

Antimicrobial Activity of *Ficus benghalensis* and *Ficus racemosa* roots L.

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Abstract: Problems statement: To evaluate the antimicrobial activity of aqueous and ethanolic extracts of *Ficus benghalensis* and *Ficus racemosa* against three bacterial strains that is *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia*. **Approach:** The different concentrations viz. 25, 50 and 75 mg mL⁻¹ of aqueous as well as ethanolic extract solutions of roots of *Ficus benghalensis* and *Ficus racemosa* were tested against above three bacterial strains. The effects were compared with that of a standard antibiotic-loaded disc, ampicillin (10 mcg/disc) of Himedia Labs. Finally, the plates were incubated with lids closed at 37°C for 24 h. Discs were observed for zones of inhibition by measuring the Diameter of Inhibition Zone (DIZ) using a ruler. **Results:** *Ficus racemosa* ethanolic extract showed maximum inhibition against *Staphylococcus aureus* when compared with *Ficus benghalensis* ethanolic extract. However standard drug ampicillin showed maximum antimicrobial activity compared with both the plants. **Conclusion:** In conclusion, it was clearly observed that ethanolic extract of both the plants were having good antimicrobial activity towards *Staphylococcus aureus*.

Key words: Antimicrobial activity, *Ficus benghalensis*, *Ficus racemosa*, saponins, tannins, flavanoids, alkaloids, therapeutic agent, Diameter of Inhibition Zone (DIZ)

INTRODUCTION

Medicinal plants have been used as an exemplary source for centuries as an alternative remedy for treating human diseases because they contain numerous active constituents of therapeutic value (Nostro *et al.*, 2000). The development of microbial resistance to antibiotics has led the researches to investigate the alternative sources for the treatment of resistant strains (Hammer *et al.*, 1999). Presently 80% of the world population relies on plant derived medicines and serves as first line of defense in maintaining health and combating many diseases (Veale *et al.*, 1992).

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 (Gordon, 2001). Over 50% of all modern clinical drugs are having their origin in natural products (Suffness and Douros, 1982). In general, bacteria have the genetic ability to transmit and acquire resistance which is utilized as therapeutic agents (Cohen, 1992).

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 (Sato *et al.*, 1996). Levels of total phenolics, total flavonol and total flavanoids compounds in *F. benghalensis* aerial roots in 70 mg g⁻¹ of extract, 3 mg g⁻¹ quercetin equivalent and 5 mg quercetin equivalent/gm extract have also been reported (Sharma *et al.*, 2009). 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 study, aqueous as well as ethanolic extracts of *Ficus benghalensis* and *Ficus racemosa* roots have been investigated for their antimicrobial activities against various microbes and interesting results have been obtained.

MATERIALS AND METHODS

Plant material:

Aqueous extract: Fresh roots of *Ficus benghalensis* and *Ficus racemosa* were collected and identified by Dr. M.K. Jangid, Taxonomist, Department of Botany, Hemchandra North Gujarat University, India. A voucher specimen has been submitted to the Institute herbarium. The roots were dried and cut into small pieces which were then mechanically crushed. 4 kg of these crushed roots were continuously extracted with

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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.

Ethanol extract: 2 kg of fresh roots of *Ficus benghalensis* and *Ficus racemosa* were shade dried for one week and then extracted with ethanol (10 lit ×3) for 72 h. The ethanolic extracts were filtered and concentrated in rotary evaporator under reduced pressure to obtain a semisolid material.

Bacterial strains, stocks and growth in vitro: Bacterial strains of *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Escherichia coli* were clinical isolates obtained from the Department of Microbiology, Vidyabharti Trust College of Pharmacy, Umrah, India. All cultures were grown at 37°C 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.

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⁻¹ of aqueous as well as ethanolic extract solutions of roots of *Ficus benghalensis* and *Ficus racemosa* 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) of Himedia Labs. Finally, the plates were incubated with lids closed at 37°C for 24 h. Discs were observed for zones of inhibition by measuring the Diameter of Inhibition Zone (DIZ) using a ruler.

RESULTS

Roots of *F. benghalensis* and *F. racemosa* aqueous and ethanolic 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⁻¹ of aqueous as well as ethanolic extract solutions was studied and comparison with standard drugs, viz. ampicillin was done. Their Zones Of Inhibition (ZOI) can be seen at a glance as follows (Table 1 and 2):

Table 1: Zone of Inhibition of different strains for ethanolic extract of *F. benghalensis* and *F. racemosa* roots at a glance

Plant used (in conc.)	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>
<i>Ficus benghalensis</i> (25 mg mL ⁻¹)	20 mm	15 mm	12 mm
<i>Ficus benghalensis</i> (50 mg mL ⁻¹)	25 mm	20 mm	18 mm
<i>Ficus benghalensis</i> (75 mg mL ⁻¹)	30 mm	24 mm	22 mm
Ampicillin (Std. drug) (10 mcg/disc)	40 mm	35 mm	30 mm
<i>Ficus racemosa</i> (25 mg mL ⁻¹)	25 mm	20 mm	15 mm
<i>Ficus racemosa</i> (50 mg mL ⁻¹)	30 mm	25 mm	22 mm
<i>Ficus racemosa</i> (75 mg mL ⁻¹)	35 mm	30 mm	25 mm
Ampicillin (Std. drug) (10 mcg/disc)	40 mm	35 mm	30 mm

Table 2: Zone of Inhibition of different strains for aqueous extract of *F. benghalensis* and *F. racemosa* roots at a glance

Plant used (in conc.)	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>
<i>Ficus benghalensis</i> (25 mg mL ⁻¹)	nil	16 mm	nil
<i>Ficus benghalensis</i> (50 mg mL ⁻¹)	18 mm	nil	nil
<i>Ficus benghalensis</i> (75 mg mL ⁻¹)	14mm	12	14 mm
Ampicillin (Std. drug) (10 mcg/disc)	40 mm	35mm	30 mm
<i>Ficus racemosa</i> (25 mg mL ⁻¹)	10 mm	15 mm	nil
<i>Ficus racemosa</i> (50 mg mL ⁻¹)	22mm	18 mm	nil
<i>Ficus racemosa</i> (75 mg mL ⁻¹)	35 mm	25 mm	10 mm
Ampicillin (Std. drug) (10 mcg/disc)	40 mm	35 mm	30 mm

All the three different concentrations (25, 50 and 75 mg mL⁻¹) of the aqueous extract (Table 2) showed sustained activity against all three bacterial strains. Though, the highest activity observed was against *Staphylococcus aureus* having DIZ 10, 22 and 30 mm at concentrations of 25, 50 and 75 mg mL⁻¹, respectively but, the activity of standard drug ampicillin observed was 40 mm DIZ against *Staphylococcus aureus* which can be practically considered as highest. Moreover, activity was quite reasonable and concentration-dependent in *Escherichia coli* bacteria having 15, 18, 25 mm DIZ at concentrations of 25, 50 and 75 mg mL⁻¹ respectively. However, the standard drug ampicillin showed maximum activity against *Escherichia coli*. In case of *Klebsiella pneumoniae*, DIZ observed was Nil, Nil and 10 mm at concentrations of 25, 50 and 75 mg mL⁻¹ respectively. The standard drug ampicillin was found to be maximum again that is 30 mm.

In contrast, ethanolic extract showed maximum inhibition against all micro-organism which can be directly observed in Table 1.

DISCUSSION

None of the different concentrations (25, 50 and 75 mg mL⁻¹) of ethanolic 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⁻¹ having DIZ 18 and 22 mm respectively. However, the activity observed of standard drug, Ampicillin was highest than these active concentrations showing only

40 mm DIZ against *Staphylococcus aureus*. Moreover, ethanolic extract has similar activity against *Escherichia coli* bacterial strains. However, the standard drug Ampicillin showed maximum against *Staphylococcus aureus* bacterial strains. The activity of ethanolic extract was found lowest against *Klebsiella pneumoniae* having DIZ 0, 0 and 14 mm at concentrations of 25, 50 and 75 mg mL⁻¹ respectively. Thus, the order of extent of antimicrobial activity against different bacterial strains was found as follows:

Aqueous roots extract:

Staphylococcus aureus>*Echerichia coli*>*Klebsiella pneumonia*

Ethanolic roots extract:

Staphylococcus aureus>*Echerichia coli*>*Klebsiella pneumonia*

The entire data suggested that the ethanolic extract of *Ficus benghalensis* and *Ficus racemosa* roots was active against all the bacterial strains against which ampicillin was found to have maximum activity. To the contrary, aqueous extract showed little activity against each bacterial strain except *Staphylococcus aureus*, depicting that the extract possessed lesser activity than that of the standard drug, Ampicillin. 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 (Carvalho *et al.*, 1988) have demonstrated the toxicity of extracts from different plants (Nascimento *et al.*, 2000). 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 (Ates and Erdogru, 2003). Therefore, our results revealed the importance of *F. benghalensis* and *F. racemosa* aqueous as well as ethanolic 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 ethanolic extracts. The results suggest that *Ficus benghalensis* roots are very promising not only as antidiabetic (Singh *et al.*, 2009) and antioxidant AGENTS but also as antimicrobial agent with special reference to *Staphylococcus aureus* infections as both, aqueous and ethanolic extracts were active against this bacterial strain. This antimicrobial profile of *Ficus benghalensis* and *Ficus racemosa* 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 (Tona *et al.*, 1998). Many reports are available on the antiviral, antibacterial, antifungal, anthelmintic, antimolluscal and anti-inflammatory properties of plants (Samy and Ignacimuthu, 2000; Nascimento *et al.*, 2000; Palombo and Semple, 2001). 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 (Cavalitto *et al.*, 1944; Cavalitto and Bailey, 1994), therefore, the presence of flavonoids identified in *Ficus benghalensis* and *Ficus racemosa* roots by phytochemical screening might be responsible for its antimicrobial activity.

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