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Antimicrobial studies and phytochemical screening of stem bark in *Syzigium cumini* (L.) and *Lannea coromentalica* Houtt (Merr.)

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ABSTRACT

The antibacterial properties of the stem bark in ethanol, methanol and aqueous extracts of two medicinal plants viz., Syzigium cumini (L.) and Lannea coromentalica Houtt (Merr.) were tested against two human pathogenic bacterial strains (Escherichia coli and Bacillus subtilis) and two fungal strains (Aspergillus niger and Aspergillus flavus) by agar well diffusion method. Lannea coromentalica of ethanol extract was exhibited maximum zone of inhibition against Bacillus subtilis (16mm) and Aspergillus niger (15mm). Aqueous extract showed minimum zone of inhibition against in Bacillus subtilis (12mm) and Aspergillus flavus (11mm). Syzigium cumini of methanol extract showed maximum zone of inhibition was against in Bacillus subtilis (15mm) and ethanol extract were Aspergillus niger (15mm). Preliminary phytochemical analysis of Lannea coromentalica showed the presence of steroids, phenols, protein, saponins, alkaloids, and coumarins were present.

INTRODUCTION

Natural products are important sources for biologically active drugs. There has been an increasing interest in the study of medicinal plants as natural products in different parts of the world. According to World Health Organization (WHO) more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. Use of herbal medicines in Asia represents a long history of human interactions with the environment. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases (Saleh Alqasoumi, 2009). Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs.

Medical plants contain large varieties of chemical sub-stances which possess important therapeutic properties that can be utilized in the treatment of human diseases. The studies of Medicinal plants used in folklore remedies have attracted the attention of many scientists in finding solution to the problems of multiple resistances to the existing synthetic antibiotics. Most of the synthetic antibiotics now available in the market have major setback due to the multiple resistance developed by pathogenic micro-organisms against there drugs (Akinpelu, *et al.,* 2008).

The medicinal value of plants lies in some chemical substances that produce a definite physiologic action on the human body. The most important of these phyto compounds of plants are alkaloids, flavanoids, tannins and phenolic compounds. The phytochemical research based on ethno-pharmacological information is generally considered an effective approach in the discovery of new anti-infective agents from higher plants (Duraipandiyan *et al.,* 2006).

There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re – emerging infectious diseases. There fore researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infection (Bandow *et al.*, 2003). The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity (Rojas *et al*., 2003). India is a varietal emporium of medicinal plants and is one of the richest countries in the world with regard to genetic resources of medicinal plants (Heuer *et al.*, 2005). It exhibits a wide range in topography and climate, which has a bearing on its vegetation and floristic composition moreover, the agro-climatic conditions are conducive for introducing and domesticating new exotic plant varieties (Edeoga *et al*., 2005).

MATERIALS AND METHODS

Plant Collection

The stem bark of *Syzigium cumini* (L.) and *Lannea coromentalica* Houtt (Merr.) were collected from Thanjavur (Dt.) brought into the laboratory for further processes.

Preparation of Plant Extracts

Two grams of sterilized plant leaves were kept in the 10ml organic solvents such as methanol, chloroform and aqueous water. Then the leaves are grind with the help of mortar and pestle. The ground plant material was subjected to centrifugation, for 10-15min (at 10,000rpm). Again, it was filtered through Whatmann No. 1 filter paper. The supernatant was collected and stored for further antimicrobial screening purposes.

Selection of Microorganisms

Totally four pathogenic microorganisms were selected for the present investigation. Among them, two were bacterial strains such as *Escherichia coli* and *Bacillus subtilis***.** The remaining two fungal strain such as *Aspergillus niger* and *Aspergillus flavus***.** The microorganisms were originally obtained from Microbial Germ Plasm Culture Collection Unit (MGPCCU), Sri Gowri Biotech Research Academy, Thanjavur and used for present investigation.

Screening for Antimicrobial Activity assay

Antibacterial Activity (Agar - well diffusion method)

The antibacterial activities of the leaves were tested against the selected bacterial strains. The 20ml of sterilized agar medium was poured into each sterile petriplates and allowed to solidify. The test bacterial cultures were evenly spread over the appropriate media by using a sterile cotton swab. Then a well of 0.5cm was made in the medium by using a sterile cork borer, 150µl of each methanol, ethanol and aqueous plant extracts were transferred into separate wells. After these plates was incubated at 37^{o}C for 24-48 hours. After incubation period, the results were observed and measure the diameter of inhibition zone around the each well.

Antifungal Activity (Agar - well diffusion method)

In the freshly prepared and sterilized Potato Dextrose Agar medium, a pinch amount of streptomycin was added and mixed well. Then these 20 ml of Potato Dextrose Agar Medium was poured into each petriplate and allowed to solidify. The test fungal cultures were evenly spread over the appropriate media by using sterile cotton swab. Then a well 0.5cm was made in the medium by using sterile cork borer, 150µl of the each methanol, ethanol and aqueous plant extracts were transferred into separate wells. Then these plates were incubated at 27^{o}C for 48-72 hours. After incubation period the results were observed and measured the diameter of inhibition zone around the each well.

Preliminary Phytochemical Screening

All the extracts such as ethanol, methanol and aqueous extract of *Lannea coromentalica* were subjected to routine qualitative chemical analysis to identify the nature of phytochemical constituents present in them (Brindha *et al*., 1982; Harborne, 1998).

Alkaloids: A 2 ml of test solution was taken with 2N HCl. Aqueous layer formed was decanted and then added with one or a few drops of Mayer's reagent. Formation of white precipitate or turbidity formed indicates the presence of alkaloids.

Sterols: A 2 ml of test solution and minimum quantity of chloroform was added with 3-4 drops of acetic anhydride and one drop of concentrated H_2SO_4. Formation of purple color changes into blue or green color that indicates the presence of steroids.

Phenols: A 2 ml of test solution in alcohol was added with one drop of neutral ferric chloride (5%) solution. Formation of intense blue color indicates the presence of phenols.

Saponins: A 2 ml of test solution was added with H_2O and shaked. Formation of foamy lather indicates the presence of saponins.

Lignins: Phloroglucinol with HCl was added with the test solution. Formation of pink color indicates the presence of lignins.

Proteins: Picric acid was added with the test solution. Formation of yellow color indicates the presence of protein.

Coumarins: A 10% NaOH was added with the test solution. Formation of yellow color indicates the presence of coumarins.

RESULTS AND DISCUSSION

In the present investigation the antimicrobial properties of ethanol, methanol and aqueous extract of two medicinal plants viz., *Syzigium cumini* and *Lannea coromentalica* were tested against four human pathogen bacterial strain and fungal strains such as *Escherichia coli*, *Bacillus subtilis*, *Aspergillus niger* and *Aspergillus flavus*.

Antimicrobial activity of stem bark extract of *Syzigium cumini*

Sajjan, *et al*., (2010) reported the ethanol and aqueous extracts of *Momordica cymbalaria* has shown significant activity against *K. pneumonia*e 18mm, 19.5mm, *E. coli* 17mm and 17mm and *S. aureus* 15mm, 17mm. Similarly chloroform extract of the plant has shown good inhibitory (37mm) activity against *A.niger*. The methanol extract was exhibited zone of inhibition against *B. subtilis* (15mm), *E. coli* (14mm), *A. niger* (14mm) and *A. flavus* (11mm). The aqueous extract was showed minimum zone of inhibition *E. coli* (11mm), *B. subtilis* (12mm), *A. niger* (10mm) and *A. flavus* (10mm) (Table 1 and Fig 1). Mohamed Sham Shihabudeen, *et al*., (2010) suggested that the methanol extracts of *Eugenia jambolana* and *Cassia auriculata* showed the highest toxicity against all the bacteria the plant extracts showed antibacterial activity but not antifungai activity against any of the fungal used. In the present study methanol extracts showed the highest antibacterial activity and antifungal activity.

Antimicrobial activity of stem bark extract of *Lannea coromentalica*

The ethanol extract of stem bark extract in *Lannea coromentalica* showed maximum zone of inhibition was observed in *B. subtilis* (16mm), *E. coli* (15mm), *A. niger* (15mm) and *A. flavus* (14mm). The methanol extract was showed moderate activity against *B. subtilis* (13mm), *A. niger* (12mm) and *A. flavus* (12mm). Aqueous extract showed minimum zone of inhibition against in *B. subtilis* (12) and *A. flavus* (12mm) (Table 2 and Fig 2). Badmanaban and Patel, (2010) investigated the results reveal significant activity against bacterial strains all fungal strains showed more in (70%) alcoholic extracts against when compared to that of aqueous extract.

Antibiotic sensitivity test (Positive control)

The antibiotic sensitivity test using standard antibiotic test such as Streptomycin, Tetracyclin and Kanamycin were tested against bacteria and fungi studied. The Kanamycin standard antibiotic showed maximum zone of inhibition of *E. coli* (23mm), and *A. niger* (13mm) (Table 3-4 an Fig 3-4). Sajjan, *et al.*, (2010) reported susceptibility test of these test organisms to traditional antibiotics was done using standard antibiotics such as ampicillin, tetracycline and streptomycin standard antibiotic showed maximum zone of inhibition of *S. aureus*, *K. pneumonia*e and *E. coli* such as 13mm, 14mm and 17mm.

Table 1. Antimicrobial activity of the stem barks extract in *Syzygium cumini* (L.)

S. No	Microbial Cultures	Solvent Extracts (Zone of inhibition in mm)		
		Methanol	Ethanol	Aqueous
1.	*Escherichia coli*	14	13	11
2.	*Bacillus subtilis*	15	12	12
3.	*Aspergillus niger*	14	15	10
4.	*Aspergillus flavus*	11	10	10

Fig 1. Antimicrobial activity of the stem barks extract in *Syzygium cumini* (L.)

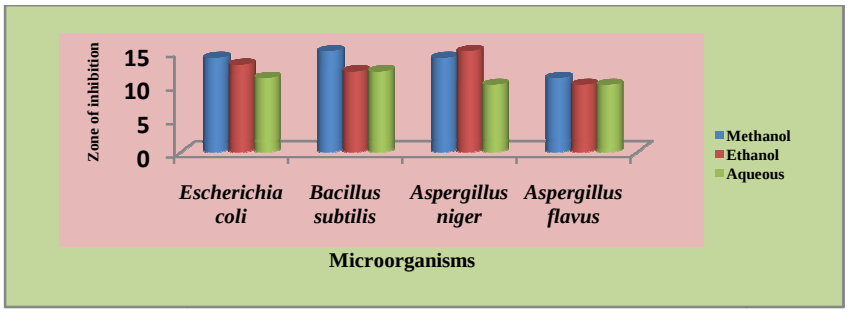


Table 2. Antimicrobial activity of the stem barks extract in *Lannea coromentalica* Houtt (Merr.)

S. No	Microbial Cultures	Solvent Extracts (Zone of inhibition in mm)		
		Methanol	Ethanol	Aqueous
1.	*Escherichia coli*	16	15	14
2.	*Bacillus subtilis*	13	16	12
3.	*Aspergillus niger*	12	15	14
4.	*Aspergillus flavus*	12	14	12

Fig 2. Antimicrobial activity of the stem barks extract in *Lannea coromentalica* Houtt (Merr.)

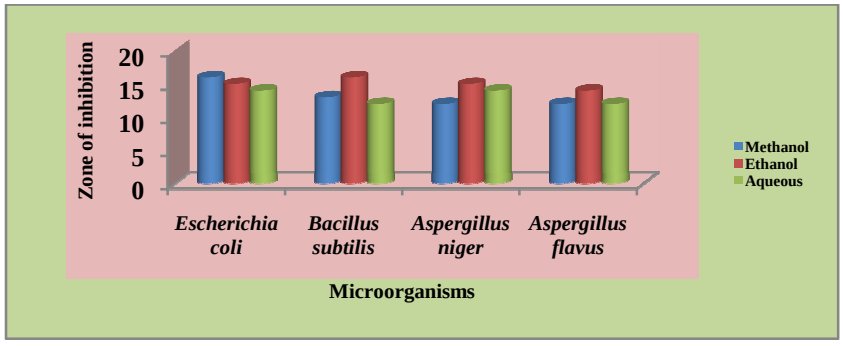


Table 3. Antibiotic sensitivity test (Positive control)

S. No	Microbial Cultures	Standard Antibiotics (Zone of inhibition in mm)		
		Streptomycin	Kanamycin	Tetracycline
1.	*Escherichia coli*	20	23	22
2.	*Bacillus subtilis*	12	20	19

Fig 3. Antibiotic sensitivity test (Positive control)

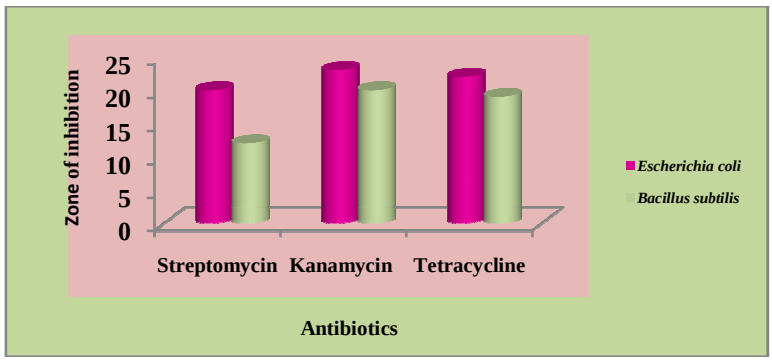
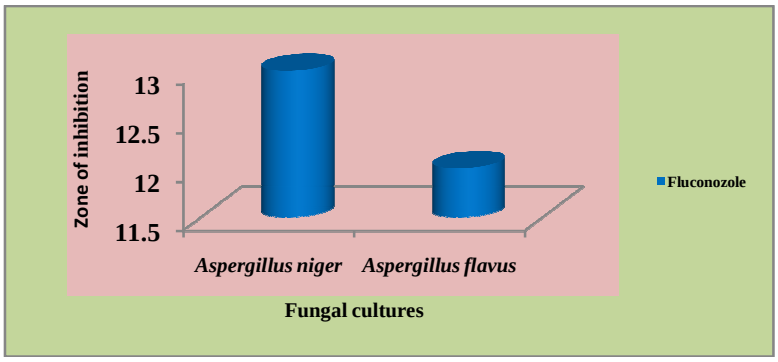


Table 4. Antibiotic sensitivity test

S. No	Fungal Cultures	Solvent Extracts (Zone of inhibition in mm)
		Fluconazole
1.	*Aspergillus niger*	13
2.	*Aspergillus flavus*	12

Fig 4. Antibiotic sensitivity test



Preliminary Phytochemical Analysis

Preliminary phytochemical analysis of the different solvent extracts such ethanol, methanol and aqueous extract of the stem bark in *Lannea coromentalica* indicated the presence of secondary metabolites in a different manner (Table 5). Phenols, protein and alkaloids are present in the ethanol extracts of stem bark. Methanol extracts of the stem bark indicated the presence of protein, alkaloids, phenols and cumarine. Sterols and lignin are absent in all the solvent extracts of ethanol, methanol and aqueous extracts of the stem bark. Mathew and Sasikumar (2007) reported the presence of alkaloids and the absence of steroids in *Pseudarthria viscida*.

Table 6. Preliminary phytochemical analysis of *Lannea coromentalica* Houtt (Merr.)

S. NO	Phytocompounds	Ethanol extracts	Methanol extracts	Aqueous extracts
1.	Proteins	+	+	+
2.	Lignins	-	-	-
3.	Saponins	-	-	+
4.	Sterols	-	-	-
5.	Alkaloids	+	+	-
6.	Phenols	+	+	+
7.	Cumarine	-	+	-

+ Present, - Absent

CONCLUSION

The antimicrobial activity of two medicinal plants stems bark extract in *Syzigium cumini* and *Lannea coromentalica* were performed. Three different organic solvent extract (ethanol, methanol and aqueous) were used for this studied. This study indicates that methanol extract of *Lannea coromentalica* showed maximum activity when compared than ethanol and aqueous extracts. The medicinal plants represent a rich source of antimicrobial agents. These plants are used medicinally in different countries and a source of potent and powerful drugs. A wide range of medicinal plants parts used for extract, as raw drugs and possess varied medicinal properties.

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