

International Journal of Pharma and Bio Sciences

PHYTOPHARMACOLOGICAL AND PHYTOCHEMICAL PROPERTIES OF THREE FICUS SPECIES - AN OVERVIEW

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ABSTRACT

Ficus plants are found throughout the world as moderate woody plants or trees. It has a vast traditional role in indigenous system of medicine like ayurveda, siddha, unani and homoeopathy. *Ficus* species, namely *F. racemosa*, *F. religiosa* and *F. benghalensis*, are important ingredients in many Ayurvedic and traditional formulations. The barks, leaves, fruits and latex are considered to be very effective in various treatments, such as diabetes, skin diseases, ulcers, dysentery, diarrhoea, stomachache, piles and as carminative, astringent, anti-inflammatory, antioxidant and anticancer agent. The present review is therefore, an effort to give a detailed survey of the literature on its phytochemical and pharmacological properties.

KEY WORDS

pharmacological, laxative, antioxidant, anticancer, antidiuretic, tannin.

INTRODUCTION

The genus *Ficus* (Moraceae) includes some 750 species of woody plants occurring in most tropical and subtropical forests throughout the world¹. The genus is remarkable for the large variation in the habits of its species². *Ficus* is one of the most loved bonsai. It is an excellent tree for beginners, as most species of *Ficus* are fast growers, tolerant of most any soil and light conditions. Most *Ficus* grow "banyan" roots naturally; this feature is often showcased by styling *Ficus* in dramatic air-root and root-over-rock styles. All plants containing active compounds are important. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. In plants, these

compounds are mostly secondary metabolites such as alkaloids, steroids, tannins, and phenol compounds, which are synthesized and deposited in specific parts or in all parts of the plant. Many *Ficus* species are commonly used in traditional medicine to cure various diseases. They have long been used in folk medicine as astringents, carminatives, stomachics, vermicides, hypotensives, antihelmintics and anti-dysentery drugs³. Many species are cultivated for shade and ornament in gardens. Several species produce edible figs of varying palatability. Some species are producing latex. The fig is a very nourishing food and used in industrial products. Figs contained water, fats, high amounts of amino acids, such as leucine, lysine, valine, and arginine, and minerals, such as potassium,

calcium, magnesium, sodium, phosphorus and vitamins ., some of the the most important species of *Ficus* are, *F.racemosa* , *F. religiosa* and *F. bengalensis*,. It is propagated by seeds.. *Ficus bengalensis* is commonly known as a Banyan tree. This tree is considered to be sacred tree in India. The bark ,leaves and fruits of this group are used as astringent, haemostatic, anti-septic, antiinflammatory,

antioxidant and anticancer agent and also in the treatment of diarrhoea, dysentery, and in the treatment of skin diseases, ulcers, vaginal disorders, leucorrhoea, menorrhagia, deficient lactation⁴.In the present study we focus on some of the phytochemical and pharmacological properties of these medicinal plants.



Ficus racemosa



Ficus religiosa



Ficus bengalensis

Taxonomy:

Kingdom : Plantae
Division : Magnoliophyta
Class : Magnolipsida
Order : Urticales
Family : Moraceae
Genus : Ficus

Habit and habitat:

Ficus racemosa grows all over India in many forests and hilly areas . It is frequently available around the water streams and is also cultivated. The tree is medium, growing 10-16 meters in height. Providing green foliage as good shade. The bark is grey and often cracked.

Ficus religiosa are ever green trees up to 30 m. Bark of trunks and older branches brown, smooth, Branchlets glabrous . Leaves: ovate , to 5 cm; petiole slender,. Leaf blade broadly ovate to ovate-orbiculate, 7-25 × 4-16 cm, thinly leathery, base rounded to truncate , margins entire,

occasionally wavy, apex abruptly long-caudate or long-acuminate, tip to 2.5-9 cm; surfaces occasionally glaucous, glabrous; basal veins 2 pairs; lateral veins 6-9 pairs, the main veins finely reticulate . Syconia paired , sessile, dark purple, nearly globose , 1-1.5 × 1-1.5 cm, glabrous; subtending bracts ovate, 3-5 mm, silky-puberulous; ostiole closed by 3 bracts 2-3 mm wide, umbonate .

Ficus bengalensis are fast growing, evergreen tree found in monsoon and rain forests,grow up to 3.0 meters, with spreading branches and many aerial roots. Leaves stalked, ovate-

cordate, 3-nerved, entire, when young downy on both sides; petiole with a broad smooth greasy gland at the apex, compressed, downy; Fruit in axillary pairs, the size of a cherry, round and downy. Hardy, drought resistance and withstands mild frost.

Traditional uses:

Ficus racemosa is a one of the herbs mentioned in all ancient scriptures of Ayurveda. Udumbara is considered sacred to God Dattaguru. It is otherwise called Udumbara. It is one of the plants from a group, called pancavalkala, meaning the thick bark skins of five herbs, viz. udumbara, vata, asvattha, parisa and plaksa. The decoction of pancavalkala is used internally or for giving enema in bleeding per rectum and vagina (Raja Nighantu). Maharishi Charka has categorized udumbara as mutra sangrahaniya anti-udumbara as mutra sangrahaniya, anti-diuretic herb. Susruta has described the properties of the plant, like astringent, promotes callus healing in fractures (bhagna sandhaniya), alleviates Rakta pitta, burning sensation and obesity, and useful in vaginal disorders. Charaka prescribed tender leaves of udumbara as astringent and styptic in diarrhoea and haemorrhages also the tender leaves and leaf buds of udumbara treating vaginal laxicity, charaka prescribed a vaginal pessary, prepared by the sesasum oil, sushruta prescribed the juice of pounded fruit in intrinsic haemorrhage; the decoction of the fruit, mixed with powdered shaali (*oryza sativa*) rice, with sugar and honey for checking miscarriage; ash of the bark mixed with honey in high cough. The tender fruits of udumbara, steamed and mixed with curd, were prescribed for dysentery. Externally the latex of udumbara was applied on boils due to diabetes. The cold infusion of ripened fruits mixed with sugar, is salutary in Rakta pitta is effectively controlled with the decoction of bark-skin. In diabetes, the ripe fruits or bark-skin decoction is beneficial, as it works well as anti-diuretic. The decoction of leaves is an effective remedy in glandular swelling, abscess, chronic wounds, cervical adenitis etc. The latex mixed with sugar is benevolent in sexual debility in males. According to Ayurveda, roots are useful in hydrophobia

whereas bark is acrid, cooling, galactagogue and good for gynaecological disorders. According to Unani system of medicine, leaves are astringent to bowels and good in case of bronchitis whereas fruits are useful in treatment of dry cough, loss of voice, diseases of kidney and spleen. Bark is useful in Asthma and piles. Latex is applied externally on chronic infected wounds to alleviate pain and to promote the healing. The tender leaf buds are applied on the skin, in the form of paste, to improve the complexion^{5,6}.

The roots, bark-skin, fruits, and leaves of *ficus religiosa* have great medicinal value. The bark is cooling and astringent and is useful in inflammations and glandular swellings of neck. Root bark is good for stomatitis, clean ulcers and it is astringent in leucorrhoea and promotes granulations. According to Unani system of medicine, root, bark is aphrodisiac and also good for lumbago. Roots are said to be good for gout. The roots are chewed to prevent gum disease. The fruit is laxative, promotes digestion, aphrodisiac and checks vomiting. Ripe fruits are alexipharmic (an antidote or defensive remedy against poison, venom or infection), are good for foul taste, thirst and heart disease. The powdered fruit is taken for asthma. The seeds are cooling, laxative and refrigerant. Seeds are useful in urinary troubles. The leaves alone are used to treat constipation. The leaves and young shoots together are purgative (strong laxative). An infusion or decoction of the bark is used with some honey for the treatment of gonorrhoea, ulcers, skin diseases and scabies. Its power bark has been used to heal the wounds for years. Charaka and Sushruta prescribed a decoction of the bark of Ashvatha in haemorrhages; leaves for covering wounds; the paste of tender roots or the bark for skin infections. Sushruta administered a decoction in urinary disorders and vaginal discharges. Milk cooked with the fruit, leaf bud, bark and the root added with sugar and honey, was prescribed as an aphrodisiac. Powder of the dried bark was dusted over burns. A paste of the bark and leaves was prescribed in

stomatitis. Ash-water of the dried bark was given for checking vomiting. decoction of the root bark with salt and jaggery induce anti diuretic property. *Ficus benghalensis* is commonly called nyagrodha. Charaka prescribed aqueous extract of leafbuds of nyagrodha, udumbara and ashvattha mixed with sugar and honey for checking diarrhoea; milk processed with the aerial roots or leaf buds of nyagrodha, in haemorrhages and bleeding piles; the paste of codhra with the decoction of nyagrodha bark for leucorrhoea and other vaginal discharges. Leaf bud of Nyagrodha was prescribed for promoting conception. It is also used as a blood purifier in skin diseases; urinary and urinogenital disorders. *Nalpamaram* is an important group of ayurvedic formulation that constitutes the barks of *Ficus racemosa* Linn, *Ficus religiosa* Linn and *Ficus benghalensis* Linn., widely used in the treatment of skin diseases with *pitta* and *rakta* predominance and also used in various ailments^{7,8}.

Phytochemical properties:

Ficus racemosa contains tannin, wax, saponin gluanol acetate, β -sitosterol, leucocyanidin-3-O- β -D-glucopyranoside, leucopelargonidin-3-O- β -D-glucopyranoside, leucopelargonidin-3-O- α -L-rhamnopyranoside, lupeol, ceryl behenate, lupeol acetate, α -amyrin acetate, leucoanthocyanidin, and leucoanthocyanin, from trunk bark, lupeol, β -sitosterol and stigmasterol were isolated⁹. Fruit contains gluanol, hentriacontane, β sitosterol, gluanolacetate, glucose, tiglic acid, esters of taraxasterol, lupeolacetate, friedelin, higher hydrocarbons and other phytosterol¹⁰. Racemosic acid were isolated from the leaves. The stem bark and fruit showed the presence of gluanol acetate¹¹.

The barks of *Ficus religiosa* species contains tannin, saponin gluanol acetate, β -sitosterol, leucopelargonidin-3-O- β -D-glucopyranoside, leucopelargonidin-3-O- α -L-rhamnopyranoside, lupeol, ceryl behenate, lupeol acetate, α -amyrin acetate, leucoanthocyanidin, and leucoanthocyanin⁹.

The bark of the *Ficus benghalensis* contains leucopelargonidin-3-O- α -L-rhamnoside and leucocyanidin-3-O- α -D-galactosyl cellobioside, glucoside, β glucoside, 20-tetraacanthene-2-one, 6-heptatriacontene-10-one, pentatriacontan-5-one, β sitosterol- α -D-glucose, and meso-inositol^{12,13}. 20-tetraacanthene-2-one, 6-heptatriacontene-10-one, pentatriacontan-5-one,¹⁴⁻¹⁹. Leaves contain, rutin, friedelin, taraxasterol, lupeol, β -amyrin along with psoralen, bergapten and β -sitosterol, quercetin-3-galactoside²⁰. Leucodelphinidin derivative²¹, bengalenoside, Aglucoside²², Leucopelargonin and leucocyanidin derivative^{23,24,25}.

Pharmacological properties:

***Ficus racemosa*:**

Anti cancer activity :

Ficus racemosa extract at a dose of 200 and 400 mg/kg when given orally a significant decrease in lipid peroxidation, xanthine oxidase, γ -glutamyl transpeptidase and hydrogen peroxide (H₂O₂) generation with reduction in renal glutathione content and antioxidant enzymes generated by Potassium bromate (KBrO₃), a nephrotoxic agent that induces renal carcinoma in rats. There was significant recovery of renal glutathione content and antioxidant enzymes.²⁶. This results suggest that *Ficus racemosa* extract is a potent chemopreventive agent and suppresses KBrO₃-mediated nephrotoxicity in rats.

Antioxidant activity:

Ethanol extract and water extract of *Ficus racemosa* were subjected to free radical scavenging both by steady state and time resolved methods such as nanosecond pulse radiolysis and stopped-flow spectrophotometric analyses. Ethanol extract exhibited significantly higher steady state antioxidant activity than water extract. Maximum radioprotection was observed at 20 μ g/ml of ethanol extract. The cytokinesis-block proliferative index indicated that ethanol extract does not alter radiation induced cell cycle delay. Based on these

results it is evident that the ethanol extract of *F. racemosa* acts as a potent antioxidant²⁷.

Hepato protective activity:

Methanol extract of *Ficus racemosa* stem bark were studied using the model of hepatotoxicity induced by carbon tetrachloride (CCl₄) in rats. Pretreatment with methanol extract resulted in significant decreases in the activities of AST, ALT and ALP, compared to CCl₄-treated rats. The results indicate that *F. racemosa* possesses potent hepatoprotective effects against CCl₄-induced hepatic damage in rats²⁸.

Hypoglycemic activity:

The ethanol extract (250mg/kg) lowered blood glucose level within 2 weeks in the alloxan diabetic albino rats confirming its hypoglycemic activity²⁹.

***Ficus religiosa*:**

Anticancer activity:

Fruit extracts exhibited antitumor activity in the potato disc bioassay. None of the tested extracts showed any marked inhibition on the uptake of calcium into rat pituitary cells GH₄C₁³⁰.

Antioxidant and antidiabetic activity:

Aqueous extract of *F. religiosa* at a dose of 100mg and 200mg/kg given orally decreased the fasting blood glucose, and also decreased the exaggerated activity of superoxide dismutase SOD in streptozotocin induced type II diabetic rats. *F. religiosa* modulated the enzymes of antioxidant defense system to combat oxidative stress. As a result glutathione was restored and inhibited the formation of malondialdehyde, proving its anti-diabetic activity along with antioxidant potential³¹.

Antihelmintic activity:

Methanol extracts of some commonly used plant materials of ethnoveterinary importance were screened for their *in vitro* anthelmintic activity. Results revealed that *Ficus religiosa* was 100% effective by 4 h post exposure, and was as good as *A. sativum* and *Z. officinale* by 6 h post

exposure. It was concluded that all the studied plants had some anthelmintic activity³².

Antimicrobial activity:

Aqueous extract of *F. religiosa* showed high antimicrobial activity against selected pathogenic organisms. High activity was found on *B. subtilis* with about 24mm inhibition zone. And also the growth of *P. aeruginosa* (multi drug resistant) was remarkably inhibited by the plant extract³³.

***Ficus bengalensis*:**

Antioxidant and hypolipidaemic activity:

Three groups of rabbits were fed with cholesterol suspended in ground nut oil to make hyper cholesterol condition (100mg/kg/day). In addition to this one group is fed with bark extract of *Ficus bengalensis* at a dose of 50 mg/kg /day. Feeding cholesterol increased serum cholesterol, triacylglycerol significantly. Treatment with bark extract decreased the serum cholesterol level by 59%, triacylglycerol by 54% and a decrease in lipid peroxidation. Further, there was significant increase in the activities of antioxidant enzymes; superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase; which were depressed in other groups after cholesterol feeding. This results shows that the water extract of the bark of *Ficus bengalensis* has significant antioxidant effect, in addition to hypolipidaemic effect³⁴.

Anticancer and anti bacterial activity:

Fruit extracts exhibited antitumor activity in the potato disc bioassay. None of the tested extracts showed any marked inhibition on the uptake of calcium into rat pituitary cells GH₄C₁. The extracts of the four tested *Ficus* species had significant antibacterial activity, but no antifungal activity. The results of this preliminary investigation support the traditional use of these plants in folk medicine³⁰.

Antihelmintic activity:

Methanolic, aqueous, chloroform, petroleum ether extracts of the roots were studied for

paralysis and death of earthworm. All the extracts were found not only to paralyze but also to kill the earthworms. The aqueous and methanolic extract was found to be more effective to execute the earthworm when compared to standard anti helminthic drugs³⁵.

Anti inflammatory and Analgesic activity :

Treatment with methanol extract during inflammatory condition both acute (carrageenan induced hind paw edema and acetic acid induced vascular permeability) and subchronic (cotton pellet induced granuloma) prevented increase in malondialdehyde formation and myeloperoxidase activity in edematous as well as granulomatous tissue. Further serum marker enzymes (AST,ALT and ALP)increased in inflammatory conditions were also inhibited with methanol extract treatment .In addition the extract also

showed significant analgesic activity in acetic acid induced writhing³⁶.

CONCLUSION

From these findings it is evident that the ethanolic extract of ficus species showed a greater effect against microbes, worms and renal carcinoma in rat compared with the standard drugs. Fruit extracts from these three species exhibited antitumor activity in the potato disc bioassay. Methanolic, aqueous, chloroform, petroleum ether extracts of the roots were studied for paralysis and death of earthworm. The present study shows the pharmacological and phytochemical properties of various bioactive compounds present in the ficus species. Further investigations should be conducted to isolate and characterize the active components of these ficus species.

REFERENCES

1. Berg CC , Classification and distribution of *Ficus*,. *Experientia* :45: 605-611. (1989).
2. Jander EA, Machado KC, CA evolutionary Ecology of figs and their associates: Recent progress and outstanding puzzles. *Ann Rev Evol.Syst.*:39:439-458(2008).
3. Trivedi, P., S. Hinde and R. C. Sharma.. Preliminary phytochemical and pharmacological studies on *Ficus racemosa*. *Journal Medicinal Research*: 56: 1070-1074,(1969).
4. C.P. Khare ,*Encyclopedia of Indian Medicinal Plants* Springer publication; pp216-217 (2004).
5. Warriar PK, *Indian medicinal plants ,A compendium of 500 species* by,Orient long man Ltd, Chennai, Vol : III , pp 34-35,(1996).
6. Chopra RN, Chopra IC and Varma BS, *Supplement to Glossary of Indian Medicinal plants*, reprinted edition,CSIR, NewDelhi, , pp.29,(1992).
7. Sivarajan VV & Balachandran I. *Ayurvedic Drugs and Their Sources*, Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi. (1994).
8. Joy PP, Thomas J, Mathew S & Skaria BP. *Medicinal Plants In: Bose TK, Kabir J, Das P & Joy PP (ed.) Tropical Horticulture - Vol. 2.* pp. 449-632. (2001).
9. Husain A, Virmani OP, Popli SP, Misra LN, Gupta MM, Srivastava GN, Abraham Z & Singh AK. *Dictionary of Indian Medicinal Plants*, CIMAP,Lucknow, India. 546.(1992).
10. Suresh C, Jawakhar L and Sabir M, *Chemical examination of the fruits of Ficus Glomerata* , *J Indian Chem Soc*, 56 (12, 1269-1270,(1979).
11. Deva raj KB, Gowda LR and Prakash V ,*An unusual thermostable aspartic protease from the latex of Ficus racemosa*, *phytochemistry* ,69(3), 647-655. (2008).
12. *The Wealth of India, Volume-(F-G)*. In: *A Dictionary of Indian Raw Materials and industrial products*. New Delhi: Council of Scientific and Industrial Research,4:24-6. (1999).

13. Subramanian PM, Misra GS. Chemical constituents of *Ficus bengalensis*. Pol J Pharmacol Pharm; (30):559-62.(1978).
14. Subramanian PM, Misra GS. Chemical constituents of *Ficus bengalensis* Indian J chemistry 15B; 762(1977).
15. Sheeja Cherian, Kumar RV, Augusti KT, Kidwai JR, Indian J Biochem biophys Antidiabetic effect of glycoside of Pelargonidin isolated from the bark of *Ficus bengalensis* linn:29:380- 382(1992).
16. Kumar R.V., Augusti K.T. Antidiabetic effect of a leucocynidin derivative isolated from the bark of *Ficus bengalensis* Linn. Indian J Biochem Biophys.: 26 (6):400-4, (1989).
17. Subramanian PM, Misra GS. Chemical constituents of *Ficus bengalensis* (Part II) Pol J Pharmacol Pharm: 30(4):559-62, (1978).
18. The Wealth of India, Volume-(F-G). In: A Dictionary of Indian Raw Materials and industrial products. Vol. 4. New Delhi: Council of Scientific and Industrial Research: p. 24- 6(1999).
19. Daniel RS, Devi KS, Augusti KT Mechanism of action of Antiatherogenic and related effects of *Ficus bengalensis* Linn. Flavonoids in experimental animals Indian Journal of Experimental Biology: 41,296-303, (2003).
20. A. Chatterjee The treaties of Indian medicinal plants: Vol.I pp. 39,(1997).
21. Geetha BS, Mathew BC, Augusti KT, Hypoglycemic effects of Leucodelphinidin derivative isolated from *Ficus bengalensis* Indian J Physiol. Pharmacol.: 38(3):220,(1994).
22. Augusti KT Hypoglycemic action of bengalenoside: Aglucoside isolated from *Ficus Bengalensis* Linn, in normal and Alloxan diabetic rabbits. Indian J Physiol Pharmacol: 19:218-20, (1975).
23. Augusti K T, Daniel RS, Cherian S, Sheela CG, Nair CR Effect of Leucopelargonin derivative from *Ficus bengalensis* Linn on diabetic dogs Indian J Med Res:82-86(1994).
24. Cherian S, Sheela and Augusti K T Insulin sparing action of Leucopelargonin derivative isolated from *Ficus bengalensis* Linn Indian Journal of Experimental Biology;33:608-611, (1995).
25. Kumar RV, Augusti KT, Insulin sparing action of a leucocynidin derivative isolated from *Ficus bengalensis* Linn Indian Journal of Biochemistry and Biophysics: 31:73-76,(1994).
26. Naghma Khan and Sarwat Sultana Modulatory Effect of *Ficus racemosa*: Diminution of potassium Bromate-Induced Renal Oxidative Injury and Cell Proliferation Response Basic Clin Pharmacol Toxicol ,97(5) , 282 – 288.(2005).
27. V.P. Veerapur, K. R. Prabhakar, Vipan kumar Parihar, Machendar Reddy Kandadi, S.Ramakrishana, B. Mishra, B. S. Satish Rao, K. K. Srinivasan, K. I. Priyadarsini and M. K. Unnikrishnan .*Ficus racemosa* Stem Bark Extract: A Potent Antioxidant and a Probable Natural Radioprotector eCAM ,6(3), 317–324.(2009).
28. Faiyaz Ahmed javascript:popRef('AF0001'), Asna Urooj javascript:popRef('AF0001') ,Hepatoprotective effects of *Ficus racemosa* stem bark against Carbon tetrachloride-induced hepatic damage in albino rats. Pharmaceutical Biology, 48(2), 210-216(2010).
29. Kar,A, Choudhary BK and Bandyopadhyay Ng, comparative evaluation of hypoglycemic activity of some Indian medicinal plants in alloxan diabetic rats, J Ethno pharmacol, 84(1), 105-108(2003).
30. Mousa O, Vuorela P, Kiviranta J, Wahab SA, Hiltohen R, Vuorela H Bioactivity of certain Egyptian *Ficus* species J Ethnopharmacol:41:71- 6,(1994).
31. H.Kirana ,SS Agarwal, B P Srinivasan, Aqueous extract of *Ficus religiosa* Linn reduce Oxidative stress in experimentally induced type 2 diabetic rats. Indian

- Journal of Experimental biology 47: pp 822-826 ,(2009).
32. Zafar Iqbal, Qazi khalid Nadeem, M.N. Khan, M.S Akhtar and Faisal Nnouman Waraich ,In Vitro Anthelmintic Activity of *Allium sativum*, *Zingiber officinale*, *Curcubita mexicana* and *Ficus religiosa* International journal of agriculture & biology 3(4)454–457(2001).
 33. R. Preethi, V.Vimal Devanathan and M. Loganathan, Antimicrobial and Antioxidant Efficacy of Some Medicinal Plants Against Food Borne Pathogens Advances in Biological Research 4 (2): 122-125, (2010).
 34. Rimi Shukla, Shweta Gupta, J.K Gambhir, K.M Prabhu and P.S Murthy,Antioxidant effect of aqueous extract of the bark of *Ficus bengalensis* in hypercholesterolaemic rabbits J Ethnopharmacol , 92: (1)47-51(2004).
 35. Aswar M, Aswar U, Watkar B, Vyas M, Wagh A., Gujar KN Anthelmintic activity of *Ficus bengalensis* IJGP;2:3,(2008).
 36. Vishnu N Thakare,Anupama A suralkar,Avinash D Deshpande,Suresh R Naik. Stem bark extraction of *Ficus bengalensis* linn for anti inflammatory and analgesic activity in animal models Indian journal of Experimental Biology 48:pp 39-45,(2010).