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EVALUATION OF ANTIMICROBIAL ACTIVITY OF *FICUS RACEMOSA* LINN LEAVES EXTRACT

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Abstract

Ficus racemosa Linn is an important traditional medicinal plant, the present study was carried out to explore the phytochemical and antimicrobial properties of leaves of this plant against clinical pathogens. The ethanol extract of *Ficus racemosa* Linn exhibited many phytochemical compounds and 10 µg/mL of the extract manifested good antimicrobial activity. The antimicrobial activity of plant contributes to the ethanopharmacological importance which indicates the pharmaceutical applications to be employed in the medicinal field against various infectious diseases.

Keywords: *Ficus racemosa* Linn, ethanopharmacology, phytochemicals, antimicrobial.

Introduction

The development of antibiotic resistant is now gathering pace and contributing more substantially to the threat of disease management. From past 10-15 years, antibiotic-resistant organisms have steadily increased, including methicillin-resistant *Staphylococci*, pneumococci resistant to both penicillin and macrolides, vancomycin-resistant *Enterococci*, multidrug resistant Gram-negative organisms, and multidrug-resistant strains of *Mycobacterium tuberculosis*¹ (Norrby, 2005). To overcome and combat increasing multidrug resistance strains, there is an urgent need to develop antimicrobial agents against multidrug-resistant strains. The search for new anti-infection agents has occupied many research groups in the field of ethnopharmacology² (Rios and Recio, 2005) which employs the use of medicinal plants for developing natural products. The recently approved natural-product based drugs (elliptinium, galantamine and huperzine) have been described extensively by various researchers including Newmann and Cragg³, Butler⁴, Chin et al.⁵ and Lam⁶. Over a 100 natural-product-derived compounds are currently undergoing clinical trials and at least 100 similar projects are in

preclinical development, mostly derived from plants and microbial sources⁴. According to Cowan⁷, plants have ability to synthesize aromatic substances, most of which are phenols or their oxygen substituted derivatives⁸. These substances serve as plant defense mechanisms against predation by microorganisms, insects and herbivore such as terpenoids give plant their odor, other (quinone and tannins) are responsible for plant pigment. Many compounds are responsible for plant flavor (terpenoid capsaicin from chili peppers) and some of the same herbs and spices used by humans to season food yield useful medicinal compounds⁷.

Ficus racemosa Linn (Moraceae) is an evergreen, moderate to large sized spreading, lactiferous, deciduous tree, without much prominent aerial roots found throughout greater part of India in moist localities and is often cultivated in villages for its edible fruit⁹. It is majorly found in Australia, India and South Asian countries, mostly found around the water streams. The tree is medium tall, growing 10-16 meters in height. The rich green foliage provides a good shade. The leaves are dark green, 7.5-10 cm long, ovate or elliptic. The fruit receptacles 2-5 cm in diameter, pyriform, in large clusters, arising from main trunk or large branches.

Ficus racemosa documented to possess anti-inflammatory¹⁰⁻¹¹, anti-pyretic¹², antidiuretic¹³. The bark and leaves of this group used as astringent, haemostatic, anti-inflammatory, antiseptic, prescribed in diarrhea, dysentery and in treatment of skin disease ulcers vaginal disorder leucorrhoea monorrhagia deficient lactation and act as urinary astringent and reduce sugar in diabetic¹⁴. The powdered leaves of *Ficus racemosa* when mixed with honey is given in bilious infections¹⁵. The plant (roots, bark-skin, fruits and the leaves) possesses great medicinal value, the present study was carried out to explore the phytochemical constituents and antimicrobial effect of *Ficus racemosa* Linn leaves against bacterial pathogens.

Material and methods

Sample collection:

The fresh leaves of plant *Ficus racemosa* Linn was collected from VIT University nursery Vellore, Tamil Nadu. The leaves were properly washed, shade dried completely for 3-4 d and was finely ground to powder by using mixer grinder.

Extraction:

Finely ground powder of leaves (50 g) of *Ficus racemosa* Linn was extracted using toluene and ethanol separately in soxhlet extractor by not exceeding the boiling point of the solvent. The obtained extracts were filtered through

Whatman Filter Paper No.1 and then concentrated under reduced atmospheric pressure. The dry extracts were stored at -20°C.

Phytochemical analysis:

The toluene and ethanol extracts from *Ficus racemosalinn* were studied for their phytochemical constituents by following Chandiraet al.¹⁶ protocol which includes test for carbohydrates, steroids, proteins, phenols, flavanoids, quinones, tannins, saponins, cardiac-glycosides and alkaloids.

Test for carbohydrate detection:

The toluene and ethanol extracts of *Ficus racemosalinn* were tested with Molisch reagent, Fehling reagent, Benedict's solution and Barfoed's test for detection of carbohydrates. A small quantity of toluene and ethanol extracts were separately dissolved in 5 mL of distilled water and filtered. The filtrate obtained from these extracts were maintained and used for phytochemical analysis.

Molisch test:

The extract filtrates were treated with few drops of alcoholic α -naphthol solution in a test tube separately and 2 ml of concentrated sulphuric acid was added carefully along the sides of the test tubes.

Fehling's test:

The extract filtrates were treated in equal volumes with 1ml Fehling A and 1ml Fehling B solutions, boiled for one minute separately. Again, the mixtures were boiled for 5-10 min on water bath. The appearance of reddish brown color indicates the presence of reducing sugar.

Benedict's test:

Equal quantity of extract filtrate was treated with Benedict's reagent, mixture was heated on water bath for few minutes. The formation of brown color indicates the presence of reducing sugars.

Barfoed's test: The extract filtrates were equally treated with Barfoed's reagent, the production of brick red color confirms the presence of monosaccharides.

Test for Glycosides: 2.5 mL of extracts were treated with 1 ml of glacial acetic acid containing few drops of ferric chloride solution. 0.5 mL of concentrated sulphuric acid was added by the sides of the test tubes. The formation of brown ring at interface indicates the presence of cardiac glycosides.

Test for Steroids:

0.5 mL of extracts was treated with 5 mL of chloroform and 5 mL of concentrated sulphuric acid. The appearance of red color at upper layer and yellow color with green fluorescence indicates the presence of steroids.

Test for Terpenoids:

2.5 mL of filtered extracts was mixed with 1 mL of chloroform and then 1.5 mL of concentrated sulphuric acid was added by the sides of test tube. The formation of reddish brown precipitate at interface indicates the presence of terpenoids.

Test for Alkaloids:

A small quantity of solvent free extracts were separately mixed with 5 mL of hydrochloric acid and filtered, the filtrate was tested with Mayer's reagent, Hanger's reagent, Dragendroff's reagent to confirm the presence or absence of alkaloids.

Test for Saponins:

The small quantity of the extracts was diluted with 20 mL of distilled water and it was agitated for 15 min. Formation of 1 cm layer of foam shows the presence of saponins.

Test for Quinones:

0.5 mL of filtered extracts was treated with 0.5 mL of concentrated sulphuric acid. The formation of red color shows the presence of quinones.

Test for Tannins:

The filtered extracts was treated with 1 mL of 5% FeCl₃, bluish-black precipitates formation indicates the presence of tannins in the solvent free extract.

Test for Phenols:

0.5 mL of the filtered extracts was treated with 10% of ferric chloride solution. The appearance of blue or green color indicates presence of phenols.

Test for Flavanoids:

A small quantity of extracts was dissolved in alcohol, few pieces of magnesium was added to it followed by drop wise addition of concentrated hydrochloric acid. The appearance of magenta color confirms the presence of flavanoids.

Thin Layer Chromatography

Thin layer chromatography was performed in order to develop the phytochemical constituents present in the toluene and ethanol extract of *Ficus racemosa* Linn. The developing solvents were used in varying ratio to provide maximum differentiation. Silica coated TLC plates were developed in the toluene: ethyl acetate: ethanol (4:0.5:0.5) and for toluene as well as ethanol extract hexane: ethyl acetate. Various spots were marked on the TLC plate and retention factor was calculated using formula: $R_f = \frac{\text{distance travelled by the compound}}{\text{distance travelled by the solvent front}}$ ¹⁷.

Antimicrobial Activity

The sensitivity of pathogenic microorganisms to toluene and ethanol extracts were tested by measuring the zone of inhibition of given concentration of *Ficus racemosa* Linn extracts by the well-diffusion method. The test organisms used were *Escherichia coli* (MTCC43), *Bacillus subtilis* (MTCC121), *Pseudomonas aeruginosa* (MTCC424), *Klebsiella pneumonia* (MTCC432), *Staphylococcus aureus* (MTCC96) and *Streptococcus mutans* (MTCC497). Clinical bacterial isolates were swabbed onto Muller-Hinton agar plates. Four wells were punctured onto the agar plate. 50 mg/ml of dry toluene and ethanol extract with different concentrations (25 μ l, 50 μ l, 75 μ l and 100 μ l) were loaded into the wells. The petriplates were incubated for 24 h, and the zone of inhibition was measured around the wells.

Results and discussion

The leaves of *Ficus racemosa* Linn were extracted using soxhlet apparatus and yielded 18% of crude extract with toluene and 26% of crude extract with ethanol. The yield of phytochemical contents from plants depends on the nature of solvent from non-polar to polar solvent. The ethanol extract of *Ficus racemosa* Linn revealed the presence of phytochemical constituents including phenols, flavanoids, quinines, tannins, saponins, steroids, alkaloids whereas toluene extract did not show presence of any phytochemical constituents. The phytochemical profile of toluene and ethanol extract of *Ficus racemosa* Linn has been presented in Table 1. The presence of phytochemicals in plant extracts supports bioactive compounds which might be responsible for therapeutic activities¹⁸. The presence of phenols in the plant contributes to the preparation of some antimicrobial compounds such as dettol and cresol¹⁹. The ethanol extract of *Ficus racemosa* Linn showed the presence of tannins and alkaloids which helps in cancer prevention as well as remarkable cytotoxic and anticancer activity²⁰. *Ficus racemosa* Linn is used as traditional medicinal plant which produces inhibitory effect on inflammation due to the presence of saponins and it the major ingredient in traditional

Chinese medicine. In the present study, Glycosides have been reported in ethanol extract of *Ficus racemosa* Linn which indicates many healing properties as it is an important class of bioactive compound containing a sugar molecule bound to non-carbohydrate.

Table-1: Phytochemical analysis of toluene and ethanol extracts of *Ficus racemosa* Linn leaves.

Phytochemical test	Toluene	Ethanol
Phenols	Absent	Present
Flavonoids	Absent	Present
Quinones	Absent	Present
Tannins	Absent	Present
Saponins	Absent	Present
Cardiac glycosides	Absent	Absent
Steroids	Absent	Present
Terpenoids	Absent	Absent
Alkaloids	Absent	Present
Molisch test	Absent	Present
Fehling's A test	Absent	Present
Fehling's B test	Absent	Present

The thin layer chromatography of *Ficus racemosa* Linn toluene extract developed two spots at R_f value of 0.4 and 0.75 whereas ethanol extract developed 4 spots with R_f value of 0.116, 0.16, 0.28 and 0.65. The ethanol extract of *Ficus racemosa* Linn manifested zone of inhibition against *E. coli* MTCC43 (13.1 mm), *Bacillus subtilis* MTCC121 (10.5 mm), *Pseudomonas aeruginosa* MTCC424 (10.4 mm), *Staphylococcus aureus* MTCC96 (11.0 mm) and *Streptococcus mutans* MTCC497 (12.4 mm) with 10 μ g/mL of the extract whereas toluene extract did not show any antimicrobial effect against pathogenic microorganisms. The antimicrobial activity exhibited by ethanol extract must be due to the presence of phytochemical compounds present in the extract which represents the medicinal importance of the plant. The present study reveals the antimicrobial importance of *Ficus racemosa* Linn which is regarded as

ethanopharmacological plant and can further used for other pharmaceutical properties. The phytochemical constituent present in this plant opens up medicinal gateway for pharmacological values. Furthermore studies should be carried to identify the particular phytochemical constituent responsible for antimicrobial activity by sophisticated analytical techniques.

Conclusion

The present investigation reveals that *Ficusracemosa* Linn leaves contain effective phytochemical constituents which are responsible for antimicrobial activity against various pathogenic microorganisms which can be used as pharmaceutical agent against infectious diseases.

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