

Antimicrobial Activity Of Ethanolic Root Extract Of *Ficus racemosa* Linn.

Goyal PK

Department of Chemistry, University of Rajasthan, Jaipur-302 055 (India).

**Corres. Author: pgoyal_chem1981@yahoo.co.in
Mob. No. +91 9726064589**

Abstract: Antimicrobial activity of ethanolic root extract were evaluated against four bacteria and four fungi at different concentration by using disc diffusion method. The test extract was found to be bacteriostatic and fungistatic in action thus can be used as a source of antibiotic substances for drug development that can be used in control of these bacterial and fungal infection.

Key words- Antimicrobial activity, disc diffusion method, *Ficus racemosa*.

Introduction

Natural product chemistry can be thought of as originating from mankind's curiosity about odour, taste, color and cures for diseases. People in all continents have long applied poultices and imbibed infusion of hundreds, if not thousands, of indigenous plants, dating back to prehistory¹ Since time immemorial, different parts of medicinal plants have been used to cure specific ailments in India. Now-a-days there is wide spread interest in evaluating drugs derived from plant sources.² one of the Indian medicinal plant used traditionally is *Ficus racemosa* Linn. syn. *F. glomerata* Roxb. (Moraceae). Several extracts (water and organic solvents) from different parts of *F. racemosa* were evaluated for blood sugar lowering activity in streptozotocin-induced diabetic rats^{3,4}. The petroleum ether extract of the stem bark of the plant reduced the blood sugar level significantly. Extracts from fruits and latex of the plant did not have any significant effect on blood sugar level of these diabetic rats. The pet. ether extract of the stem bark completely inhibited the enzyme glucose-6-phosphate dehydrogenase from rat liver⁴. However, extract from fruits and latex

inhibited only glucose 6-phosphate but not arginase from rat liver⁴. The bark exhibited hypoglycemic effect in normal and alloxan-induced hyperglycemic animals⁵⁻¹¹ and β -sitosterol-D-glucoside was identified as the active principle¹². The glucoside rich fraction of the leaf extract showed hypotensive and cardiac depressant activity⁶. Anti-inflammatory, analgesic, antipyretic, antibacterial, antidiarrhoeal and hepatoprotective activities of the various extracts from the leaves have also been evaluated in rats and mice⁷⁻¹¹. A uterine tonic prepared using the aqueous extract of fruits was found to show effect similar to oxytocin¹³. Its latex was found to exhibit protease activity¹⁴. Ethanolic extract of its bark showed significant inhibitory activity against castor oil induced diarrhea and PGE₂ induced enter pooling in rats^{10,15}. The Kwath douche of its stem bark can be used for the treatment of leucorrhoea and vaginitis¹⁶. Gluanol acetate isolated from bark is useful in bilious affection¹⁵.

Experimental

Plant material

The plant material, roots of *F. racemosa* Linn., was collected from the road side of Ganesh Marg, Bapu Nagar, Jaipur and carefully identified in the Department of Botany, University of Rajasthan, Jaipur (Herbarium sheet No. RUBL 19764).

Processing and extraction of plant material

Powdered roots of *F. racemosa* were extracted with ethanol for antibacterial and antifungal activity. The extract was filtered, the residue re-extracted (2x) for complete exhaustion. The combined ethanolic extract was evaporated under reduced pressure using a rotary vacuum evaporator. The extract was stored at 4°C in a refrigerator until screened for a particular activity.

Test microorganism

Pure cultures of test bacteria, namely *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Enterobacter cloacae* were obtained through the courtesy of SMS Medical College, Jaipur, which were maintained on Nutrient Broth Medium. Likewise, pure cultures of test fungi, namely *Penicillium chrysogenum*, *Aspergillus niger*, *Trichophyton rubrum* and *Candida albicans* were obtained from the Seed Pathology Laboratory, Department of Botany, University of Rajasthan, Jaipur, which were maintained on Potato Dextrose Agar (PDA) medium.

Media preparation

For the cultivation of bacteria, Nutrient Broth Medium (NBM) was prepared using 8% Nutrient Broth (Difco) in distilled water and agar-agar and sterilized at 15 lbs for 25-30 min. The agar test plates were prepared by pouring ~15 ml of NBM into the petri-dishes (10 mm) under aseptic conditions. The peptone saline solution was prepared (by mixing 3.56 g KH_2PO_4 + 7.23 g NaH_2PO_4 + 4.30 g NaCl + 1.00 g peptone in 1000 ml distilled water, followed by autoclaving) and the bacterial cultures were maintained on this medium by regular subculturing and incubation at 37°C for 24 hrs. However, for the cultivation of fungi, potato dextrose agar (PDA) medium was prepared by mixing 1000 ml potato infusion prepared from 200 g potatoes, 20 g agar and 20 g dextrose, followed by autoclaving. The test fungi were incubated at 27°C for 48 hrs and the cultures were maintained on the same medium by regular subculturing.

To prepare the test plates, in both bacteria and fungi, 10-15 ml of the respective medium was poured into the petri dishes and used for screening. For assessing the antibacterial efficacy, a fresh

suspension of the test bacteria was prepared in saline solution from a freshly grown agar slant, while for fungicidal efficacy, a uniform spread of the test fungi was made using sterile swab.

Paper disc diffusion method

For both, antibacterial and antifungal assays, Disc diffusion method was adopted, because of re-productivity and precision. The different test organisms were preceded separately using a sterile swab over previously sterilized culture medium plates and the zone of inhibitions were measured around sterilized dried discs of Whatman No.1 paper (6 mm in diameter), which were containing 8, 6, 4 and 2 mg/disc of the test extracts and control streptomycin (for bacteria) and ketoconazole (for fungi) as reference drugs separately. Such treated discs were air-dried at room temperature, to remove any residual solvent which might interfere with the determination, sterilized and inoculated. Before incubation, these plates were placed at low temperature for 1 hr so as to allow maximum diffusion of the compound from the test discs into the agar plate and later, incubated at 37°C for 24 hrs in case of bacteria and 48 hrs of fungi, after which the zone of inhibition could be easily observed. Three replicates of each test extract were examined and the mean values were taken.

Result and discussion

Investigating the antimicrobial effect of *F. racemosa* roots involved a comparison with commercially available antibiotics showed a larger inhibitory effect than the *F. racemosa* root ethanolic extract. This is not surprising and reinforces the position that commercially perfected and tested antibiotics should be used in treatments whenever available.

It was found that the ethanolic extract exhibited good activity (Table I) against *E. coli* and *E. cloacae*, moderate activity against the *P. aeruginosa*, while exhibited trace activity against *B. subtilis* at all concentration.

Evaluation of the test extract for antifungal activity (Table II) indicated that the ethanolic root extract were moderately active against *P. chrysogenum*, *T. rubrum* and *C. albicans* at all concentration and found non active against *A. niger* at all concentration.

The demonstration of activity of the extract against bacteria and fungi is an indication of the broad spectrum of activity and thus can be used to source antibiotic substances for drug development that can be used in control of these bacterial and fungal infection¹⁷.

Table No. 1: Antibacterial activity of ethanolic root extract of *Ficus racemosa* Linn.

Dose mg/disc	Zone of inhibition(mm) mean \pm SD							
	<i>E. coli</i>		<i>B. subtilis</i>		<i>P. aeruginosa</i>		<i>E. cloacae</i>	
	<i>F. racemosa</i>	Streptomycin	<i>F. racemosa</i>	Streptomycin	<i>F. racemosa</i>	Streptomycin	<i>F. racemosa</i>	Streptomycin
8	24.4	30.2	7.2	26.0	9.1	31.8	16.1	22.2
6	22.8	28.8	6.8	24.8	8.7	29.2	14.7	19.8
4	21.5	27.3	-	22.4	8.1	27.17	13.5	17.12
2	20.7	25.2	-	21.6	7.1	26.6	12.6	16.6

The above mentioned readings are inclusive of disc diameter. Observation are expressed as mean \pm standard deviation, n=3.

Table No. 2: Anti fungal activity of ethanolic root extract of *Ficus racemosa* Linn.

Dose mg/disc	Zone of inhibition(mm) mean \pm SD							
	<i>P. chrysogenum</i>		<i>A. niger</i>		<i>T. rubrum</i>		<i>C. albicans</i>	
	<i>F. racemosa</i>	Ketoconazole	<i>F. racemosa</i>	Ketoconazole	<i>F. racemosa</i>	Ketoconazole	<i>F. racemosa</i>	Ketoconazole
8	15.4	28.9	8.2	25.1	16.5	30.1	14.2	24.9
6	13.9	27.1	7.1	23.9	14.2	28.3	12.1	23.2
4	11.6	25.2	-	22.2	12.3	26.1	10.5	21.0
2	10.8	24.4	-	20.8	11.2	25.3	9.6	19.7

The above mentioned readings are inclusive of disc diameter. Observation are expressed as mean \pm standard deviation, n=3.

The phytochemical screening results indicated the presence of carbohydrates, flavonoidal glycoside, steroids and tannins as the main constituents that might be responsible for antimicrobial activity of the test extract. Flavonoids are hydroxylated phenolic substances occur as C3-C6 unit linked to an aromatic ring. They are known to be synthesized by plant in response to microbial infection. Their activity is probably due to their ability to complex with bacterial cell walls. More lipophilic flavonoids may disrupt the microbial membrane¹. The flavonoids extracted from impatiens roots were believed to be responsible for the antimicrobial activity¹⁸.

Tannins have received a great deal of attention in recent years, since it was suggested that the consumption of tannin containing beverages, especially green teas and red wines, can cure and prevent a variety of ills. Their mode of antimicrobial action may be related to their ability to inactivate microbial adhesion, enzyme, cells envelop transport proteins etc. They also combine with polysaccharides¹.

The mode of action of antimicrobial effects of saponins seems to involve membranolytic

properties, rather than simply altering the surface tension of the extracellular medium.

Conclusion

As the rapid emergence of drug-resistant organism necessitates the continuous search of new antimicrobial substances, natural product may act as alternative for antibiotics and chemotherapeutic agent in certain circumstances. The results showed that the ethanolic extract of *F. racemosa* root was able to inhibit all of the bacteria and fungi used in this study with different degree of inhibition. The information obtained may provide validation for its reported medicinal uses. In conclusion, the ethanolic root extract of *F. racemosa* is more effective against the tested bacteria than the fungi.

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