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ORIGINAL ARTICLE

**PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITY ON MEDICINAL
PLANT *LANNEA COROMANDALICA* (LINN) BARK EXTRACT**

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ABSTRACT

The present study is to investigate the phytochemical and antibacterial activity of *Lannea coromandalica* bark extract. The *Lannea coromandalica* (Anacardiaceae) is medicinal plant used in the treatment of various diseases (Wound, cancer, diabetics, rheumatic and diarrhea). The water, chloroform and methanol extract from the bark of *lannea coromandlica* were screened on their phytochemicals analysis and the *lannea coromandalica* bark against the antibacterial like *E. coli*, *B. subtilis*, *S. aureus* and *E. faecalis* and *E. pnemonia*. The phytochemical screening showed that all (water, chloroform and methanol) the extract contained at lease trace amount of steroids, terpenoids, saponins, quino nes, tannins and flavonoids. Further, GC-MS analysis shows the presence of following bioactive compounds like 1, 2 – Benzenediol, 4-1 Isopropyl-5-methyl-hexa-2, 4-dienoic acid methyl ester, vitamin A aldehyde and methoprene. These are compound may presence of antimicrobial properties. This study which is the primary report on the biological activities, phytochemicals and antibacterial properties of *Lannea coromandalica* supports its traditional uses in the treatment of infectious and non-non infectious diseases.

Keywords: *Lannea coromandalica*, rheumatic, diarrhea, E.coli, extract, bark and methanol.

1.INTRODUCTION

Life on earth mainly depends on plants and it is very important for survival of human beings. Plant and it products are used by human beings from time immemorial. But very few people realize the importance of plants and it is also a part of our environment. The use of plants as medicine is widespread throughout the world because of increase in the side effects caused by synthetic drugs. Nearly 80% of world population depends on herbal medicines for primary healthcare (Kamboj 2000). The Indian Matera medical includes 2000 drugs are plant base, which are derived from different Indigenous knowledge and folklore practices (Narayana *et al.*, 1998).

Medicinal plants represent a rich source of antimicrobial, antidiabetic and anticancer properties plants are used medicinally in different countries and are a source of many potent and powerful drugs (Srivastava,1996). A wide range of medicinal plant parts

is used for extract as raw drugs and they possess varied medicinal properties. The different parts used include root, stem and flower, fruit twigs exudates and modified plant organs. Which some of these raw drugs are collected in smaller quantities by the local communities and folk healers for local used many other raw drugs are collected in larger quantities and traded in the raw material for many herbal industries (Uniyal, 2006).

A lot of studies have been done on *Lannea coromandelica* on various activities but some important areas like anti-rheumatic or anticancer activities (Shaikh, *et al.*, 2005). Nature has always been a great contributor towards this goal. Plant-derived natural products such as flavonoids, terpenoids and steroids have received considerable attention due to their diverse pharmacological properties, which include cytotoxic and chemopreventive effects (Abdullaev 2001, Cragg *et al.*, 2005).

Lannea coromandelica which is commonly known as “The Indian Ash Tree” (Dinesh Valke.) is a deciduous tree which grows up to 14 meters high. It belongs to the family Anacardiaceae (Jackson, 2000), the bark of the tree has astringent effect and it is used as stomachic in traditional

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medicine. It is used as a lotion in impetiginous eruptions, leprosy and obstinate ulcers. It is known to cure sprains, bruises, skin eruptions, heart diseases, dysentery and mouth sores. The decoction of the bark can be used to alleviate toothache traditionally used to treat impotency (Jyothi *et al.*, 2011). The present study was investigated the phytochemical, GC-MS analysis and antibacterial activity of *Lannea coromandelica* bark extract.

2. MATERIALS AND METHODS

COLLECTION OF PLANT MATERIAL:

The bark of *Lannea coromandelica* Linn was collected from the Nannikuppam village, Pantruti Taluk, Cuddalore District in Tamilnadu, India.

CHEMICALS:

All the chemicals used for this work, were purchased from Precision Scientific Supplies, Trichy.

EXTRACT PREPARATION:

The collected bark of *Lannea coromandelica* were dried at room temperature. Dry bark were uniformly ground using a mechanical grinder to yield fine powder.

PREPARATION OF AQUEOUS EXTRACT:

10 grams of the bark powder was added to 100 ml of the aqueous until super saturation for a period of 24 hours at room temperature and it was heated at 60°C in water bath (temperature do not exceeding the boiling point of aqueous). The bark extract obtained was protected from sunlight and stirred several times with a sterile glass rod. The resultant suspension was then filtered using Whatman no.1 filter paper. The filtrates were then evaporated under reduced pressure.

PREPARATION OF CHLOROFORM EXTRACT:

10 grams of the bark powder was added to 100ml of the chloroform until super saturation for a period of 24 hours at room temperature and it was heated at 60°C in water bath (temperature do not exceeding the boiling point of chloroform). The bark extract obtained was protected from sunlight and stirred several times with a sterile glass rod. The resultant suspension was then filtered using what man no.1 filter paper. The filtrates were then evaporated under reduced pressure. Suspension was then filtered using whatman no.1 filter paper. The filtrates were then evaporated under reduced pressure.

PREPARATION OF METHANOL EXTRACT:

10 grams of the bark powder was added to 100 ml of the methanol until super saturation for a period of 24 hours at room temperature and it was heated at 60°C in water bath (temperature do not exceeding the boiling point of methanol). The bark extract obtained was protected from sunlight and stirred several times with a sterile glass rod. The resultant suspension was then filtered using Whatman No.1 filter paper. The filtrates were then evaporated under reduced pressure.

PHYTOCHEMICAL SCREENING

Phytochemical evaluation for major phytochemicals was done using standard qualitative methods (Sowofora, 1993; Tiwari *et al.*, 2011). Tests for presence of reducing sugars, alkaloids, anthraquinones, tanins, terpenoids, saponins, oils and fats, flavonoids and cardiac glycosides were carried out on both extracts.

GC-MS ANALYSIS:

The GC-MS analyses of these extracts were carried out by following the method of Hema *et al.*, (2010). GC-MS analysis were performed using a Perkin-Elmer GC clauses 500 system and Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with an Elite-I fused silica capillary column (30m×0.25mm ID × 1µdf), composed of 100% Dimethyl polysiloxane. For GC-MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1ml/min and an injection volume of 2µl was employed split ratio of 10:1 injector temperature 250°C; ion-source temperature 2800°C. The oven temperature was programmed from 110°C (isothermal for 2 min) with an increase of 100°C/min to 2000°C, then 50°C/min to 2800°C, ending with a 9 min isothermal at 2800°C. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 36 minutes. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas, software adopted to handle mass spectra and chromatograms was a Turbo mass.

MICROORGANISMS USED FOR ANTIBACTERIAL ACTIVITY:

The microorganisms are collected from Pondicherry, Center for Biological Science. The name of the culture for *staphylococcus aureus*, *Bacillus subtilis*, *Escherichiacoli*, *Enterococcus faecalis*, *Klebsiella pneumonia*.

ANTIBACTERIAL ACTIVITY BY DISC DIFFUSION METHODS:

In this method 0.1ml of 24 hour old culture of test pathogen was spread on the surface of nutrient agar plate. The sterile antibiotic disc into 4mm diameter, impregnated and the medicinal that is respective solvent extract was loaded in the disc at equal distance in a concentration of 5µ the plate were kept at room temperature for 30 min, which helps to diffuse extracts on the medium. The antibacterial activity was studied in the various solvent extract of *Lannea coromandelica* bark extract.

3. RESULTS AND DISCUSSION

The bark of *L. coromandelica* contained a number of phytochemicals such as alkaloids, glycosides, steroids, terpenoids, reducing sugars and amino acids (Table.1). Among these phytochemicals are maximum present in all extracts (Water, Chloroform and Methanol). Above the three extracts aqueous extract has more phytochemical when compared with other extracts.

Table 1: Phytochemicals analysis of *Lannea coromandelica* bark

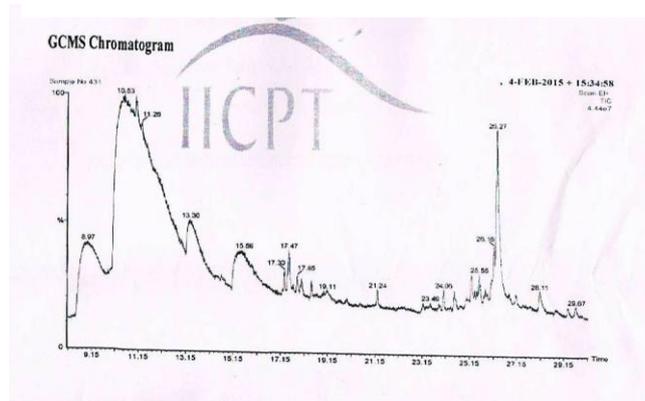
S. No	Compound Name	Solvents		
		Water	Chloroform	Methanol
1.	Alkaloids	-	+	-
2.	Volatile oils	-	-	-
3.	Fatty acids	-	-	-
4.	Emodins	+	-	-
5.	Flavonoids	-	-	-
6.	Steroid Terpenoids	+	-	+
7.	Anthracene glycosides	-	-	-
8.	Phenolics	-	-	-
9.	Saponins	-	-	-
10.	Tannins	+	-	+
11.	Carbohydrates	+	+	+
12.	Cardiac glycosides	+	+	+
13.	Reducing sugars	+	+	+
14.	Amino acids	+	+	-

Table 2: GC-MS analysis report for ethanol extract of *Lannea coromandelica* bark

S. No	RT	Name of the compounds	Molecular Formula	MW	Peak Area
1.	8.97	1,2-Benzenediol	C ₆ H ₆ O ₂	110	11.74
2.	10.53	1,2,3-Benzenetriol	C ₆ H ₆ O ₃	126	69.80
3.	13.30	2,4-Octadienoic acid, 7-hydroxy-6-methyl-, [r-[r*,s*-(E,E)]]-	C ₉ H ₁₄ O ₃	170	9.12
4.	15.59	4-Decyenoic acid, methyl ester	C ₁₁ H ₁₈ O ₂	182	2.98
5.	17.30	Benzenepropanoic acid, 10-undecenyl ester	C ₂₀ H ₃₀ O ₂	302	0.15
6.	19.11	Cyclohexanepropanoic acid, 1,2-dimethyl-6-methylene-	C ₁₂ H ₂₂ O	182	0.38
7.	23.49	4-Isopropyl-5-methyl-hexa-2,4-dienoic acid, methyl ester	C ₁₁ H ₁₈ O ₂	182	0.15
8.	25.55	Octadecatrienoic acid, phenyl ester, (Z,Z,Z)-	C ₂₅ H ₃₆ O ₂	368	0.38
9.	26.27	Vitamin A aldehyde	C ₂₀ H ₂₈ O	284	4.61
10.	28.11	1,2-Cyclopentanedicarboxylic acid, 4-(1,1-dimethylethyl)-, dimethyl ester, (1a',2a', 4a')-	C ₁₃ H ₁₄ O ₄	242	0.53
11.	29.67	Methoprene	C ₁₉ H ₃₄ O ₃	310	0.15

Table 3: Antibacterial activity of *Lannea coromandelica* bark

S. No	Name of the organisms	Extracts		
		Water	Chloroform	Methanol
1.	<i>E. coli</i>	2	7	3
2.	<i>B. subtilis</i>	-	4	7
3.	<i>S. aureus</i>	1	6	1
4.	<i>E. faecalis</i>	1	-	2
5.	<i>K. pneumoniae</i>	-	7	9

Figure 1. GC-MS Histogram of *Lannea*

In antibacterial testing, the methanol, chloroform and Aqueous extract of *Lannea coromandelica* were tested against five different human pathogenic bacteria (shown in table 2). Among these extracts, methanol, chloroform will possess antibacterial activity against *Enterococcus faecalis*. Chloroform extract showed maximum inhibition observed in *E. coli*, *K. pneumoniae* when compared with other bacteria. The methanol the maximum activity observed in *K. pneumoniae* and *E. coli*. Water extract showed activity against *Bacillus subtilis*, *Staphylococcus aureus*, showed maximum inhibition in aqueous extract.

The leaves of *Lannea coromandelica* bark were subjected to GC-MS studies. The various plant phytochemical compounds found in the bark of *Lannea coromandelica* methanol extract are listed in table-2, interpreted on mass spectrum GC-MS was conducted using the data base of (IICPT) (Kumaravel, 2010). The name, molecular weight, and molecular formula of the components of the test material were ascertained in table-3. According to the result, the phytochemicals are screened and most of the medicinal properties of the plant may be the presence of these following phytoconstituents: 1,2-Benzenediol (peak area is 11.74%), 1, 2, 3-Benzenetriol (peak area is 69.80%), 2,4-Octadienoic acid, 7-hydroxy-6-methyl-, [r-[r*,s*-(E,E)]]- (peak area is 9.12%), 4-Decyenoic acid, methyl ester (peak area is 2.98%), 6,9,12-Octadecatrienoic acid, phenylmethyl ester, (Z,Z,Z)- (peak area is 0.38%), Vitamin A aldehyde (peak area is 4.61%), 1,2-Cyclopentanedicarboxylic acid, 4-(1,1-dimethylethyl)-, dimethyl ester, (1a',2a', 4a')- (peak area is 0.53%), Methoprene (peak area is 0.15%).

In the present study preliminary phytochemical screening revealed that the presence of alkaloids, emodins, steroid, triterpenoids, tannins, carbohydrates, cardiac glycosides, reducing sugars and amino acids as probable active compounds present in the crude extracts of *Lannea coromandelica*. It is well known that numerous members of these phytochemical groups have already demonstrated antibacterial activity (Cowan, 1999). In many cases, these substances serve as plant defense mechanisms against aggression by microorganisms, insects, and herbivores either synthesized only after contact with the pathogen induced resistance factor (Castro, 2005).

The presence of phytochemicals in *L. coromandelica* bark extract revealed that, tannins are known to be useful in the treatment of inflamed or ulcerated tissues and they have remarkable activity in cancer prevention and anticancer (Ruch *et al.*, 1998). Flavonoids have been shown to exhibit their actions through effects on membrane permeability, and by inhibition of membrane-bound enzymes such as the ATPase and phospholipase A2 (Li *et al.*, 2003). Flavonoids serve as health promoting compound as a results of its anion radicals (Hausteen, 1983). These observations support the usefulness of this plant in folklore remedies in the treatment of stress related ailments and as dressings for wounds normally encountered in bruises, cuts and sores (Ferguson *et al.*, 2001 and Grierson, 1999). The plant extract was also positive for steroids which are very import and compounds especially due to their relationship with compounds such as sex hormones (Okwu, 2001). The presence of these phenolic compounds in this plant contributed to their anti-oxidative properties and thus the usefulness of these plants in herbal medicament. Alkaloid was detected in this plant study. Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity (Nobori *et al.*, 1995).

The presence of these secondary metabolites in plants, produce some biological activity in man, animals and it is responsible for their use as herbs. These compounds also serve to protect the plant against infection by microorganisms, predation by insects and herbivores, while some give plants their odors and or flavors and some still are responsible for their pigments. In some cases, the activity has been associated with specific compounds or classes of compounds . these active constituents can be used to search for bioactive lead compounds that could be used in the partial synthesis of more useful drugs (Ogbonnia *et al.*, 2008 and Mahmood *et al.*, 2010).

In a study with *Lannea coromandelica* bark it has been pointed out that the pattern of inhibition largely depends upon extraction procedure, plant parts, physiological and morphological state of plant, extraction solvent and microorganism tested. It has been demonstrated that extracts prepared using dried plant material is much more effective than the fresh plant materials (Goyal *et al.*, 2008). Infectious diseases are a major causes of morbidity and mortality in India. The number of multiple drug resistant strains and the appearance of the strains with reduced susceptibility to antibiotics are continuously increasing. This situation provided the impetus to the search for new antimicrobial substances from various sources like medicinal plants. The *Lannea coromandelica* bark extracts has shown remarkable antibacterial activity. It is important to investigate scientifically these plants which have been used in traditional medicines as potential source of novel antibacterial compounds. The first step towards this goal is the in-vitro antibacterial activity assay. In the bark extracts of *Lannea coromandelica* was tested against various human pathogenic bacteria species like E.coli, K. pneumonia *Bacillus substilis* *Staphylococcus aureus* and other disease caused pathogens (Avians Kumar Reddy *et al.*, 2011)

4.CONCLUSION

The present work concluded that phytochemical analysis and different extracts such as methanolic, chloroform extracts except aqueous extract inhibited bacterial growth and growth of gram negative and gram positive bacteria. Plant *Lannea coromandelica* bark extract was ineffective against *staphylococcus aureus*, *Bacillus substills*, *Escherichiacoli*, *Enterococcus faecalis*, *Klebsiella pneumonia*. The antimicrobial activity has been attributed to the presence of some active constituents in the extracts. This study is a substantial step and it further requires a long term study to evaluate therapeutic efficacy and toxicity of leaf, stem and flower of *Lannea coromandelica*. This result may provide a basis for the isolation of compounds from this plant component and establish the mechanism of action for antimicrobial activity of different parts of the plant with different extracts.

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