



## Preliminary phytochemical analysis of bark of *Ficus racemosa* Linn and antibacterial activity of its bark oil.

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### ABSTRACT:

The leaves, fruits and bark of *Ficus racemosa* Linn, are used in the Ayurveda for the treatment of dysentery, skin diseases, diabetes, asthma and inflammatory swellings. Phytochemical screening of the bark showed positive tests for sterols, saponins and tannins. Agar well diffusion method proved that methanolic extracts of the oil prepared from the bark possess good antibacterial activity against the tested Gram positive strains and has shown intermediate action against tested Gram negative strains.

Key words: *Ficus racemosa*, Anti-bacterial activity, Agar well diffusion assay, Phytochemical analysis

### INTRODUCTION

Medicinal plants are natural sources of compounds that can be used against many diseases today [1]. A considerable amount of literature deals with the action of plant on micro organisms, pathogenic to other plants or animals. Although a number of antibiotics were widely used in medicine, the search for anti-bacterial substances from plants will continue because better and safer drugs to combat Gram positive and Gram negative bacterial infections are still needed. On the other hand, the development of resistant strains of pathogenic bacteria to antibiotics currently in use is a problem of containing concern to public health [2]. *Ficus racemosa* Linn of Moraceae family is an erect tall monoecious tree growing 25-30m tall or more height with greyish brown bark. Various extracts of its bark are used in herbal medicine to treat diverse ailments. The bark is antiseptic, antipyretic and vermifugal, and the decoction of bark is used in the treatment of various skin diseases, and ulcers. *F. racemosa* possess various biological effects such as chemo preventive, antidiabetic [3], anti inflammatory [4] and antidiuretic [5]. Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents. The problem of microbial resistance is growing and outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore it is essential to find new compounds that have antimicrobial properties and it is worthwhile to screen plant species which have the above properties to synthesize new drugs [6]. The purpose of the present work was to analyse the actives present in the bark of *Ficus racemosa* by preliminary phytochemical analysis and to evaluate the antibacterial activity of the methanolic extract of bark oil of *Ficus racemosa* Linn. Antibacterial study was carried out by well diffusion assay method. Here the oil was unable to diffuse through the well. Oil is miscible in organic solvents only. Since methanol is having polarity, the oil cannot be miscible in it. Almost all the phytochemical actives can be extracted with methanol. So in the present

study methanolic extract of the bark oil was freeze and the solidified oil at the bottom was removed by filtering through Whatman No.40 filter paper. The top methanolic layer containing actives was taken for the study.

### MATERIALS AND METHOD

#### Collection of Plant Material and Phytochemical analysis

The stem barks of the plant *Ficus racemosa* Linn. (Moraceae) were collected from Herbal Garden Division of Kerala Ayurveda Limited (KAL), Aluva and authenticated at the Department of Botany, KAL and voucher specimen of the plant were deposited at KAL herbarium for future references.

The bark was dried separately under shade. Medicated oil of the powdered bark of *Ficus* was prepared in the laboratory of Kerala Ayurveda Limited. Preliminary phytochemical analysis was carried out using standard analytical procedure [7] Crude methanolic extract of the oil was used for the antibacterial studies, obtained by using Soxhlet extraction method using methanol as the solvent.

Table 1 : Preliminary Phytochemical screening of *Ficus racemosa* Linn bark extract

Secondary metabolites	Bark extract	
	Methanol	Water
Alkaloids	-	-
Tannins	+	+
Flavonoids	+	-
Sterols	+	-
Glycosides	-	-
Saponins	-	+

(+ Presence and - Absence)

**Table 2: Antibacterial activity of methanolic extract of the oil prepared from bark of *Ficus racemosa* Linn.**

Organism	Gentamycin	Methanol	Methanolic extract of oil of <i>Ficus racemosa</i>	
	+ve control	-ve control	Zone size	Inference
<i>Staphylococcus aureus</i>	32mm	Nil	19mm	S
<i>Bacillus subtilis</i>	32 mm	Nil	19 mm	S
<i>Salmonella typhimurium</i>	24 mm	Nil	17 mm	S
<i>Escherichia coli</i>	24mm	Nil	Nil	R
<i>Klebsiella pneumoniae</i>	22mm	Nil	16 mm	S

S – Sensitive; R - Resistant

#### Test microorganisms

*Bacillus subtilis* (MTCC 3053), *E.coli* (MTCC 727), *Staphylococcus aureus* (MTCC 3160), *Salmonella typhimurium* (MTCC 98), *Klebsiella pneumoniae* (MTCC 3384) were procured from IMTECH Chandigarh. All these organisms were further confirmed by Gram staining and biochemical tests, and maintained in nutrient broth at 4°C.

#### Medium used:

Medium used was Muller Hinton Agar and Nutrient Broth. All media was supplied by HiMedia.

#### Antibiotic disc:

Antibiotic disc used was Gentamycin (10mcg/disc) from HiMedia.

#### Preparation of extract for Antibacterial studies:

The extract was prepared by dissolving 10g of the *Ficus* oil in 40ml of methanol and extracted for 30min under reflux. The supernatant was filtered and the filtrate was freeze-dried for 2 days. The top methanol layer was filtered through Whatman No.40 filter paper from the solidified oil. This methanol extract was concentrated.

#### EVALUATION OF ANTIBACTERIAL ACTIVITY

Evaluation of antibacterial activity of the methanolic extract of the *Ficus* oil was performed by agar well diffusion method. The plates were prepared by using Muller Hinton Agar. 18h culture of test organisms *Salmonella typhimurium*, *E.coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Bacillus subtilis* in Nutrient broth were taken for the study. Sterile cotton swabs were dipped in the culture broth and pressed around the side walls of the tubes in order to remove excess inoculum and swabbed it evenly on the Muller Hinton Agar plates. The wells of 5.0mm diameter were made in the agar plate by using sterile cork borer. Aliquots of 200µl of the methanolic extract of the oil of *Ficus racemosa* bark were applied aseptically to the wells using the micropipette and sterile tips. Methanol was used as negative control. The antibiotic disc Gentamycin (10mcg/disc), used as positive control was placed aseptically over the medium at a sufficient distance between the wells containing the sample using sterile forceps. The plates were kept undisturbed at room temperature for 2h for the proper diffusion of test-drug into agar. Then the plates were incubated at 37°C for 24 hrs and the zone of inhibition was observed. The zone of clearance around the well was measured in millimeters (mm) using Himedia zone size checking scale. The experiment was repeated and took the average zone size.

#### RESULTS & DISCUSSION

In the traditional system of medicine, *Ficus racemosa* is used for various health problems and diseases [8]. The medicinal value of a plant lies in some chemical substances that produce a definite physiological action on human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavanoids and phenolic compound[9]. In the present work, the preliminary phytochemical analysis on *Ficus racemosa* bark extract reveals the presence of sterols, saponins and tannins which was shown in Table 1. Tannins have been reported to be bacteriostatic or bactericidal against *Staphylococcus aureus* [10]. In another study by Arunachalam *et al.*, phytochemical analysis of *Ficus racemosa* Linn showed the presence of saponins, flavanoids, tannins and alkaloids [11].  $\beta$  sosterol isolated from the stem bark was found to possess potent hypoglycemic activity when compared to other isolated compound[12]. The data pertaining to the antimicrobial potential of the plant extract are presented in Table 2. The methanolic extract of the oil of *Ficus racemosa* bark showed that it had strong activity against all the tested Gram-positive organism, *Bacillus subtilis* and *Staphylococcus aureus*. *Salmonella typhimurium* and *Klebsiella pneumoniae* were found to have intermediate action to the methanolic extract of the oil of *Ficus racemosa* bark. The methanolic extract had no activity against *E.coli*. A study by Mandal SC showed that leaves of *Ficus racemosa* had good antibacterial activity against *Escherichia coli* ATCC 10536, *Bacillus fusiformis* ATCC 14884, *Bacillus subtilis* ATCC 6633, *Pseudomonas aeruginosa* ATCC 25619 and *Staphylococcus aureus* ATCC 29737 [13]. No antibacterial activity was observed in negative control. The positive control used for comparison was Gentamycin (10µg/disc).

#### CONCLUSION

The phytochemical screening on qualitative analysis showed that the bark of *Ficus* is rich in popular phytochemical substances like flavanoids, sterols, saponins and tannins. The results obtained from the antibacterial activity reveals that the methanolic extract of the oil of *Ficus racemosa* bark have good antibacterial activity against the tested Gram-positive strains and have intermediate action to the tested Gram-negative organisms except *E.coli*. The phytochemical compounds present in the bark have great potential as compounds against microorganisms and is responsible for the antibacterial activity. Thus it can be summarised that, the oil of *Ficus racemosa* bark can be used in the treatment of infectious diseases.

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