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Antibacterial activity of *Ficus microcarpa* L. extract on gram positive and gram negative bacteria

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Abstract
In view of increasing resistance to existing antimicrobial agents, herbal drugs are being looked as very important source for discovery of new agents for treating various ailments related to bacterial infections. These drugs have been used in India as folk remedy in the form of decoctions and infusions to treat bacterial infections and also claimed to be effective against variety of skin diseases. The present investigation was carried out to study the antibacterial efficacy of *Ficus microcarpa* L. It is a popular ornamental tree, grown widely, both indoors and outdoors. Bark, root, leaves, fruit and latex of this plant are frequently used for the treatment of various illnesses Aqueous and acetone extracts of the plant was subjected to antibacterial evaluation against both gram positive and gram negative bacteria. Disc diffusion and Minimum Inhibitory Concentration (MIC) studies were carried out to assess the antibacterial effect. Cefotaxime was used as the reference standard. Aqueous extracts were found to be more effective against all the bacteria. Antibacterial activity obtained in this study varied with solvents used for extraction. *F. microcarpa* extracts showed moderate inhibition activity with the zone range of 7–14 mm. Maximum inhibition was observed against *P. aeruginosa* (18 mm) and minimum inhibition against *E. coli* (7 mm). Based on the MIC result *P. aeruginosa* was found to be more sensitive to the plant extract when compared with the other bacteria.

Keywords: Agar diffusion, Minimum Inhibitory concentration, antibacterial activity

1. Introduction
Plants have universal role in the treatment of diseases in all systems of medicine. There has been considerable emphasis on the research on medicinal plants especially those which are used in traditional system of medicine. Because of greater emphasis on ethno botanical surveys, more plants have been added to the list of medicinal plants already reported in India during the last decades.

The forest in India is the principal repository of large number of medicinal and aromatic plants, which are largely collected as raw materials for the manufacture of drugs and perfumery products. Plant derived medicines have made large contribution to human health and well-being. Medicinal property of plant based medicines depends on its active constituents of the drug which are effective against specific ailments. It is necessary that drugs are subjected to the dose screening of their phytochemical characters prior to their use by pharmacies for producing medicine. Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effect that are often associated with synthetic antimicrobials (Iwu MW; 1991) [1].

The genus *Ficus* (Moraceae) constitutes one of the largest genera of angiosperms includes with more than 800 species and 2000 varieties of Ficus genus, occurring in most tropical and subtropical forests worldwide (Hamed, 2011) [2]. *Ficus microcarpa* is a large glabrous evergreen tree with a rounded dense crown, smooth gray bark, milky sap, and long, thin, dangling aerial roots. Flowers tiny, unisexual, numerous, hidden within the "fig" a fleshy specialized receptacle that develops into a syconium. The bark and leaves are used in wounds, ulcers, hepatopathy, diarrhoea, dysentery, diabetes, hyperdipsia, burning sensation, haemaorrhages, leucorrhoea and colporrhagia (Warrier PK, 1995) [3]. All *Ficus* species possess latex- like material within their vasculatures that provide protection and self- healing from physical assaults (Sirisha N, 2010) [4]. The antibacterial activities of ethanolic extracts of *F. sycomorus* L. and *F. platyphylla* Del. in the treatment of ailments have been previously reported (Adeshina, 2010) [5]. Other scientists, however, reported that the *F. benghalensis* and *F. racemosa* ethanolic extract of roots at different concentrations (25, 50 and 75 mg/ml) showed moderate anti-bacterial activity against *S. aureus, P. aeruginosa* and *K. pneumonia*

isolates (Murti K, 2011) [6]. The present investigation is to evaluate the antibacterial efficacy of *Ficus microcarpa* L. against gram positive and gram negative bacteria

Materials and Methods

Preparation of plant extract

Leaf, stem bark and root bark from fully grown plant was used in the present study. It was cleaned thoroughly with water to remove dirt and other microorganisms. The plant materials were dried in the shade and ground into powdered form and stored for future use. One gram of dried powder were weighed and dissolved in 10ml of the solvents and left for 24hrs with occasional shaking. Filtered off using sterile filter paper (Whatman No. 1) into a clean conical flask and centrifuged and filtrate was collected for further study. In the case of acetone extract the filtrate was allowed to evaporate. The dried residue was collected and dissolved in same amount of distilled water and used to study the antibacterial property.

Micro-organisms used

Gram- Positive: *Staphylococcus aureus* (ATCC 25923)
Gram-Negative: *Pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 25922) were procured from PSG Medical College, Coimbatore.

Culture media

Mueller-Hinton Agar was prepared according to the manufacturer's instruction, autoclaved and dispensed at 20ml per plate in 12x12cm Petri dishes. Set plates were incubated over night to ensure sterility before use.

Antibacterial Assay

20 ml of sterilized Muller Hinton Agar was poured into sterile petriplates, after solidification, fresh cultures of human pathogen were swabbed on the respective plates. The discs were kept over the agar plates using sterile forceps.The plates were subsequently incubated at 37 °C for 24 Hrs. After incubation the growth inhibition zone were quantified by measuring the diameter of the zone of inhibition in mm. The antibiotics Cefotaxime and blank (water and acetone) discs were used as positive and negative controls respectively.

Minimum Inhibitory Concentration

Minimum inhibitory concentrations (MICs) are defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. The bacteria inoculums were prepared in 3 ml nutrient broth and incubated at 37 °C, for 2-4 hours. The cultures were adjusted to a 0.5 McFarland turbidity standard [1 to 2 $x10^8$ colony forming units (CFU)/ml].

Different concentrations of the plant extracts such as 150 mg/ml, 75 mg/ml, 37.5 mg/ml, 18.75 mg/ml, 9.37 mg/ml, 4.68 mg/ml,2.34 mg/ml, 1.17 mg/ml and 0.586 mg/ml were prepared. The Nutrient Broth, which contained logarithmic serially two fold diluted amount of test compound and controls, was inoculated with actively dividing bacteria cells. The cultures were incubated for 24 h at 37 °C and the growth was monitored visually and spectrophotometrically. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as minimum inhibitory concentration.

Results and Discussion

Recently much attention has been directed towards the biologically active compounds isolated from popular plant species. The use of medicinal plants play a large role in covering the basic health needs in developing countries. The antibacterial activity has been done by two methods. Dilution method was used to determine the minimal concentration of an antimicrobial agent required to inhibit or kill a microorganism and is usually expressed in mg/ml. Diffusion method was used to test the bacterial susceptibility to antimicrobial agent and may be measured *in-vitro* by using the principles of agar diffusion. In the present study the *in vitro* antibacterial activity and Minimum Inhibitory Concentration of crude extract of leaf, stem, root and total plant extract of *Ficus microcarpa L.* was assessed.

The acetone extract of leaf and aqueous extract of stem bark and root bark of *Ficus microcarpa* L. showed no activity against *Staphylococcus aureus*. The aqueous extract of leaf and stem bark showed minimum activity and root bark extract possessed moderate inhibitory effect against *Escherichia coli* (14mm). The stem bark extract exhibited no activity against *Pseudomonas aeruginosa* (Table-1).The latex of the plant has been reported to contain chitinase, giving rise to antifungal properties (Taira T, 2005) [7]. Liu *et al*., reported antitussive and expectorant potential of *F. microcarpa.* Traditionally, the bark has a reputation of being efficient in the treatment of diabetes, ulcers, burning sensation, hemorrhages, leprosy, itching, liver disease, and toothache (Kirtikar, 1987) [9].

Whole plant extract of *Ficus microcarpa* L showed considerably good activity compared to individual plant part extracts. The aqueous extract of the whole plant showed moderate inhibitory activity against *Pseudomonas aeruginosa* (18mm) followed by *Staphylococcus aureus and Escherichia coli.* The acetone extract showed comparatively less activity against the tested bacteria. It was observed that the aqueous and acetone extract of *Ficus microcarpa* showed same inhibition zone against *Staphylococcus aureus* (10mm).

The results indicated that the plant extracts showed antibacterial activities at variable degrees against the tested bacteria, with MICs values ranging from 4.68 to 37.5mg/ml. The present study indicated that *P. aeruginosa* was more sensitive to the whole plant extract of *F. microcarpa* with an MIC value of 4.68mg/ml (Table 2)

F. carica has been reported to include antiviral, antibacterial, hypoglycemic, and anthelmintic effects (Wang G, 2004; Solomon A, 2006; Jeong, M. R., 2005) [10, 11, 12]. Medicinal plants possess immunomodulatory and antioxidant properties, leading to antibacterial activities. They are known to have versatile immunomodulatory activity by stimulating both non-specific and specific immunity (Pandey, 2006) [13]. These techniques played a significant role in the search for additional resources of raw material for pharmaceutical industry.

It is concluded that plant extract possess antibacterial activity against tested organisms. The zone of inhibition varied suggesting the varied degree of efficacy and different phytoconstituents of herb on the target organism.

Table 1: Antibacterial activity of different parts of *Ficus microcarpa* L. against *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*

Bacteria	Zone of inhibition (mm)							
	Leaf		Stem		Root		Whole extract	
	Water	Acetone	Water	Acetone	Water	Acetone	Water	Acetone
S. aureus	7	-	-	7	-	10	10	10
E. coli	10	6	10	-	14	10	8	7
P. aeruginosa	7	8	-	-	12	10	18	10

Table 2: Minimum Inhibitory concentration (mg/ml) of the plant parts tested

	Leaf	Stem	Root	Whole extract
S. aureus	9.37	9.37	37.5	9.37
E. coli	9.37	9.37	9.37	9.37
P. aeruginosa	9.37	37.5	9.37	4.68

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