for 1 month, and coarsely powdered by using mixer grinder and were packed separately in air-tight containers.

Extraction of plant material

The bark powder was subjected to continuous extraction in a Soxhlet apparatus. Two different solvents ethanol and distilled water were used for extraction. For each extraction 10-g powder was packed in soxhlet apparatus separately with 100 ml of ethanol and 100 ml of distilled water. The solvent was heated to boil on heating mantle and was subjected to extraction for 12 h. Each extract was filtered through a sterilized Whatman filter paper and the filtered extracts were concentrated to a dry mass by concentrating on rota evaporator, and keeping it in dessicator. Percentage yield for ethanolic and aqueous extract were found to be 10.44% and 14.58%, respectively. It was then autoclaved at 121°C and 15 lbs pressure and then stored at 4°C.

Microbial strains

The micro-organisms used for antimicrobial screening were selected which are common in female genital tract infection. Two bacteria *S. pyogenes* (MTCC 1928), *S. aureus* (MTCC 3160), and fungi *C. albicans* (MTCC 183) were selected. These organisms were procured from Institute of Microbial Technology (IMTECH-CSIR), Chandigarh, India. The microorganisms were sub-cultured on the specific media recommended for different microorganisms such as Nutrient agar for both bacteria and Sabouraud Dextrose Agar (SDA) for *C. albicans*.

Chemicals

Ciprofloxacin (CDH, New Delhi) and Amphotericin B was obtained from Bio basic Inc. Yeast extract, Beef extract, Peptone, Dextrose (Himedia, Mumbai, India), Agar, and Ethanol (S.D. Fine, Mumbai India) were procured for the present study. All the chemicals used in the present study were of analytical grade.

Antimicrobial assay

In vitro antibacterial activity and antifungal activity of aqueous and ethanol extract were assessed by the agar well diffusion method.^[22] In this method, pure isolate of each microbe was subcultured on the recommended specific media for each microorganism at 35-37°C for 25 h. Inoculums of each test organisms were spread on the specific media to achieve a confluent growth. The agar plates were allowed to dry and wells of 6 mm were made with a sterile cork borer in the inoculated agar plates. Then mother stocks of sample 200 mg/ml were prepared and then further dilutions of 100% (200 mg/ml), 75% (150 mg/ml), and 50% (100 mg/ml) were prepared. An 80- μ l volume of each dilution of each extract 100% (16 mg), 75% (12 mg), and 50% (8 mg) was propelled directly into

the wells (in triplicates) of the inoculated specific media agar plates for each test organism. The plates were then incubated at 37°C in an incubator for 16-18 h (for antifungal activity the plates were incubated for 22-24 h).

Ciprofloxacin was used as standard for antibacterial (stock 10 µg/ml) and amphotericin B for antifungal (stock 10 µg/ml). After incubation period, each plate was examined. The diameters of the zones of complete inhibition (as judged by the unaided eye) are measured, including the diameter of the disc. Zones were measured to the nearest whole millimeter, using sliding calipers. This was held on the back of the inverted Petri plate. The experiments were performed in triplicates and the mean values of the diameter of inhibition zones with standard deviation were calculated.

RESULTS AND DISCUSSION

The results of antimicrobial activity of the ethanolic and aqueous extracts of *L. coromandelica* on three microbes (two bacteria and one fungi) by agar well diffusion method have been shown in Tables 1 and 2.

Activity against *S. aureus*

From the evaluation of data, it is evident that ethanolic extract has higher antibacterial activity against *S. aureus*. One hundred percent, 75%, 50% concentrations have 124.97%, 119.98%, 106.72% activity, respectively, [with the mean diameter of the zone of inhibition (ZI) being 19.21 mm,

18.45 mm, 16.41 mm] as compared with control drug i.e., 100% (Ciprofloxacin, zone of inhibition 15.37 mm) [Figure 1]. While aqueous extract has higher activity only in 100% concentration i.e., 126.38% (ZI-19.76 mm) as compared with control drug (ZI-15.64 mm). However, 75%, 50% concentrations showed sensitivity 92.92% and 79.45% (ZI-14.53 mm and 12.42 mm), respectively.

Activity against *S. pyogens*

Analysis of agar well plates showed that ethanolic extract has higher activity against *S. pyogens*. 100%, 75%, 50%, concentrations have 117.09%, 112.08%, 105.66% (with the mean diameter of the zone of inhibition being 18.12 mm, 17.35 mm, 16.35 mm) activity, respectively, as compared with control drug (ZI-15.48 mm) and aqueous extract have higher activity only in 100%, 75% concentration i.e., 119.97%, 101.93% (ZI-19.18 mm, 16.29 mm), respectively, while 50% concentration showed 84.34% (ZI-13.48 mm) activity [Figure 2].

Activity against *C. albicans*

From the data present in Tables 1 and 2 it was concluded that only 100% concentration (ZI 16.97 mm) of aqueous extract was higher than the control (Amphotericin-B, with the mean diameter being 14.17 mm), and in 75%, 50% concentrations showed 93.48%, 76.88% (ZI-13.25 mm, 10.89 mm) activity. However, ethanolic extract showed lesser activity as compared with control drug i.e., 95.58%, 80.02%, 66.24% (ZI-13.20 mm, 11.05 mm, 9.15 mm), respectively for 100%, 75%, and 50% concentrations [Figure 3].

Table 1: Zone of inhibition of aqueous ext. of *Lannea coromandelica* on microbes

Aqueous extract				
<i>Staphylococcus aureus</i>	100%	75%	50%	Control (ciprofloxacin)
1.	19.79	14.53	12.45	15.16
2.	19.68	14.48	12.32	16.14
3.	19.83	14.59	12.51	15.62
Mean	19.76667	14.53333	12.42667	15.64
SD	0.077675	0.055076	0.097125	0.490306
<i>Streptococcus pyogens</i>	100%	75%	50%	Control (ciprofloxacin)
1.	19.15	16.13	13.55	15.82
2.	19.27	16.35	13.42	16.02
3.	19.12	16.41	13.48	16.12
Mean	19.18	16.29667	13.48333	15.98667
SD	0.079373	0.147422	0.065064	0.152753
<i>Candida albicans</i>	100%	75%	50%	Control (Amphoterecin B)
1.	16.78	13.32	11.82	14.26
2.	16.89	12.97	10.73	14.23
3.	17.24	13.46	10.14	14.03
Mean	16.97	13.25	10.89667	14.17333
SD	0.240208	0.252389	0.852311	0.125033

SD=Standard deviation

Table 2: Zone of inhibition of Ethanolic ext. of *Lannea coromandelica* on microbes

Ethanolic extract				
<i>Staphylococcus aureus</i>	100%	75%	50%	Control (ciprofloxacin)
1.	19.15	18.43	16.45	15.17
2.	19.26	18.53	16.41	15.24
3.	19.24	18.39	16.37	15.72
Mean	19.21667	18.45	16.41	15.37667
SD	0.058595	0.072111	0.04	0.299388
<i>Streptococcus pyogens</i>	100%	75%	50%	Control (ciprofloxacin)
1.	18.02	17.44	16.34	15.34
2.	18.21	17.36	16.46	15.28
3.	18.15	17.25	16.27	15.82
Mean	18.12667	17.35	16.35667	15.48
SD	0.097125	0.095394	0.09609	0.295973
<i>Candida albicans</i>	100%	75%	50%	Control (Amphoterecin B)
1.	15.62	12.73	9.83	14.16
2.	12.14	10.62	8.96	13.24
3.	11.86	9.82	8.67	14.05
Mean	13.20667	11.05667	9.153333	13.81667
SD	2.094692	1.503341	0.603683	0.502427

SD=Standard deviation

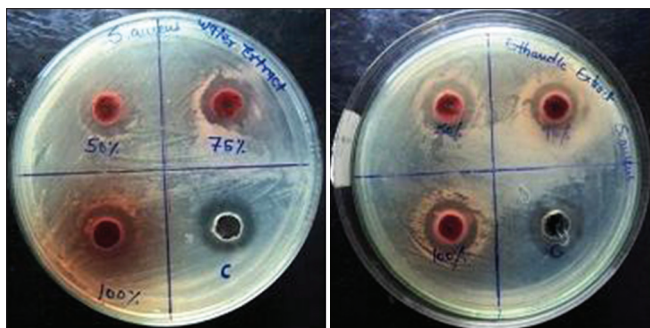


Figure 1: Zone of inhibition of *Lannea coromandelica* against *Staphylococcus aureus*

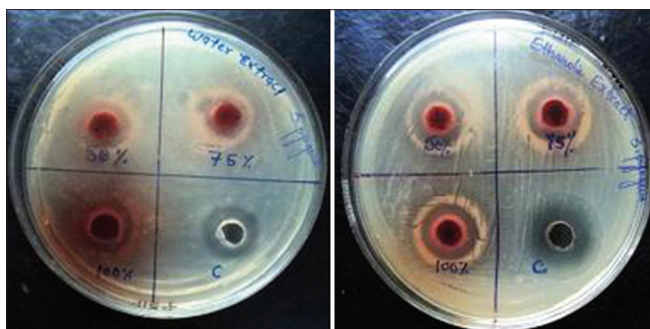


Figure 2: Zone of inhibition of *Lannea coromandelica* against *Streptococcus pyogenes*

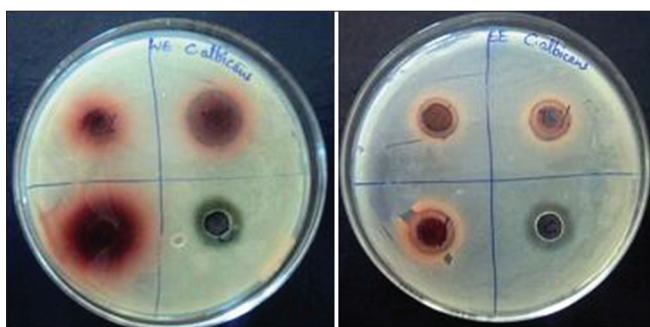


Figure 3: Zone of inhibition of *Lannea coromandelica* against *Candida albicans*

The preliminary phytochemical analysis revealed the presence of flavonoids, triterpenoids, phenols, gallic tannins, coumarins in *L. coromandelica*.^[23-25] The major active ingredient responsible for antimicrobial activity of *L. coromandelica* is flavonoids, the major group of phenolic compounds reported for their antimicrobial,^[26] while coumarins reported for antibacterial^[27] and antifungal.^[28]

CONCLUSIONS

Based on our findings, it is concluded that *L. coromandelica* bark extracts have immense potential as antimicrobial and antifungal compounds against microorganisms and they can be used in the treatment of female RTI caused by microorganisms. Ethanolic bark extract

showed stronger activity against all the tested bacterial strains. However, aqueous extract demonstrated higher antifungal activity. Therefore, *L. coromandelica* can be selected for further analysis. It can be used to discover bioactive natural products that may serve as leads in the development of new antimicrobials that address unmet therapeutic needs.

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