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ANTIMICROBIAL ACTIVITY OF CHLOROFORM AND METHANOL EXTRACT OF *LENNEA COROMANDELICA* Merr.

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ABSTRACT. The antimicrobial activity of chloroform and methanol extracts of *Lennea coromandelica* were screened for their was studied against gram positive bacteria strains *Staphylococcus aureus, Streptococcus pyogenes* and *Bacillus subtilis* gram negative bacteria strains *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Escherichia coli* and fungal strains such as *Candida albicans, C. parapsilosis, C. krusei* and *C. glabrata* using disc diffusion method, determination of Minimum Inhibitory Concentrations (MIC), Minimum Bacterial Concentrations (MBC) and Minimum Fungicidal Concentrations (MFC). The antimicrobial activity for different concentrations like 250 µg, 500 µg and 1000 µg of different solvent extracts of *Lannea cormandelica.* bacterial strains and recorded in highest mean zones of inhibition ranged from 19.6 mm and *Candidal* strains and the exhibited the highest mean zones of inhibition ranged from 10.6 mm. Methanol extracts showed the best results as inhibition zones against tested organisms. Results showed also that, the greatest effect was towards *Staphylococcus aureus* and the lowest was against *Candida krusei*. The present study reported the great effect of *Lannea cormandelica* extracts against some of most important pathogens.

1. INTRODUCTION

Medicinal plants are major source of great economic value in the Indian subcontinent. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. India is rich in all the three levels of biodiversity, namely species diversity, genetic diversity and habitat diversity. In India thousands of species are known to have medicinal value and the use of different parts of several medicinal plants to cure specific aliments has been in vogue since ancient times (Jigha and Sumitra 2006). A number of new antibiotics have been produced by pharmacological industries but the toxic effects and the global emergence of multi-drug resistant (MDR) of microbes is limiting the effectiveness of these drugs (Hancock, 2005). Medicinal plants have been used to treat human diseases for thousands of years because they have vast and diverse assortment of organic compounds that can produce a definite physiological action on the human body. Most important of such compounds are alkaloids, tannins, flavonoids, terpenoids, saponins and phenolic compounds. Pharmacists are interested in these compounds because of their therapeutic performance and low toxicity (Inayatullah et al., 2012). A number of such compounds have been isolated from plants which could be used for the development of new drugs to inhibit the growth of bacterial and fungal pathogens and to quench ROS with possibly novel mechanisms of action and low toxicity to the host cell (Ahmad and Aqil, 2007).

Lennea coromandelica Merr. Coming under Anacardiaceae family. It is a medium sized deciduous tree. Bark thick, ashy grey. Leaves crowded at the end of branches. Flowers small, greenish yellow in compact fascicles of racemes, at the end of the leafless branches. Drupes, reniform, produced in clusters from the end of leafless branches. The plant used in astringent and stomachic; used as a lotion in impetigenous eruptions, leprous and obstinate ulcers; cures sprains, bruises, skin eruptions, heart diseases, dysentery and mouth sores. In the present investigation, various solvent extracts viz. chloroform and methanol of leaf and bark *Lannea coromadelica* were studied for its antimicrobial activity against gram positive bacteria *viz., Staphylococcus aureus,*

Streptococcus pyogenes and *Bacillus subtilis,* gram negative bacteria *viz., Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Escherichia coli* and fungal strains such as *Candida albicans, C. parapsilosis, C. krusei* and *C. glabrata.*

2. MATERIALS AND METHODS:

2.1. Plant Collection:

The healthy leaves and bark of *Lannea coromadelica* were collected from Akkaramangalam, Cuddalore district, Tamilnadu, India.

2.2. Preparation of plant extracts:

The collected plant parts were immediately brought to the laboratory, washed with tap water, surface sterilized with 10% sodium hypochlorite solution and rinsed with sterile distilled water and shade dried. After shade drying the leaves and barks were packed in brown cover and kept in an oven at 60˚C for an hour to make grinding easy. The samples were ground into powder using electric blender. Two hundred grams of powder of leaves/barks of *Lannea coromadelica* were loaded in separate Soxhlet apparatus and extracted with solvents, chloroform and methanol. The solvent was evaporated using rotary evaporator under reduced pressure at 40˚ C and the crude extracts were kept at 4˚C in refrigerator for antimicrobial screening.

2.3. Antimicrobial assay

2.3.1. Collection of test organisms

Ten clinical microbial strains *viz.,* gram positive bacteria such as *Staphylococcus aureus, Streptococcus pyogenes, Bacillus subtilis,* gram negative bacteria such as *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Escherichia coli*, fungal strains such as *Candida albicans*, *C. parapsilosis, C. krusei* and *C. glabrata* obtained from Rajah Muthiah Medical College and Hospital, Annamalai University, Tamilnadu, India. Stock cultures were maintained on nutrient agar medium (for bacteria) and Sabouraud Dextrose agar medium (for fungi) at 4˚C.

2.3.2 Disc diffusion assay

The antibacterial and anticandidal activity of crude extracts was determined by disc diffusion method according to Bauer *et al*., (1966) with modifications. Petri plates were prepared by pouring 20 ml of MHA for bacteria and SDA for candidal fungi. Then the plates were allowed to solidify and used in susceptibility test. 100 µL of bacterial and fungal suspension containing 10^8CFU/ml of bacteria and 10^4spore/ml of fungi were swabbed on the top of the solidified respective media and allowed to dry for 10 minutes. The crude extracts was dissolved in 10 per cent Dimethyl sulphoxide (DMSO) and under aseptic conditions Whatmann No.1 paper disc (6 mm) were impregnated with 20 µL of different concentrations (1000, 500 and 250 µg/disc). The discs with extracts were placed on the surface of the medium with sterile forceps and gently pressed to ensure contact with inoculated agar surface. Ciprofloxacin (10 µg/disc) for bacteria, Amphotericin B (100 units/disc) for *Candida* species were used as positive controls and 10 per cent DMSO was used as blind control in all the assays. Finally, the inoculated plates were incubated at 37 °C for 24 h for all bacterial strains, 28 °C for 24-48 h for *Candida* sps. The zone of inhibition was observed and measured in millimeters. The experiment was repeated three times.

2.3.3. Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentrations of the crude extracts were tested in Muller Hinton Broth for bacteria and Sabouraud Dextrose Broth for mycelia fungi to get the concentrations of 1000 – 15.6 µg/ml by the broth macro dilution method. The culture tubes were incubated at 37 °C for 24 h (for bacteria) and 28 °C for 48 h (for fungi).

2.3.4. Determination of the Minimum Bactericidal Concentration (MBC) and the Minimum Fungicidal Concentration (MFC)

MBC and MFC were determined by plating a loopfull of samples from each MIC assay well with growth inhibition in to freshly prepared MHA for bacteria and SDA for fungal strains. The plates were incubated at 37 °C for 24 h for all bacterial strains, 28 °C for 24-48 h for *Candida* species. The MBC and MFC were recorded as the lowest concentration of the crude extracts that did not permit any visible bacterial and fungal growth after the period of incubation.

3. RESULT

In the present study, variuos concentrations of chloroform and methanol extracts of leaves and bark of *Lannea cormandelica* were tested against gram positive bacteria *viz., Staphylococcus aureus, Streptococcus pyogenes* and *Bacillus subtilis,* gram negative bacteria *viz., Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Escherichia coli* and fungal strains such as *Candida albicans, C. parapsilosis, C. krusei* and *C. glabrata.* The Isolated test organisms exhibited the antimicrobial activity for different concentrations like 250 µg, 500 µg and 1000 µg of different solvent extracts of *Lannea cormandelica.*

The minimum inhibitory concentration and minimum fungicidal concentration were from 125 to 500 µg/mL and 250 to 1000 µg/mL respectively. The standard drug, Ciprofloxacin (10 µg/disc) was used for bacterial strains and recorded mean zones of inhibition ranged from 27.0 ± 0.5 to 31.5 ± 0.3 mm and Amphotericin B (100 units/disc) was utilized for *Candidal* strains and the exhibited the mean zones of inhibition ranged from 15.5 ± 0.8 to 17.8 ± 0.2 mm.

The results of antimicrobial activity of chloroform extract of leaves of *Lannea coromandelica* is presented in Table 1. The mean zone of inhibition for bacteria ranged between 9.0 ± 0.5 and 16.3 ± 0.2 mm on the other hand, for *Candidal* strains, the mean zone of inhibition values were from 7.2 ± 0.2 to 9.6 ± 0.5 mm. For bacteria, the highest mean zone of inhibition (16.3 ± 0.2 mm) was observed against *Staphylococcus aureus.* The lowest zone of inhibition (9.0 ± 0.5 mm) was recorded against *E. coli.* With regard to fungal strains tested, the highest zone of inhibition was recorded against *Candida glabrata* with a mean zone of inhibition of 9.6 ± 0.5 mm. The lowest zone of inhibition (7.2 ± 0.2 mm) was recorded against *Candida krusei.*

The antimicrobial activity of methonal extracts of leaves of *Lennea coromandelica* was carried out and the results is presented in Table 2. The mean zone of inhibition for bacteria ranged from 9.6 ± 0.7 to 19.6 ± 0.2 mm. On the other hands, for *Candidal* strains, the mean zone of inhibition values was from 7.1 ± 0.5 to 10.6 ± 0.5 mm. For bacteria, the highest mean zone of inhibition (19.6 ± 0.2 mm was recorded against *Staphylococcus aureus. E. coli* was less susceptible towards the extract in the mean zone of inhibition 9.6 ± 0.7 mm. With regard to fungal strains tested, the highest mean zone of inhibition (10.6 ± 0.5 mm) was observed against *Candida glabrata* and the lowest mean zone of inhibition (7.1 ± 0.5 mm) was recorded against *C. krusei.*

Table 1. Antimicrobial activity of chloroform extract of leaves of *Lannea coromandelica*

Microbial strains	Mean zone of inhibition (mm)[b]			Ciprofloxan (10 µg/disc)/ AmphoteriB (100 units/disc)	Minimum Inhibitory Concentra-tion (µg/mL)	Minimum Bactericidal concentration/ Minimum Fungicidal concentration (µg/mL)
	Concentration the disc (µg/mL)					
	1000	500	250			
Staphylococcus aureus	16.3 ± 0.2	13.1 ± 0.2	11.0 ± 0.8	30.0 ± 0.5	125	250
Streptococcus pyogenes	13.5 ± 0.5	11.8 ± 0.7	9.8 ± 0.76	29.3 ± 0.28	250	500
Bacillus subtilis	15.6 ± 0.1	13. 3 ± 0.2	10.1 ± 0.57	30.3 ± 0.57	250	500
Klebsiella pneumoniae	16.0 ± 0.86	14.3 ± 0.28	12.5 ± 0.28	28.5 ± 0.50	250	500
Pseudomonas aeruginosa	14.6 ± 0.86	11.6 ± 0.28	9.8 ± 0.28	30.0 ± 1.00	250	500
Escherichia coli	13.1 ± 0.28	11.8 ± 0.57	9.0 ± 0.50	27.3 ± 0.28	250	500
Candida albicans	9.5 ± 0.50	8.1 ± 0.28	7.3 ± 0.28	16.8 ± 0.28	500	1000
Candida krusei	9.6 ± 0.28	8.3 ± 0.76	7.2 ± 0.28	17.0 ± 1.0	500	1000
Candida paprapsilosis	9.3 ± 0.57	8.1 ± 0.28	7.8 ± 0.28	16.0 ± 0.5	500	1000
Candida glabrata	9.1 ± 0.28	8.3 ± 0.57	7.5 ± 0.50	15.5 ± 0.86	500	1000

a-diameter of zone of inhibition (mm) including the disc diameter of 6 mm
b-mean of three assays; ± standard deviation.

Table 2. Antimicrobial activity of methanol extract of leaves of *Lannea coromandelica*

Microbial strains	Mean zone of inhibition (mm)[b]			Ciprofloxacin (10 µg/disc)/ Amphotericin B (100 units/disc)	Minimum Inhibitory Concentra-tion (µg/mL)	Minimum Bactericidal concentration/ Minimum Fungicidal concentration (µg/mL)
	Concentration the disc (µg/mL)					
	1000	500	250			
Staphylococcus aureus	19.6 ± 0.2	17.6 ± 0.2	15.5 ± 0.5	30.6 ± 0.8	125	250
Streptococcus pyogenes	15.8 ± 0.28	13.3 ± 0.28	10.6 ± 0.57	29.0 ± 0.5	250	500
Bacillus subtilis	18.1 ± 0.28	15.6 ± 0.28	12.5 ± 0.50	28.3 ± 0.28	125	250
Klebsiella pneumoniae	18.5 ± 0.56	16.8 ± 0.28	14.5 ± 0.50	31.3 ± 0.57	125	250
Pseudomonas aeruginosa	16.5 ± 0.50	13.5 ± 0.50	10.6 ± 0.76	30.0 ± 1.30	250	500
Escherichia coli	15.5 ± 0.50	13.3 ± 0.76	9.6 ± 0.76	28.0 ± 1.00	250	500
Candida albicans	9.0 ± 0.50	8.6 ± 0.57	7.3 ± 0.28	17.3 ± 0.28	500	1000
Candida krusei	9.3 ± 0.28	8.0 ± 0.50	7.1 ± 0.57	16.3 ± 0.28	500	1000
Candida paprapsilosis	9.6 ± 0.28	8.5 ± 0.76	7.2 ± 0.28	17.3 ± 0.28	500	1000
Candida glabrata	10.6 ± 0.57	8.6 ± 0.86	7.4 ± 0.76	17.5 ± 0.50	500	1000

a-diameter of zone of inhibition (mm) including the disc diameter of 6 mm
b-mean of three assays; ± - standard deviation.

Table 3. Antimicrobial activity of chloroform extract of bark of *Lannea coromandelica*

Microbial strains	Mean zone of inhibition (mm)[b]			Ciprofloxacin (10 µg/disc)/ Amphotericin B (100 units/disc)	Minimum Inhibitory Concentra-tion (µg/mL)	Minimum Bactericidal concentration/ Minimum Fungicidal concentration (µg/mL)
	Concentration the disc (µg/mL)					
	1000	500	250			
Staphylococcus aureus	18.6 ± 0.5	15.8 ± 0.2	13.3 ± 0.28	28.6 ± 0.28	125	250
Streptococcus pyogenes	15.8 ± 0.2	13.1 ± 0.2	10.8 ± 0.28	29.8 ± 0.28	250	500
Bacillus subtilis	17.1 ± 0.5	13.6 ± 0.2	11.4 ± 0.58	29.6 ± 1.15	125	250
Klebsiella pneumoniae	14.3 ± 0.2	12.5 ± 0.5	10.0 ± 0.50	30.0 ± 0.50	250	500
Pseudomonas aeruginosa	17.3 ± 0.2	15.0 ± 0.5	13.7 ± 0.28	30.0 ± 0.28	125	250
Escherichia coli	15.0 ± 0.5	13.5 ± 0.5	11.6 ± 0.57	27.0 ± 0.50	125	250
Candida albicans	9.0 ± 0.5	8.1 ± 0.5	7.0 ± 0.50	16.5 ± 0.50	500	1000
Candida krusei	9.8 ± 0.5	8.0 ± 0.5	7.3 ± 0.28	15.8 ± 0.76	500	1000
Candida paprapsilosis	10.0 ± 0.5	8.6 ± 0.2	7.5 ± 0.50	17.6 ± 0.28	500	1000
Candida glabrata	10.3 ± 0.2	8.8 ± 0.5	7.5 ± 0.50	17.1 ± 0.28	500	1000

a-diameter of zone of inhibition (mm) including the disc diameter of 6 mm
b-mean of three assays; ± - standard deviation.

Table 4. Antimicrobial activity of methanol extract of bark of *Lannea coromandelica*

Microbial strains	Mean zone of inhibition (mm)[b]			Ciprofloxacin (10 µg/disc)/ Amphotericin B (100 units/disc)	Minimum Inhibitory Concentration (µg/mL)	Minimum Bactericidal concentration/ Minimum Fungicidal concentration (µg/mL)
	Concentration the disc (µg/mL)					
	1000	500	250			
Staphylococcus aureus	18.6 ± 0.28	16.8 ± 0.3	14.6 ± 0.3	30.3 ± 0.3	62.5	125
Streptococcus pyogenes	16.7 ± 0.2	14.5 ± 0.3	12.0 ±0.50	29.8 ± 0.2	125	250
Bacillus subtilis	17.3 ± 0.3	15.8 ± 0.2	13.5 ± 0.2	27.7 ± 0.2	125	250
Klebsiella pneumoniae	15.5 ± 0.3	13.6 ± 0.3	11.3 ± 0.2	31.5 ± 0.3	250	500
Pseudomonas aeruginosa	14.2 ± 0.2	12.8 ± 0.4	10.9 ± 0.2	30.1 ± 0.2	250	500
Escherichia coli	16.9 ± 0.4	14.5 ± 0.4	11.8 ± 0.3	27.9 ± 0.3	125	125
Candida albicans	10.2 ± 0.3	9.1 ± 0.2	7.8 ± 0.3	17.8 ± 0.2	500	1000
Candida krusei	9.9 ± 0.2	8.2 ± 0.3	7.1 ± 0.28	17.1 ± 0.2	500	1000
Candida paprapsilosis	10.3 ± 0.2	8.7 ± 0.3	7.3 ± 0.3	16.9 ± 0.3	500	1000
Candida glabrata	10.9 ± 0.3	8.5 ± 0.2	7.6 ± 0.3	16.1 ± 0.2	500	1000

a-diameter of zone of inhibition (mm) including the disc diameter of 6 mm
b-mean of three assays; ± - standard deviation.

The antimicrobial activity of chloroform extract of bark of *Lannea coromandelica* against tested microbes was shown in Table 3. The results showed mean zone of inhibition for bacteria ranged were 10.0 ± 0.5 to 18.6 ± 0.5 mm. On the other hand, for *Candidal* strains, mean zone of inhibition were from 7.0 ± 0.5 to 10.3 ± 0.2 mm. For bacteria, the maximum zone of inhibition (18.6 ± 0.5 mm) was observed against *Staphylococcus aureus.* The minimum mean zone of inhibition (10.0 ± 0.5 mm) was recorded against *K. pneumonia.* In fungi, the maximum zone of inhibition (10.3 ± 0.2 mm) was observed against *Candida glabrata* while minimum inhibitory zone (7.0 ± 0.5 mm) was shown against *C. albicans.*

The antimicrobial activity of methonal extracts of bark of *Lennea coromandelica* was screened against bacterial and fungal stains and the results is presented in Table 4. The mean zone of inhibition for bacteria ranged between 10.9 ± 0.2 and 18.6 ± 0.2 mm. On the other hand, for inhibition values of fungi were from 7.1 ± 0.2 to 10.9 ± 0.3 mm. For bacteria, the highest mean zone of inhibition (18.6 ± 0.2 mm) was recorded against *Staphylococcus aureus.* The lowest

inhibitory activity was recorded against *P. aeruginosa* with mean zone of inhibition 10.9 ± 0.2 mm. With regard to fungal strains tested, the highest mean zone of inhibition (10.9 ± 0.3 mm) was observed against *Candida glabrata* and the lowest mean zone of inhibition (7.1 ± 0.2 mm) was recorded against *C. krusei.* All the *Candidal* strains susceptible with MIC of 500 μg/mL and MFC of 1000 μg/mL.

4. DISCUSSION

The plants usually involve approaches; ethno botanical approach is one of the common methods that are employed in choosing the plant for pharmacological study. Traditionally plant parts, extracts, infusions etc., were used for treatment of various diseases. The plants have traditionally provided a source of hope for novel drug compounds as plant herbal mixtures have made large contribution to human health and well being the use of plant extracts with known antimicrobial properties can of great significance for therapeutic treatment (Iwu *et al.,* 1999).

The present research work showed the chloroform and methanol extracts of *Lennea coromandelica* leaf and bark possess antimicrobial activity against most of the tested pathogens. The result of this study showed that the methanol extracts of *Lennea coromandelica* was more effective than the chloroform extracts demonstrated the highest activity. These results support earlier studies which observed that plant extracts in organic solvent provide more consistent antimicrobial activity (Ahmad *et al.,* 1998). Among the Gram – positive bacteria and Gram - negative bacteria tested against the leaf extract of *Lennea coromandelica* have most sensitive organism was *Staphylococcus aurers.* The tested plant extracts were most active against gram positive bacteria than the gram negative bacteria (Ayyappan *et al.,* 2010; Parekh *et al.,* 2005; Rajakaruna *et al.,* 2002). Methanol provided more consistent antimicrobial activity compared to other extracted in chloroform. These activities might depend on the compounds being extracted by each solvent, the polarity of the solvints, and their intrinsic bioactivity. Which have been associated to antibacterial activities and thus have curative properties against pathogens (Nweze *et al.,* 2004).

The antifungal activity of both the extract of *Lennea coromandelica* leaf and bark extracts were determine against four fungal (Candidal) strains and recorded. The highest antifungal activity was observed in methanolic leaf extracts of *Lennea coromandelica* against the tested Candidal strains. Chareprasert *et al.,* (2006) isolated endophytes fungi from *Tectona grandis* L. and *Samanea saman* Merr., from 37 isolated fungi, 18 shown antimicrobial activity against *Bacillus subtilis, staphylococcus arureus, Escherichia coli* and three isolates inhibited the growing of *Candida albicans.* Weber *et al.,* (2007) demonstrated antifungic activity from metabolities of endophyte fungi group ascomicete against *Candida albicans.* Similar results were observed in previous studies (Josephin Sheeba and Selva Mohan, 2012; Hema *et al.,* 2013; Vinoth and Manivasagaperumal, 2015). Further pharmacological evaluations, toxicological studies and possible isolation of the therapeutic drug from the plant are the future challenges.

5. CONCLUSION

Finaly it can be concluded that methanol extract of *Lennea coromandelica* had a potential antimicrobial activity against all the microorganisms tested. Based on this study, isolation and identification of antimicrobial compound from methanol extract of *Lennea coromandelica* will fetch a new natural antimicrobial agent.

6. STATISTICAL ANALYSIS

All the data of microbial activities were examined as mean ± SD. One-way analysis of variance (ANOVA) was carried out to determine the significant difference ($P < 0.005$) between the means. The analyses were carried out using SPSS package software, 11.5 (SPSS Inc., Chicago, IL).

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