

Anti-tuberculosis Property of Various Extracts of *Lannea* against multi-drug resistant *Mycobacterium tuberculosis*

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ABSTRACT

Tuberculosis had been a stubborn disease that human beings are combating with from ages. It is a disease that affects the pulmonary system, basically the trachea and upper respiratory organs. It is caused by bacteria called *Mycobacterium tuberculosis*. Bacteria in the respiratory tract will cause infection and inflammation in the respiratory passages. It leads to narrowing and pain, which results in difficulty in breathing. There had been raising the confidence of the safety and potency of the herbs and medicinal plants. We have been using herbs to treat many diseases. The diseases that are caused primarily due to the viruses and bacteria are effectively treated using herbs in the traditional systems of medicine. One such plant is *Lannea coromandelica* which is proven to treat many diseases and is rich in polyphenols and flavonoids. The leaves of *lannea* were extracted using various solvents based on the polarity. It was then investigated for the anti-bacterial property against *mycobacterium tuberculosis*. All the extracts showed potency by inhibiting the bacteria, but Methanol and aqueous extracts showed a significantly better activity compared to the standard.



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INTRODUCTION

Tuberculosis had been a stubborn disease that human beings are combating with from ages [1]. It is a disease that affects the pulmonary system, basically the trachea and upper respiratory organs [2]. It is caused by bacteria called *Mycobacterium tuberculosis* [3]. Bacteria in the respiratory tract will cause

infection and inflammation in the respiratory passages [4]. It leads to narrowing and pain, which results in difficulty in breathing [5]. The patient was suffering from tuberculosis experiences often shortness of breath, pain with inspiration, inflamed and sore tract and difficulty for swallowing [6]. Slowly his condition deteriorates, and eventually, the patient dies [7].

There had been many drugs that are used to treat bacteria [8] successfully. They act upon the bacterial RNA by altering their structure and replication process [9]. They interfere with the cellular processes of the bacterial multiplication and results in the inhibition of the disease [10]. They are potent drugs like Rifampicin and Isoniazid that were proven effective and influential against the disease [11]. These drugs have been used to treat TB from the time the drugs were invented. They were very successful in completely curing the disease, but recent trends in the bacterial resistance is an unsolved question.

The bacteria are gaining resistance to the potent drugs leading to the increase in the dose of the drugs. It results in the rise in resistance, and finally, the bacteria will no longer respond to the existing drugs. There is also a concern that synthetic drugs have side effects and adverse effects. Besides bacterial resistance making the drugs ineffective, the side effects also pose a problem in the effective use of drugs. So there is an urgent necessity to search for alternative treatments to the drugs [12].

There had been raising confidence in the safety and potency of the herbs and medicinal plants. We have been using herbs to treat many diseases [13]. The diseases that are caused primarily due to the viruses and bacteria are effectively treated using herbs in the traditional systems of medicine. One such plant is *Lannea coromandelica* which is proven to treat many diseases and is rich in polyphenols and flavonoids as investigated by Avinash Kumar Reddy et al. [14]. Having in mind the anti-bacterial property of *Lannea* various extracts had been studied on the potency of *lannea* on *Mycobacterium tuberculosis* and establishing which extract shows a better activity in TB [15].

Preparation of extracts

The fresh leaves of the plant *Lannea coromandelica* were collected from the local area in Nellore and adequately authenticated by a botanist. The reference herbarium sample is stored in the college library for any future reference. The plant leaves were dried under direct sunlight for two days and powdered finely. This fine powder was weighed, and 50g of this was extracted with Soxhlet using various solvents like Pet ether, N-Hexane, Benzene, Chloroform, Ethyl acetate, Acetone, Ethanol, Methanol and distilled water. The extracts were collected and dried to remove moisture. The dried extracts were stored in a desiccator and names serially as follows; LPE, LNE, LBE, LCE, LEE, LACE, LETE, LME and LWE. These extracts were used for further experiments.

The extracts were then dissolved in respective solvents in the specific quantities to achieve 5microgram/ml, 10microgram/ml, 20microgram/ml and 40microgram/ml. which were marked and subjected for centrifugation at 4000rpm. All the solid undissolved matter was filtered off, and the clear liquid was collected and used in the experiments.

Bacterial Cultures

The microbes tested were *mycobacterium tuberculosis*, and they were collected from the swabbing of the TB patient. The swabbing was performed in the presence of a registered medical practitioner who

authenticated the patient is suffering from TB. A sterile cotton swab was taken, and the bacterial strain was collected by swabbing his throat secretions. His sputum was also collected and stored in the college laboratory in nutrient broth medium. This culture was let to incubate for 24 hr in an oven at 35°C. from this mother culture; sub cultures were taken and used for further experiments.

Antitubercular activity

The antitubercular activity was tested for all the extracts that were resulted from various solvents were tested in disc diffusion method. Theivasanthia [4] proposed the standard procedure. Petri plates were sterilized in the oven, and freshly prepared agar medium was poured in the plates. It was let to cool and solidify. The nutrient broth medium with the culture was poured on to the agar medium and ensured a thin film was spread evenly in the plate. The plates were then incubated. The diffusion discs of about 0.5 cm in diameter were dipped in the extracts each different in different extracts. They were gently placed on the agar plates which were spread in equal distances from one another.

They were appropriately marked to future identification. Plates were then incubated in an oven at 40°C for about 24hrs and taken out to measure the zone of inhibitions. The agar plates were taken out and regulated for the zone of inhibition but calculating the distance from edge of diffusion disc to the edge of the clear zone where the bacteria were inhibited. It was noted against each of the extracts.

RESULTS AND DISCUSSION

The plant leaves of *Lannea* were extracted using various solvents, and the colour of the extracts was given in Table 1. The polar solvents, Water, Methanol and ethanol nad acetone yielded extracts that are brown to green in colour. They are dark and clear. They had a very bitter taste and a characteristic odour. Non-polar or organic solvents like Pet ether, chloroform, hexane and benzene yielded extracts that are light greasy textured with dark green to yellow colour. They had a little slimy texture to them and had a solvent odour.

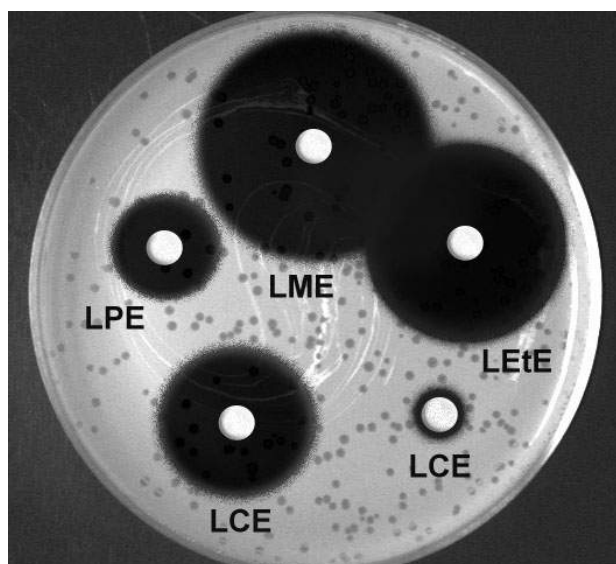
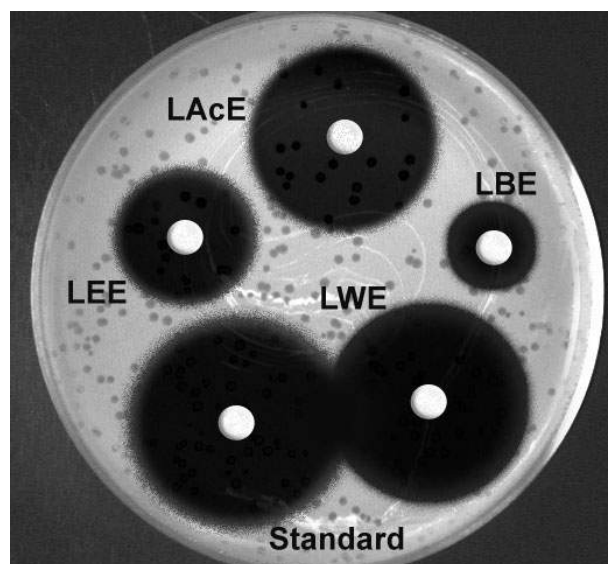
The percentage of yields were also tabulated in Table 1. The Methanol extract and aqueous extracts were highest in the yields, and the least yield was achieved with hexane extracts. It means the leaves contained polar soluble chemical constituents in a higher ratio than the organic chemical components. The polar classes of drugs that are isolated from the plant earlier are flavonoids and polyphenols. So the extraction solvent might have an effect in the

Table 1: Extract parametres

S.NO	Solvent	Extract colour	Extractive weight	Extractive value
1.	Aqueous extract	Dark brown	780mg	15.6%
2.	Pet ether	Dark green	350mg	7%
3.	Methanol	Dark green	810mg	16.2%
4.	Chloroform	Dark green	290mg	5.8%
5.	Ethanol	Brownish green	640mg	12.8%
6.	N-Hexane	Pale olio green	70mg	1.4%
7.	Ethyl acetate	Green	110mg	2.2%
8.	Benzene	Brownish green	140mg	2.8%

Table 2: Antitubercular activity of Extracts

Extract	Zone of inhibition			
	5microgram/ml	10microgram/ml	20microgram/ml	40microgram/ml
LME	10.2±0.7	12.5±0.8	15.5±0.8	17.2±0.3
LAE	9.5±0.3	11.3±0.1	13.1±0.5	15.6±0.6
LETE	7.3±0.8	10.0±0.4	12±0.9	13.1±0.2
LACE	5.6±0.2	8.2±0.6	11.0±0.3	12.3±0.1
LCE	4.4±0.1	6.1±0.3	10±0.2	9.7±0.4
LEE	1.7±0.9	5.6±0.7	8.3±0.1	7.4±0.8
LPE	0.8±0.6	4.3±0.5	6.2±0.4	5.0±0.7
LBE	0.2±0.4	2.0±0.9	4±0.6	3.2±0.5
LNE	0.1±0.5	1.4±0.2	1±0.7	1.5±0.4
Standard	9.9±0.5	11.9±0.5	14.2±0.6	16.4±0.4

**Figure 1: Antitubercular activity of extracts****Figure 2: Antitubercular activity of extracts**

antitubercular property of the plant too, which will be evident from the results.

The antitubercular activity of the extracts was investigated, and zones of inhibition were calculated for all extracts. Four concentrations of the

extracts were investigated, and the zones of inhibitions were tabulated in Table 2. The zones of inhibition of the extracts yielded with Methanol and water had shown the highest inhibition and least were given with hexane and benzene extracts.

All the extracts showed a dose-dependent activity were in the extracts at 40microgram/ml showed the highest activity inhibiting all the organisms. It reveals the antitubercular activity was mainly due to the presence of polar chemical constituents which were extracted using polar solvents like water and Methanol.

As discussed the polar chemical constituents like flavonoids and phenols might be responsible for the antitubercular activity. These chemicals might have inhibited the growth of mycobacterium bacteria. So this extracts, when consumed orally, might also inhibit the growth of bacteria in the body which should be investigated in future research. The organic solvent extracts had also shown a little activity which was not comparable to the standard but exhibited a smaller zone of inhibition. It might be due to steroids that were reported in the plant leaves earlier and also the steroids will be extracted in the non-polar solvents like pet ether, chloroform and hexane. The plant also contains resins that are usually extracted with benzene which might be responsible for the inhibition of mycobacteria.

CONCLUSION

Tuberculosis is one of the dangerous diseases in the world now. *Mycobacterium tuberculosis* is the bacteria that causes TB in the majority of the cases. The leaves of lannea were extracted using various solvents based on the polarity. It was then investigated for the anti-bacterial property against *mycobacterium tuberculosis*. All the extracts showed potency by inhibiting the bacteria, but Methanol and aqueous extracts showed a significantly better activity compared to the standard. Extracts were also to be investigated for a chemical constituent profile of plant and to establish the mechanism of action of those showed potent activity.

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Conflict of Interest

Authors declared no conflict of interest.

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