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ANTIMICROBIAL POTENTIAL OF *FICUS BENGALENSIS* AERIAL ROOTS

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ABSTRACT

Herbal preparations of *Ficus bengalensis* L. (Moraceae) have been considered as safe, effective and economical for various ailments in Indian traditional system of medicine. The present study was aimed to scientifically explore the antimicrobial potential of *F. bengalensis* aerial roots. Effect of variable concentrations of aqueous and hexane extracts of *F. bengalensis* aerial roots was studied on three different non-pathogenic bacterial strains. All the three different concentrations of 25, 50 and 75 mg/ml of aqueous and hexane aerial root extracts showed sustained activity against all the bacterial strains. The sensitivity of the extracts against each bacterial strain was determined by identifying the diameter of inhibition zone (DIZ) for each concentration and the highest activity was observed against *Staphylococcus aureus* for each of the three concentrations in case of both the extracts.

KEYWORDS

Antimicrobial, Aerial roots, Bacterial strains*, Ficus bengalensis,* Hexane and Moraceae

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Even today, plant materials continue to play a major role in primary health care in therapeutic remedies in many developing countries. Plants still continue to be almost the exclusive source of drugs for majority of the world's population. The World Health Organization reported that 80% of the world's population rely predominantly on traditional medicine and a major part of the traditional therapies involve the use of plant extracts or their active constituents.

In the past 20 years, there has been a lot of interest in the investigation of natural materials as sources of new antibacterial agents. Throughout the history of mankind, many infectious diseases are known to be treated with herbal medicines [1]. Infectious diseases are the number one cause of death accounting for approximately one-half of all deaths in tropical countries. For many centuries, plants and their products have been used in the treatment of infections. However, their scientific study has been made possible only after the development of microbiology.

Natural antimicrobials can be derived from barks, stems, leaves, flowers and fruits of plants, various animal tissues or from microorganisms [2]. Over 50% of all modern clinical drugs are having their origin in natural products [3]. In general, bacteria have the genetic ability to transmit and acquire resistance which is utilized as therapeutic agents [4]. The widespread distribution of drug-resistant microorganisms has imposed serious limitations on successful antibiotics and antifungal therapy. Bacterial resistance is a growing problem worldwide [5]. Even though pharmacological industries have produced a number of new antibiotics in the last three decades but, microorganisms have developed resistance to these drugs in the consecutive years [6]. Such a fact is a cause for concern.

Since, the problem of microbial resistance is growing worldwide, therefore actions must be taken to reduce this problem either by controlling the use of antibiotics or by developing the research to better understand the genetic mechanisms of resistance and by developing new drugs, either synthetic or natural. The ultimate goal should be to offer appropriate and efficient antimicrobial drugs to the patients.

According to the World Health Organization [6], medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use medicines, which have compounds derived from medicinal plants. Thus, plants could be investigated to identify their antimicrobial profile, safety and efficacy. Such studies can be of great significance in therapeutic treatments against microbes. In the last few years, a number of plant studies have been conducted in different countries to prove their antimicrobial efficiency [7-13]. The antimicrobial traits of the plants may be primarily due to the essential oils [14], as well as tannins [15].

Secondary plant products can have a variety of functions in plants. It is likely that their ecological function may have some bearing on potential medicinal effects for humans. For example, secondary products involved in plant defense through cytotoxicity towards microbial pathogens could prove useful as antimicrobial agents in humans, if not too toxic. The role of secondary products as defense chemicals would not only ensure effectiveness against a wide range of pathogens but would also decrease the chances of these organisms developing resistance or adaptive responses.

In Argentina, a research tested 122 known plant species used for microbial treatments [16]. It was documented that among the compounds extracted from these plants, twelve inhibited the growth of *Staphylococus aureus*, ten inhibited *Escherichia coli*, and four inhibited *Aspergillus niger* and, also reported that the most potent compound was the one extracted from *Tabebuia impetiginosa*. The antimicrobial properties of compounds isolated from *Parthenum argentatum* were found active against a number of bacterial strains such as *Candida albicans, Torulopsis hansemula, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* [17,18].

Flavonoid compounds exhibit inhibitory effect against bacteria. Flavonoids, hydroxyl groups on their β-rings are more active against microorganisms and have also been found that the more hydroxylation, the greater the antimicrobial activity[19]. Levels of total phenolics, total flavonol and total flavanoid compounds in *F. bengalensis* aerial roots in 70 mg/g of extract, 3 mg/g quercetin equivalent and 5 mg quercetin equivalent/gm extract have also been reported[20].

Though, the antimicrobial properties of plants have been investigated time to time by a number of researchers worldwide, but recently it has gained much importance globally after the development of molecular biology. Thus, in the present work, aqueous as well as hexane extracts of *Ficus bengalensis* aerial roots have been investigated for their antimicrobial activities against various microbes and interesting results have been obtained.

MATERIALS AND METHODS

(i) Plant material:

Aqueous extract:

Fresh aerial roots of *Ficus bengalensis* were collected and identified by Prof. Satya Narayan, Taxonomist, Department of Botany, University of Allahabad, Allahabad, India. A voucher specimen has been submitted to the University herbarium. The roots were dried and cut into small pieces which were then mechanically crushed. 4 kg of these crushed aerial roots were continuously extracted with distilled water using soxhlet up to 48 h. The extract was filtered and concentrated in rotary evaporator at 35-40°C under reduced pressure to obtain a semisolid material, which was then lyophilized to get a powder (12.32%, w/w).

Hexane extract:

2 kg of fresh aerial roots of *Ficus bengalensis* were shade dried for one week and then extracted with hexane (10 lit × 3) for 72 hours. The hexane extract was filtered and concentrated in rotary evaporator under reduced pressure to obtain a semisolid material, which was then dried in vacuum desiccator to obtain a powder (12.4% w/w).

(ii) Bacterial strains, stocks and growth *in vitro*:

Bacterial strains of *Klebsiella pneumonia*, *Staphylococcus aureus* and *Escherichia coli* were clinical isolates obtained from the Department of Biotechnology, All India Institute of Medical Sciences (AIIMS), New Delhi, India and the microbiologist of the department confirmed the identity based on microscopic examination, Gram's character, and biochemical test profile. Bacterial stocks were maintained and stored as 1 ml aliquots at -80ºC in Luria Bertani (LB) broth for *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Escherichia coli* strains. Bacterial stocks were revived from -80ºC and grown in Luria Bertani (LB) broth for *Klebsiella pneumonia*, *Staphylococcus aureus* and *Escherichia coli.* All cultures were grown at 37ºC in a shaker incubator (190-220 rpm) overnight.

Luria Bertani broth (Himedia), Luria Bertani agar (Himedia) and sterile discs as well as Ampicillin disc (Himedia) were used in antimicrobial sensitivity testing.

(iii) Determination of Zone of Inhibition (ZOI):

The freshly prepared inoculum of *Klebsiella pneumoniae, Staphylococcus aureus and Escherichia coli* were swabbed all over the surface of the LB Agar plates. Three sterile discs of 6 mm diameter were placed on the inoculated media with the help of a disc dispenser and were numbered properly. The different concentrations viz. 25, 50 and 75 mg/ml of aqueous as well as hexane extract solutions of aerial roots of *Ficus bengalensis* were poured on the discs with the help of sterilized micropipette. Discs were left for some time till the extract solutions diffused in them. The effects were compared with that of a standard antibiotic-loaded disc, ampicillin (10 mcg/disc) and cefepime/tazobactum (30/10 mcg/disc), of Himedia. Finally, the plates were incubated with lids closed at 37ºC for 24 hours. Discs were observed for zones of inhibition by measuring the Diameter of Inhibition Zone (DIZ) using a ruler.

RESULTS

Aerial roots of *F. bengalensis* aqueous and hexane extracts have been screened for their antimicrobial activity and very interesting profiles have been found against *Klebsiella pneumoniae*, *Staphylococcus aureus and Escherichia coli* bacterial strains. Effect of varied concentrations of 25, 50 and 75 mg/ml of aqueous as well as hexane extract solutions was studied and comparison with standard drugs, viz. ampicillin and cefepime/tazobactum, respectively was done. Their zones of inhibition (ZOI) can be seen at a glance as follows:

Table 1

Zone of Inhibition of different strains for aqueous extract of *F. bengalensis* aerial roots at a glance

Plant used (in conc.)	Zone of Inhibition		
	Staphylococcus aureus	***Escherichia coli***	***Klebsiella pneumoniae***
Ficus bengalensis (25 mg/ml)	18 mm	10 mm	10 mm
Ficus bengalensis (50 mg/ml)	22 mm	14 mm	12 mm
Ficus bengalensis (75 mg/ml)	24 mm	16 mm	16 mm
Ampicillin (Std. drug) (10 mcg/disc)	10 mm	Nil	Nil

All the three different concentrations (25, 50 and 75 mg/ml) of the aqueous extract showed sustained activity against all three bacterial strains. Though, the highest activity observed was against *Staphylococcus aureus* having DIZ 18, 22 and 24 mm at concentrations of 25, 50 and 75 mg/ml, respectively but, the activity of standard drug ampicillin observed was only 10 mm DIZ against Staphylococcus *aureus* (Fig. 1a & 1b), which can be practically considered as Nil. Moreover, activity was quite reasonable and concentration-dependent in *Escherichia coli* bacteria having 10, 14, 16 mm DIZ at concentrations of 25, 50 and 75 mg/ml respectively. Though, the DIZ of 10 mm with concentration of 25 mg/ml can again be practically considered as Nil. However, the standard drug ampicillin did not show any activity against *Escherichia coli* (Fig. 2a & 2b). In case of *Klebsiella pneumoniae*, DIZ observed was 10, 12 and 16 mm at concentrations of 25, 50 and 75 mg/ml respectively. The standard drug ampicillin was found to be inactive again and DIZ of concentration of 25 mg/ml was also too low to be considered (Fig 3a & 3b).

Table 2

Zone of Inhibition of different strains for hexane extract of *F. bengalensis* at a glance

Plant used (in conc.)	Zone of Inhibition		
	Staphylococcus aureus	***Escherichia coli***	***Klebsiella pneumonia***
Ficus bengalensis (25 mg/ml)	Nil	16 mm	Nil
Ficus bengalensis (50 mg/ml)	18 mm	Nil	Nil
Ficus bengalensis (75 mg/ml)	22 mm	Nil	14 mm
Cefepime/Tazobactum (Std. drug) (30/10 mcg/disc)	14 mm	12 mm	10 mm

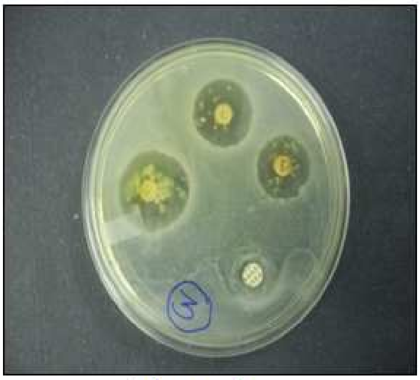

Figure-1a

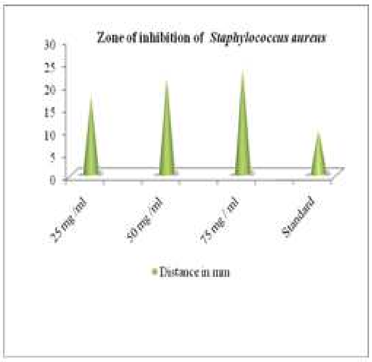

Figure-1b
Concentrations of aq. root extract against *Staphylococcus aureus*

Figure-2a

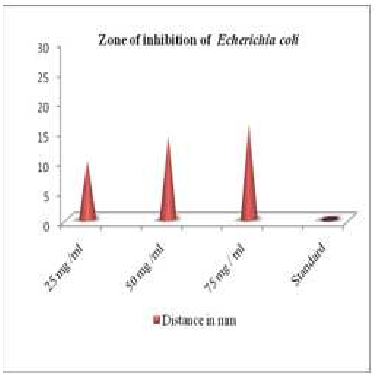

Figure-2b
Concentrations of aq. root extract against *Escherichia coli*

Figure- 3a

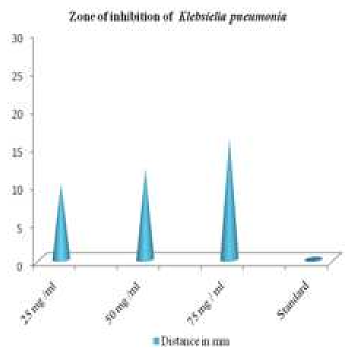

Figure- 3b
Concentrations of aq. root extract against *Klebsiella pneumoniae*

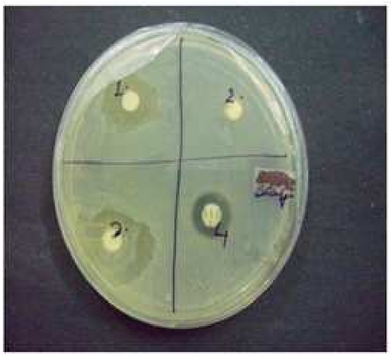

Figure-4a

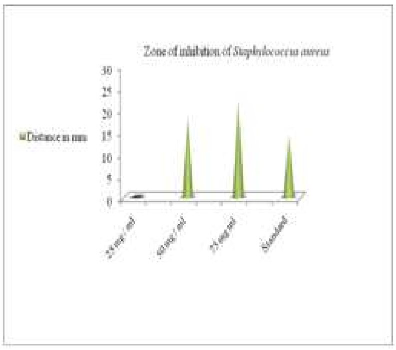

Figure- 4b
Concentrations of hexane extract against *Staphylococcus aureus*

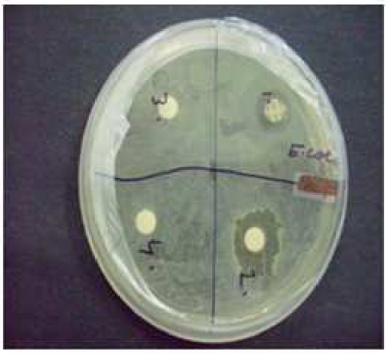

Figure-5a

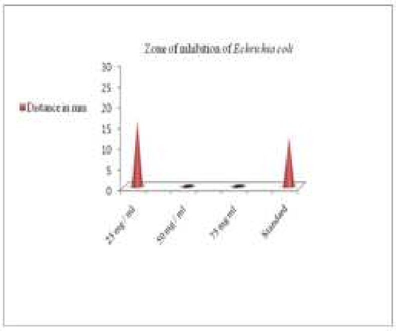

Figure- 5b
Concentrations of hexane extract against *Escherichia coli*

Figure-6a

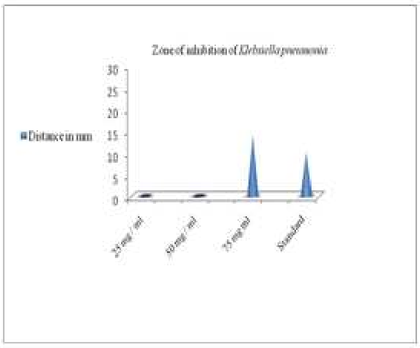

Figure- 6b
Concentrations of hexane extract against *Klebsiella pneumoniae*

DISCUSSION

None of the different concentrations (25, 50 and 75 mg/ml) of hexane extracts showed sustained activity against all three bacterial strains. Though, the highest activity observed was against *Staphylococcus aureus* but, only at concentrations of 50 and 75 mg/ml having DIZ 18 and 22 mm respectively. However, the activity observed of standard drug, cefepime/tazobactum was less than these active concentrations showing only 14 mm DIZ against *Staphylococcus aureus* (Fig. 4a & 4b). Moreover, hexane extract has similar activity against *Escherichia coli* bacterial strains.

It is interesting to note that the activity was high at low concentration and nil at high concentration showing 16, 0 and 0 mm DIZ at concentration of 25, 50 and 75 mg/ml respectively. However, the standard drug cefepime/tazobactum showed DIZ 12 and 10 mm against *Escherichia coli* (Fig. 5a & 5b) bacterial strains. The activity of hexane extract was found lowest against *Klebsiella pneumoniae* having DIZ 0, 0 and 14 mm at concentrations of 25, 50 and 75 mg/ml respectively. The activity of standard drug, cefepime/tazobactum was found to be practically nil having DIZ 10 mm (Fig 6a & 6b) against the same bacterial strain.

Thus, the order of extent of antimicrobial activity against different bacterial strains was found as follows:

Aqueous aerial roots extract:

Staphylococcus aureus **>** *Echerichia coli* **>** *Klebsiella pneumoniae*

Hexane aerial roots extract:

Staphylococcus aureus **>** *Echerichia coli* **>** *Klebsiella pneumoniae*

The entire data suggested that the aqueous extract of *Ficus bengalensis* aerial roots was active against all the bacterial strains against which ampicillin was found to be inactive, though, the pattern of zone of inhibition of *Echerichia coli* and *Klebsiella pneumoniae* were almost the same. To the contrary, hexane extract showed little activity against each bacterial strain except *Staphylococcus aureus*, depicting that the extract possessed slightly more activity than that of the standard drug, cefepime/tazobactum. It is important to note that the pattern of ZOI of *Escherichia coli* was exactly the same as the standard drug, cefepime/tazobactum.

The use of medicinal plants to heal diseases, including infectious ones, has been extensively applied by people worldwide. Data from literature as well as our results reveal the great potential of plants for therapeutic treatment, in spite of the fact that they have not been completely investigated. Therefore, more studies need to be conducted to search for new compounds responsible for their antibacterial activity. Once extracted, and before being used in new therapeutic treatments, they should have their toxicity tested *in vivo*. Bioassays [21,22] have demonstrated the toxicity of extracts from different plants [23].

It is not surprising that there are differences in the antibacterial effects of plant groups, due to the phytochemical differences between species. To better evaluate the plants growing naturally that are potentially useful resources, additional studies are necessary from both the medicinal and economic stand points [24].

Therefore, our results revealed the importance of *F. bengalensis* aqueous as well as hexane extracts when associated with antibiotics, to control resistant bacteria, which are becoming a threat to human health. Furthermore, in a few cases, these plant extracts were active against antibiotic resistant bacteria under very low concentration, thus minimizing the possible toxic effects.

CONCLUSION

The activity was highest against *Staphylococcus aureus* and lowest against *Klebsiella pneumoniae* in both the aqueous and hexane extracts. The results suggest that *Ficus bengalensis* aerial roots are very promising not only as antidiabetic[25] and antioxidant agents but also as antimicrobial agent with special reference to *Staphylococcus aureus* infections as both, aqueous and hexane extracts were active against this bacterial strain. This antimicrobial profile of *Ficus bengalensis* aerial roots can be exploited for developing a novel antimicrobial agent of high potential.

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity assay[26]. Many reports are available on the antiviral, antibacterial, antifungal, anthelmintic, antimolluscal and anti-inflammatory properties of plants [27,28]. Some of these observations have helped in identifying the active principle responsible for such activities and in the developing drugs for the therapeutic use in human beings.

Since, most of the plants, rich in flavonoids, possess antimicrobial activity [29], therefore, the presence of flavonoids identified in *Ficus bengalensis* aerial roots by phytochemical screening might be responsible for its antimicrobial activity.

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AUTHORS' STATEMENTS

Competing Interests

The authors declare no conflict of interest.

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