

Screening of antimicrobial potential of medicinal plant (*Ficus racemosa* L.) against different species of pathogenic microbes

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Abstract

Ficus racemosa L. is an important medicinal plant found in India, Australia, and Southeast Asia. It is popularly known as 'gular.' Many active constituents that have been isolated from various parts of this plant possess useful pharmacological activities. The literature survey proposed that it has multiple pharmacological actions that include antidiabetic, antioxidant, antidiarrhoeal, anti-inflammatory, antipyretic, antifungal, antibacterial, hypolipidemic, antifilarial, and hepatoprotection. The emergence and spread of antibiotic resistance, as well as the evolution of new strains of disease causing agents, are of great concern to the global health community. Effective treatment of a disease entails the development of new pharmaceuticals or some potential source of novel drugs. The objective of the study is to screen the antimicrobial activity of different leaf and fruit extracts against four different pathogens. Streptomycin was used as a positive control. In agar well diffusion method the petroleum ether fruit extract of *Ficus racemosa* was found to be most potent, which showed highest zone of inhibition (18mm in 1000 µg) against *Streptococcus mutans* and the acetone leaf extract of *Ficus racemosa* showed highest activity (16 mm in 1000 µg) against *Escherichia coli*.

Key words : *Ficus racemosa*, Antimicrobial, Flavonoid, Coumarin, Streptomycin, Aminoglycosides.

Phytochemicals are non-nutritive components present in a plant-based diet that exert protective or disease-preventing effects⁵. Carbohydrates are a common source of energy in living organisms. Plant contains a variety of chemically active compounds such as flavonoid, terpenoids, alkaloids, coumarin, saponin, polysines, sulphides and furyl compounds.

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Phytochemicals provide colours to plants and an array of flavors both pleasant and unpleasant when consumed. They contain active constituents that are used in the treatment of many human diseases¹. They are effective in the treatment of infectious diseases, while simultaneously alleviating many of the side effects that are often connected with synthetic antimicrobial³. The World Health Organization (WHO) has listed 20,000 medicinal plants utilized worldwide²¹. *Ficus* is a very large genus of tropical plants. It has more than 700 species that grow in warm parts of Asia, Africa, America, and Australia^{15,12}. Ayurveda and Unani, the traditional Indian medical system, has employed the popular medicinal plant *Ficus racemosa* for many years to treat a variety of illnesses and disorders, including skeleton diseases, diabetes, inflammatory, hyperlipidemia, hemorrhoids, respiratory, liver dysfunction, antitussive, hepatoprotective, antimicrobial, and various GIT disorders²³. *Ficus racemosa* L. is a moderate sized avenue plant, commonly called as cluster fig tree is a member of family moraceae. It is unusual in that its figs grow on or close to the tree trunk, termed cauliflory. The tree is upto 18m high, bark 8-10mm thick and leaves are ovate, ovate-lanceolate or elliptic, subacute, entire and petiolate². The fruit receptacles are 3-6 cm in diameter, pyriform, large clusters, arising from main trunk or large branches. The fruits resemble the figs and are green when raw, turning orange, dull reddish or dark crimson on ripening⁷. A number of studies have reported the antibacterial potential of *Ficus racemosa* against different bacterial strains. Stem and bark ethanol extract was found to be very effective against *Pseudomonas aeruginosa*, *Proteus mirabilis*,

Staphylococcus aureus, *Bacillus cereus*, *Alcaligenes faecalis*, and *Salmonella typhimurium* bacterial strains, indicating the scope to discover bioactive natural products that may serve as leads in the development of new pharmaceuticals in order to address unmet therapeutic needs¹⁴. An antimicrobial is an agent that kills microorganisms or stops their growth of microorganisms such as bacteria and fungi. They contain active constituents that are used in the treatment of many human diseases. They are effective in the treatment of infectious diseases, while simultaneously alleviating many of the side effects that are often connected with synthetic antimicrobial. Natural products have been a rich source of bioactive compounds, some of these compounds showed significant antibacterial activity¹³. Antibacterials are used to treat bacterial infections. Antibiotics are classified generally as beta-lactams, macrolids quinolones, tetracycline or aminoglycosides. Their classification within these categories depends on their antimicrobial spectra, pharmacodynamics, and chemical composition. Prolonged use of certain antibacterials can decrease the number of enteric bacteria, which may have a negative impact on health⁶. Bacteria are considered as a group of the microorganisms that cause the most deadly diseases and widespread epidemics of human civilization. Infectious diseases caused by pathogenic bacteria, have prevalence rate and morbidity more than other pathogenic microorganisms¹⁰. To overcome this problem, researchers studied on the antibacterial properties of various plants against antibiotic-resistant bacteria. Medicinal plants may offer a new source of antibacterial agents for use. Biologically, active compounds from natural sources has always been of great

interest to scientists working on infectious diseases. Plant-derived drugs serve as a prototype to develop more effective and less toxic medicines¹⁸. The use of herbal medicine is drastically increasing in wealthy nations^{4,21}. The present study aims to screen the antimicrobial potential of four different solvent extracts of *Ficus racemosa* leaf and fruit against four different bacterial pathogens such as *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus mutans*.

Collection of plant sample :

Fresh leaves and fruit of *Ficus racemosa* were collected from local area, Trivandrum district in a plastic bag and cleaned with distilled water.

Preparation of extract :

For each extract preparation, fresh fruit and leaves of 20gm were weighed and grinded with 100ml of petroleum ether, hexane, acetone and methanol. Then the solutions were centrifuged at 5000rpm for 15 minutes, supernatant were collected and filtered through whatsmann number 1 filter paper.

Bacterial cultures :

The microorganisms (Gram positive and Gram negative) were obtained from Microbial Type Culture Collection (MTCC), institute of microbial technology, Chandigarh. The bacterial cultures were maintained in nutrient broth as turbid growth and kept in refrigerator.

Determination of antimicrobial activity :

Petriplates containing 20ml Muller Hinton Agar Medium were seeded with bacterial culture of *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus mutans* (growth of culture adjusted according to McFarland Standard, 0.5%). Wells of approximately 10mm was bored using a well cutter and different concentrations of sample such as 250µg, 500µg and 1000µg were added. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well. Streptomycin was used as a positive control. The plates were incubated for 24 hours at 37°C. After incubation, the diameter of inhibitory zones (in mm) formed around each well was measured.

The petroleum ether, hexane, methanol and acetone fruit and leaf extracts of *Ficus racemosa* were screened for *In vitro* antibacterial property by Agar well diffusion method against *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus mutans*. The antibacterial activity was compared with that of the standard antibacterial drug Streptomycin. The antibacterial activity of various fruit and leaf extracts of plant were assessed and compared with that of the Streptomycin. In the present study the antibacterial potential of *Ficus racemosa* fruit and leaf extracts were plated in plate (1) and plate (2). The results were tabulated in table (1) and table (2). The plant extracts exhibited good antimicrobial properties against the microorganism tested, as exhibited by agar well diffusion method. At higher concentration

(1000 μg), activity was remarkable in comparison to lower one (250 μg). Petroleum ether fruit extract and methanol fruit extract of *Ficus racemosa* showed good antibacterial activity against *Streptococcus mutans* with zone of inhibition of 18mm and 16mm at concentration of 1000 μg . Extract of Petroleum ether leaf and Acetone leaf showed most promising result against *Pseudomonas aeruginosa* with similar zone of inhibition of 15mm respectively at 1000 μg concentration. *Staphylococcus aureus* was found highly sensitive to the action

of acetone fruit extract (16mm at 1000 μg) followed by acetone leaf and methanol leaf with 11 and 12mm zone of inhibition respectively. At 1000 μg concentration extracts of Petroleum ether leaf and Acetone fruit were effective against *E.coli* with significant zone of inhibition 14, 12mm respectively. Among the eight *Ficus racemosa* plant extracts, Petroleum ether fruit and leaf extract, Methanol fruit and leaf extract, showed good antimicrobial activity against all tested microorganisms.



A: Petroleum ether fruit extract

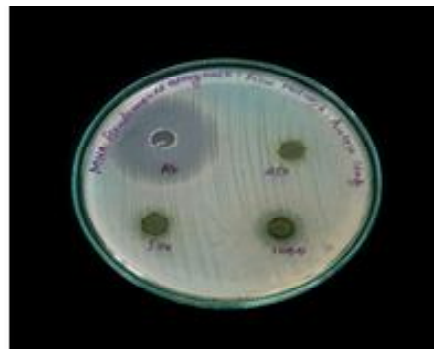


B: Methanol fruit extract

Plate 1. Shows the antibacterial activity of petroleum ether and methanol fruit extract of *Ficus racemosa* against *Streptococcus mutans*.



A: Petroleum ether leaf extract



B: Acetone leaf extract

Plate 2. Shows the antibacterial activity of petroleum ether and acetone leaf extract of *Ficus racemosa* against *Pseudomonas aeruginosa*.

Table-1. Antibacterial potential of petroleum ether, hexane, methanol and acetone fruit extracts of *Ficus racemosa* against pathogenic microbes.

Extract	Concentra- tion (µg)	<i>E. coli</i> (mm)	<i>Pseudo- monas aerugi- nosa</i> (mm)	<i>Staphylo- coccus aureus</i> (mm)	<i>Strepto- coccus mutans</i> (mm)
Petroleum ether fruit extract	250 µg	-	-	13	15
	500 µg	-	-	15	16
	1000 µg	13	12	16	18
Hexane fruit extract	250 µg	-	-	-	-
	500 µg	-	12	-	-
	1000 µg	13	17	3	11
Methanol fruit extract	250 µg	-	11	12	12
	500 µg	11	13	14	14
	1000 µg	13	17	15	16
Acetone fruit extract	250 µg	-	-	12	14
	500 µg	-	-	15	15
	1000 µg	12	12	16	16

Table 2: Antibacterial potential of petroleum ether, hexane, methanol and acetone leaf extracts of *Ficus racemosa* against pathogenic microbes.

Extract	Concentra- tion (µg)	<i>E. coli</i> (mm)	<i>Pseudo- monas aerugi- nosa</i> (mm)	<i>Staphylo- coccus aureus</i> (mm)	<i>Strepto- coccus mutans</i> (mm)
Petroleum ether leaf extract	250 µg	-	-	-	-
	500 µg	-	12	-	-
	1000 µg	14	15	11	11
Hexane leaf extract	250 µg	-	-	-	-
	500 µg	-	11	-	-
	1000 µg	13	14	-	-
Methanol leaf extract	250 µg	-	-	-	-
	500 µg	-	-	-	-
	1000 µg	14	12	12	11
Acetone leaf extract	250 µg	-	-	-	-
	500 µg	-	12	-	-
	1000 µg	16	15	11	-

Medicinal plants are considered as a rich resource of ingredients which can be used in drug development and synthesis. The compounds found in plants are of many kinds, but most are in four major biochemical classes, alkaloids, glycosides, polyphenols, and terpenes. Some plants consider as important source of nutrition while others are recommended for their therapeutic values¹¹. Several studies reported that phytochemicals like terpenoids, flavonoids, tannins, alkaloids, steroids and some phenolic compounds are responsible for the antibacterial activity of the plant extract^{17,20}. The mode of action of antimicrobial agents also depends on the type of microorganisms and is mainly related to their cell wall structure and the outer membrane arrangement. While screening medicinal plants for antibacterial activity, it is generally expected that a greater number of compounds would be active against Gram positive rather than Gram negative bacteria⁹. This study confirms that the fruit and leaf extracts of *Ficus racemosa* possess antibacterial activities. In the present study, the highest inhibition zone was determined by petroleum ether fruit extract and acetone leaf extract against *Streptococcus mutans* and *E. coli* (Table-1 and 2). In another study it is also recorded the antibacterial activity of *Ficus racemosa* aqueous fruit leaf extract against *E. coli*¹⁶. Similarly, evaluated the antibacterial activity of aqueous bark leaf extract of *Ficus religiosa* and *Ficus benghalensis* against enterotoxigenic *E. coli* and observed that both plants bear antibacterial properties²². *Ficus carica* has been reported the antiviral, antibacterial, hypoglycemic and anthelmintic effects^{8,19,24}. Medicinal plants possess immunomodulatory and antioxidant properties, leading to antibacterial activities. The

contradiction in the zone of inhibition may be due to concentration variation, various test environment and methods. On the basis of this finding, the petroleum ether fruit extracts of plant possess a good candidate in the search for a natural antimicrobial agent against infections or diseases caused by the test organisms. The extracts of *Ficus racemosa* plant should be further analyzed to isolate the specific antibacterial properties in them. Clinical trials should be carried out to explore the potential of these plant extracts in the treatment of the infectious diseases.

The antimicrobial activity of *Ficus racemosa* extracts was probably due to of some important secondary metabolites. In the current study, the fruit and leaf extract of *F. racemosa* has been analyzed for antimicrobial inhibition properties. Therefore, this property of chemical component in these herbs can be used to discover natural bioactive products that potentially enhance the therapeutic value in the development of novel pharmaceutical research activities and suggesting for using potential antibacterial agents to cure bacterial infection. *Ficus racemosa* is a herb which possess lots of medicinal value to treat many diseases. The study suggest that this extract possess significant antimicrobial potential and could be used in clinical trials for exploring it to commercial use.

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