Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Der Pharmacia Sinica, 2011, 2 (2): 270-275

Der Pharmacia Sinica

ISSN: 0976-8688
CODEN (USA): PSHIBD

Phytochemical investigation and *in vitro* antimicrobial activity of different parts of *Ficus racemosa* L

Abhishek Mathur*[1], Gautam K. Singh[1], Satish K. Verma[1], Sajad Yousuf[1], Aprajita Bhardwaj[1], Santosh K. Singh[2], GBKS Prasad[3] and V.K. Dua[4]

[1]*Sai Institute of Paramedical & Allied Sciences, Dehradun (U.K), India*
[2]*SBS (PG) Institute of Biomedical Sciences, Dehradun (U.K), India*
[3]*School of Studies in Biochemistry, Jiwaji University, Gwalior (M.P), India*
[4]*National Institute of Malaria Research, Hardwar (U.K), India*

ABSTRACT

In the present investigation different parts of Ficus racemosa L. were screened for their in vitro antimicrobial activity by well diffusion method and phytochemical screening by different conventional methods. The hydro-alcoholic extracts (50 % v/v) were prepared according to cold percolation method. The extracts were assayed against different pathogenic microorganisms. The hydro-alcoholic extracts of different parts of the tree showed maximum activity against bacterial strains in comparison to fungal strains. It was found that hydro-alcoholic extracts of leaves of the tree showed maximum potency against Lactococcus sp. at MIC value 0.5 mg/ml, but showed almost similar activity against E.coli and Aspergillus niger. The leaves extract showed no potency against Klebsiella pneumoniae, Streptococcus pyogenes and Saccharomyces cerevisae. The hydro-alcoholic extracts of the bark also showed potent activity against Lactococcus sp. at MIC value 0.3 mg/ml and showed minimum activity against Candida albicans. The extracts also showed no antimicrobial activity against E.coli, Klebsiella pnemoniae, Streptococcus pyogenes, Aspergillus niger and Saccharomyces cerevisae. The hydro-alcoholic extracts of the fruits of the plant showed maximum potency against E.coli at MIC value 0.6 mg/ml while moderate activity against Lactococcus sp. and minimum activity against Aspergillus niger. The extracts of the fruit showed no activity against Klebsiella pnemoniae, Streptococcus pyogenes, Candida albicans and Saccharomyces cerevisae. The extracts of different parts of the tree were further assayed for phytochemical screening and cellular toxicity. The extracts of different parts of the tree showed no cellular toxicity against sheep fresh blood erythrocytes.

Keywords: *Ficus racemosa* L., antimicrobial activity, phytochemical screening, pathogens.

INTRODUCTION

Ficus racemosa L. is a large deciduous tree distributed throughout India particularly in evergreen forests and moist localities [1]. Root, bark, leaves and fruits of the tree are used for medicinal

purposes [2]. Antibacterial activity of *Ficus racemosa L.* leaves on *Actinomyces viscous* were reported [3]. In developing countries and particularly in India low income people such as farmers, people of small isolated villages and native communities use folk medicine for the treatment of common infections [4]. These plants are ingested as decoctions, teas and juice preparations to treat respiratory infection. They are also made into a poultice and applied directly on the infected wounds or burns. When people from these remote communities get an infectious disease, they are usually treated by traditional healers and shamans because of their expertise in such procedures as making diagnoses, treating wounds, setting bones and making herbal medicines. Traditional healers claim that their medicine is cheaper and more effective than modem medicine. Patients of these communities have a reduced risk to get infectious diseases from resistant pathogens than people from urban areas treated with traditional antibiotics. One way to prevent antibiotic resistance of pathogenic species is by using new compounds that are not based on existing synthetic antimicrobial agents. Traditional healers claim that some medicinal plants are more efficient to treat infectious diseases than synthetic antibiotics. It is necessary to evaluate, in a scientific base, the potential use of folk medicine for the treatment of infectious diseases produced by common pathogens. Medicinal plants might represent an alternative treatment in non-severe cases of infectious diseases [5]. They can also be a possible source for new potent antibiotics to which pathogen strains are not resistant. Latest and previous studies have concluded the beneficial aspects of plant derived drugs as good source of antibiotics, antioxidants and anti-inflammatory agents [6- 12].

MATERIALS AND METHODS

Experimental Section

All the chemicals and reagents used were from C.D.H and Ranchem. Glass wares used were from Borosil. The media and broth used for microbial culture were from Hi-Media Pvt. Limited, Bombay, India.

Plant material

The authenticated sample was collected from Forest Research Institute, Dehradun (U.K), India and was further confirmed by the taxonomist.

Preparation of plant extracts

The method [13] was adopted for preparation of plant extracts with little modifications. Briefly three 20 g portions of each of the powdered plant material of the different parts of the tree were soaked separately in 100 ml of hydro-alcohol (50 % v/v) for 72 h. Each mixture was stirred after every 24 h using a sterile glass rod. At the end of extraction, each extract was passed through Whatmann filter paper no1 (Whatmann, England). The filtrate obtained were concentrated in vacuo using water bath at 30^{o}C.

Determination of Antibacterial and Antifungal activity

Culture Media

The media used for antibacterial test was Nutrient agar/broth and Sabouraud's dextrose agar/broth of Hi media Pvt. Bombay, India.

Inoculum

The bacteria were inoculated into soyabean casein digest agar/broth and incubated at 37^{0} C for 4 h and the suspension were checked to provide approximately 10^{5} CFU/ml. Similar procedure is done for fungal strains by inoculating in Sabouraud's dextrose broth for 6 h.

Microorganisms used

The bacterial test organisms (*E.coli, Klebsiella pneumoniae*, *Lactococcus sp.*, and *Streptococcus pyogenes*) and the fungal test organisms used for study were *Candida albicans*, *Aspergillus niger* and *Saccharomyces cerevisae*, procured from pure lab cultures of National Chemical Laboratory (NCL), Pune, India.

Determination of antimicrobial activity

The agar well diffusion method [14] was modified. Nutrient agar medium (NAM) was used for bacterial cultures. The culture medium was inoculated with the microorganism separately suspended in nutrient broth. Sabouraud's dextrose agar (SDA) was used for fungal cultures. The culture medium was inoculated with the fungal strains separately suspended in Sabouraud's dextrose broth. A total of 8 mm diameter wells were punched into the agar and filled with plant extracts (1mg/ml) and solvent blank (hydro-alcohol as the case may be). Standard antibiotic (Chloramphenicol, concentration 1mg/ml) was simultaneously used as positive control. The bacterial plates were then incubated at 37^{o}C for 18 h. The antibacterial activity was evaluated by measuring the diameter of zone of inhibition observed. The same procedure was done for determining antifungal activity but in this case standard antibiotic (Fucanazole, concentration 1 mg/ml) was used as positive control and fungal plates were incubated at 37^{o}C for 72 h. Here also the diameter of zone of inhibition observed was measured.

Determination of MIC and MBC

The broth dilution method was adopted for determination of MIC and MLC values against the pathogens. The plant extracts (1 mg/ml) were serially diluted in different aliquots and the final volumes of the aliquots were made up to 1 ml with N-saline (0.85 % NaCl). Equal amount of the specific pathogen were added in different aliquots and the test tubes were kept for 48 h at 30^{0} C. The minimum dilution of the plant extract that kills the bacterial and fungal growth was taken as MLC (Minimum lethal count) while the minimum dilution of plant extract that inhibits the growth of the organism was taken as MIC.

Determination of cellular toxicity using sheep erythrocytes

The method [15] was employed to study cellular toxicity. Briefly 10 fold serial dilution of the extract were made in phosphate buffered saline. A total volume of 0.8ml for each dilution was placed in an ependroff tube. A negative control tube (containing saline only) and a positive control tube (containing tap water) were also included in the analysis. Fresh sheep erythrocytes were added to each tube, to give a final volume of 1 ml. Solutions were incubated at 37^{o}C for 30 minutes and all tubes were centrifuged for 5 minutes and then observed for hemolysis.

Phytochemical screening of the different parts of the tree

Different conventional methods were followed to determine qualitatively the presence of phytochemical constituents present in the extract [16, 17].

RESULTS AND DISCUSSION

Determination of antimicrobial activity

The antimicrobial activity of the hydro-alcoholic extracts of leaves, bark and fruits of *Ficus racemosa* L. were investigated by well diffusion method. The extracts of each of the part(s) of the tree were found to be most potent antibacterial agent in comparison to fungal strains. All the parts of the tree used showed antimicrobial activity against bacterial and fungal strains but in varying proportion. The leaf extracts were found to be most potent against *Lactococcus sp*. (diameter of zone of inhibition: 18 mm; MIC value: 0.5 mg/ml) but showed moderate activity

against *E.coli* (diameter of zone of inhibition: 10 mm; MIC value: 0.6 mg/ml). The leaf extracts showed no antibacterial activity against *Klebsiella pneumoniae* and *Streptococcus pyogenes*. The leaf extracts showed potent activity against *Aspergillus niger* (diameter of zone of inhibition: 10 mm; MIC value: 0.7 mg/ml) and showed moderate activity against *Candida albicans* (diameter of zone of inhibition: 08 mm; MIC value: 0.7 mg/ml). The leaf extracts showed no antifungal activity against *Saccharomyces cerevisae*. Bark extracts showed maximum antimicrobial activity against *Lactococcus sp*. (diameter of zone of inhibition: 15 mm; MIC value: 0.3 mg/ml) but showed no activity against *E.coli*, *Klebsiella pneumoniae* and *Streptococcus pyogenes*. The extracts of the bark were found to be potent only against *Candida albicans* (diameter of zone of inhibition: 08 mm; MIC value: 0.4 mg/ml) but showed no activity against *Aspergillus niger* and *Saccharomyces cerevisae*. The fruit extracts of the tree showed potent activity against *E.coli* (diameter of zone of inhibition: 18 mm; MIC value: 0.6 mg/ml) in comparison to *Lactococcus sp*. (diameter of zone of inhibition: 10 mm; MIC value: 0.7 mg/ml). The fruit extracts showed no antibacterial activity against *Klebsiella pneumoniae* and *Streptococcus pyogenes*. The fruit extracts showed no activity against *Candida albicans* and *Saccharomyces cerevisae* amongst fungal strains. The results are depicted in **Table 1 (a, b)** and **Figure 1**.

Table 1 (a): Minimum Inhibitory Concentration (MIC) of different part(s) of *Ficus racemosa* L.

Pathogens	MIC (mg/ml)			MLC (mg/ml)		
	Leaves	Bark	Fruit	Leaves	Bark	Fruit
E.coli	0.6	NA	0.6	0.7	NA	0.8
Klebsiella pneumoniae	NA	NA	NA	NA	NA	NA
Streptococcus pyogenes	NA	NA	NA	NA	NA	NA
Lactococcus sp.	0.5	0.3	0.7	0.7	0.5	0.8
Candida albicans	0.7	NA	NA	0.8	NA	NA
Aspergillus niger	0.7	NA	0.3	0.8	NA	0.4
Saccharomyces cerevisae	NA	NA	NA	NA	NA	NA

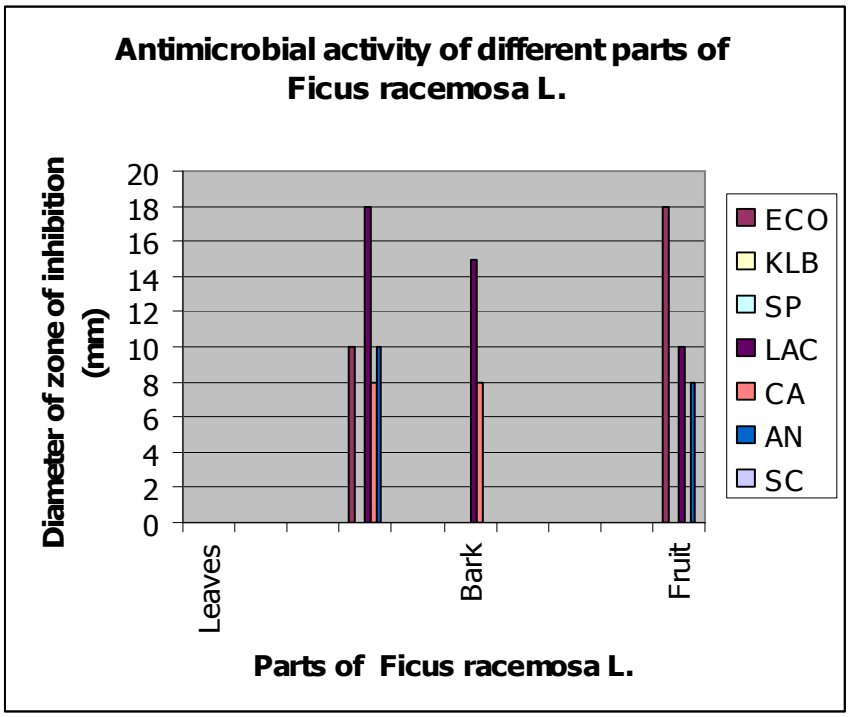

Figure 1: Antimicrobial activity of different part(s) of *Ficus racemosa* L.

Table 1 (b): Antimicrobial activity of different part(s) of *Ficus racemosa* L.

Plant/ Positive control	Part(s) Used	Solvent Extract	Diameter of zone of inhibition (mm)						
			ECO	KLB	SP	LAC	CA	AN	SC
Ficus racemosa L.	Leaves	Hydro-alcoholic	10.0	NA	NA	18.0	8.0	10.0	NA
	Bark	Hydro-alcoholic	NA	NA	NA	15.0	8.0	NA	NA
	Fruit	Hydro-alcoholic	18.0	NA	NA	10.0	NA	8.0	NA
Chloramphenicol (1mg/ml)			25.0	26.0	20.0	25.0	NT	NT	NT
Fucanazole (1mg/ml)			NT	NT	NT	NT	28.0	26.0	25.0

ECO-E.coli; KLB-Klebsiella pneumoniae; SP-Streptococcus pyogenes; LAC-Lactococcus sp.; CA-Candida albicans; AN-Aspergillus niger; SC- Saccharomyces cerevisae; NA-No activity; NT- Not tested.

From the present investigation it is observed that *Lactococcus sp.* is the most sensitive organism against the extracts of different parts of the tree. *Klebsiella pneumoniae*, *Streptococcus pyogenes* and *Saccharomyces cerevisae* were found to be the most resistant organisms which have no effect against any of the extract.

Phytochemical screening

Various conventional methods were followed to determine qualitatively the presence of alkaloids, flavanoids, steroids, saponins, glycosides, tannins, anthraquinones and sugars in the hydro-alcoholic extracts of leaves, bark and fruit of *Ficus racemosa* L. The results are recorded in **Table 2**.

Table 2: Phytochemical constituents of different part(s) of *Ficus racemosa* L.

Part(s)	Alkaloids	Flavanoids	Steroids	Saponins	Glycosides	Tannins	Anthraquinones	Sugars
Leaf	NP	NP	P	NP	NP	P	NP	NP
Bark	P	P	P	NP	P	P	P	NP
Fruit	NP	NP	P	P	NP	NP	NP	NP

NP, Not present; P, Present

The results of phytochemical screening confirmed the presence of phytochemical constituents in different parts of the tree. Further studies are however needed to isolate and characterize the active compound responsible for antimicrobial activity in each of the part of the tree.

CONCLUSION

The hydro-alcoholic extracts of the plant part(s) used showed prominent antimicrobial activity. The use of these parts in the treatment of pathogenic diseases associated with the infection of these pathogens is validated, scientifically supported by the results obtained in this work. The studies may lead to the formulation of antibiotic which may be used against the treatment of diseases associated with these microbial infections.

Acknowledgement

Sincere thanks for the cooperation to Dr. V.D. Sharma, Professor and Dean Life Sciences and Dr. Sandhya Dogra, Principal & HOD, Dept. of Microbiology, Sai Institute of Paramedical & Allied Sciences, Dehradun (U.K), India. The authors felt their heartiest thanks to Mr. Harish Arora and Mrs. Rani Arora, Chairman and Vice Chairperson, Sai Institute of Paramedical & Allied Sciences, Dehradun (U.K), India for supporting us to carry out the investigation. Sincere thanks to Mr. S.N. Uniyal, Mr. Akhilesh and Research Staff of Sai Institute for enabling the research successful.

REFERENCES

[1] The Ayurvedic Pharmacopoeia of India, Government of India, Ministry of Health & Family Welfare, Department of Indian System of Medicine and Homeopathy, New Delhi, **1990**, 1st edition, Part I, Vol. I, 117-118.
[2] A.K. Nadkarni, Indian Materia Medica, **2000**, 3rd edition, 548-550.
[3] T. Shaikh, R. Rub, K. Bhise, R.B. Pimprikar, A. Sufiyan, *Journal of Pharmaceutical Sciences and Research*, **2010**, 2, 1, 41-44.
[4] D.S. Fabricant, N.R. Farnsworth, Environmental Health Perspectives Supplements, **2001**, 109, 69-75.
[5] J. Gonzalez, *J Ethnopharmacology*, **1980**, 2, 43-47.
[6] A. Mathur, G.B.K.S. Prasad, V.K. Dua, *Flora and Fauna*, **2010**, 16, 281-285.
[7] A. Mathur, V.K. Dua, G.B.K.S Prasad, *Int. Journal Chem. Env. Pharm. Res*., **2010**, 1, 1, 12-16.
[8] A. Mathur, S.K. Verma, S.K. Singh, G.B.K.S Prasad, V.K. Dua, *J. Pharmacogonasy and Herbal Form.*, **2010**, 1,1, 1-7.
[9] A. Mathur, G.B.K.S. Prasad, V.K. Dua, *Int. J. Pharm. Biosciences*., **2011**, 2,1, 541-544.
[10] A. Mathur, S. Yousuf, S.K. Verma, S.K. Singh, G.B.K.S. Prasad, V.K. Dua, *Int. J. Pharm. Biosciences*., **2011**, 2, 1, 545-548.
[11] A. Mathur, R. Purohit, D. Mathur, G.B.K.S. Prasad, V.K. Dua, *Der Chemica Sinica*, **2011**, 2, 1, 174-181.
[12] A. Mathur, R. Purohit, D. Mathur, G.B.K.S. Prasad, V.K. Dua, *Der Pharmacia Sinica*, **2011**, 2, 1, 208-216.
[13] P.I. Alade, O.N. Irobi, *J. Ethnopharmacol*., **1993**, 39, 171-174.
[14] C. Perez, C. Anesini, *J. Ethnopharmacol*., **1993**, 44, 41-46.
[15] He Xian-guo, M. Ursula, *J. Ethnopharmacol.*, **1994**, 43, 173-177.
[16] G.E. Trease, W.C. Evans, Pharmacogonasy, **1989**, Brown Publication, 14th Edition,
[17] J.B. Harborne, Phytochemical method, **1993**, Chapman and Hall, London, 3rd Edition, 135-203.