WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 7, 493-502. Research Article ISSN 2277– 7105

DEVELOPMENT OF HPTLC-UV METHOD FOR COMPARATIVE PHYTOCHEMICAL STUDY OF STEM BARK *VERSUS* SMALL BRANCHES OF *FICUS RACEMOSA* LINN

S. C. Verma*, E. Vashishth, S. Subhani, R. Singh, P. Pant, S. Gaidhani, M. M. Padhi, K. S. Dhiman

Central Council for Research in Ayurvedic Sciences, 61-65, Institutional Area, Opp.-D-Block, Janakpuri, New Delhi-110058, India.

Article Received on
30 April 2015,

Revised on 20 May 2015,
Accepted on 10 June 2015

***Correspondence for Author**
S. C. Verma
Central Council for Research in Ayurvedic Sciences, 61-65, Institutional Area, Opp.-D-Block, Janakpuri, New delhi-110058, India.

ABSTRACT

Ficus racemosa Linn. belongs to the family Moraceae. It is a moderate to large size scattering laticiferous tree growing 10-16 m in height without major aerial roots. It is considered as an Ayurvedic medicine to treat several diseases. It possesses various biological effects such as hepatoprotective, chemopreventive, anti-diabetic, anti-inflammatory, antipyretic, and anti diuretic. The plant is used in dysentery, pectoral complaints, applied in mumps, other inflammatory glandular enlargements and hydrophobia. The official part (stem bark) is reported to use in the indigenous systems of medicine for a variety of purposes, including coughs and colds etc. The unique patterns of the chromatographic fingerprint of stem bark and small branches of *F. racemosa* were developed and validated for comparison of phytochemicals. Our results revealed that the chromatographic fingerprint combined with similarity measurement could efficiently identify and distinguish *F. racemosa* stem bark and small branches. The phytochemical fingerprint profiling of stem bark and small branches of *F. racemosa* were found similar as an official part of *F. racemosa* plant i.e. stem bark, therefore small branches may be used in place of stem bark and vice-versa after comparison and confirmation of same pharmacological activities. The method can also be used for identification of different *F. racemosa* species and adulterants.

KEYWORDS: *Ficus racemosa* Linn, HPTLC–UV detection, phytochemical fingerprint profiling analysis.

ABBREVIATIONS: HPTLC–UV, high performance thin layer chromatography-ultra violet detection; R_f, retention factor; **min**., minutes; **St. Bk.,** stem bark., **Sm. Br.**, small branches;

INTRODUCTION

Ficus racemosa Linn belongs to the family Moraceae. It is a moderate to large size scattering laticiferous tree growing 10-16 m in height without major aerial roots.[1,2] It is commonly known as Gular fig, Cluster fig or Country fig. It has various synonyms like yajnanga, yajniya, yajnayoga, yajnyasara etc.[3] The plant grows all over India in many forests and areas up to 1200 m altitude on hilltop from outer Himalayan ranges, Khasia mountain, Punjab, Rajasthan, Chota Nagpur, Bihar, Orrisa, West Bengal, Deccan and South India,[2] This is native to Australia, South East Asia and the Indian subcontinent. It is frequently found around the water streams and is also cultivated.[3] It is having quite rich green foliage that provides good shade,[4] For the proper growth it requires well-drained and medium to heavy soils. It also grows in all kinds of soils except in water logged and clay soil.[3]

The colour of bark is varies from rusty brown to grayish green having soft touch from outside while inner surface is light brown, with a thickness from 0.5-2 cm according to the age of trunk or bark.[1,2] The leaves are dark green, ovate-lanceolate or elliptic, sub acute, glabrous, entire, petiolate, and 7.5-10 cm long, receptacles are small and sub globose or piriform, arrange in large clusters from old nodes of main trunk. They produce a pleasant smell resembling that of cidar apples.[2,3] The fruits look like the figs. They are green in colour when raw but on ripening they were turning orange, dull reddish or dark crimson. The udumbara flower is enclosed within a fig-like fruit structure.[2] Figs have been traditionally used by children to play.[3] According to Buddhist mythology, the flower was said to bloom only once every 3,000 years.[2] The tree are shed by December and replenished by January and April, it becomes bare for a short period,[3] The plant is propagated by using cuttings of stem and root suckers. Seeds can also be used for propagation. The flowers are pollinated by very small wasps.[3]

The root contains cycloartenol, euphorbol and its hexacosanoate, taraxerone, tinyatoxin.[5] Stem contains campesterol, hentriacontane, hentriacontanol, kaempferol, stigmasterol, methyl ellagic acid.[5] The stem bark contains gluanol acetate, β-sitosterol, leucocyanidin-3-O-β-D-glucopyrancoside, leucopelargonidin-3-O-β-D-glucopyranoside, leucopelarg- onidin -3-O-α-L-rhamnopyranoside, ceryl behenate, lupeol acetate and α-amyrin acetate two leucoanthocyanins: leucocyanidin-3-O-α-glucopyranoside, leucopelarogonidin - 3 – O

eucopelarogonidin - 3 - O - α- L - rhamnopyranoside, unidentified long chain ketone [1,3,6]. From trunk bark, lupeol, β-sitosterol, and stigmasterol were isolated.[1,2] The literature reported that the leaves contain sterols, triterpenoids in petroleum ether extract and alkaloids, tannins and flavonoids in ethanolic extract. A tetracyclic triterpene glauanol acetate which is characterized as 13α, 14β, 17 β H, 20 α H lanosta-8, 22-diene-3 β-acetate and racemosic acid was also isolated from the leaves.[1,3,5,6] The fruit contains glauanol, hentriacontane, β sitosterol, lupeol-OAc, sterol, gluanol acetate, glucose, tiglic acid, esters of taraxasterol, lupeol acetate, friedelin, higher hydrocarbons and other phytosterol,[7,8] The plant latex contains a-amyrin, β-sitosterol, cycloartenol, cycloeuphordenol, 4-deoxyphorbol and its esters, euphol, euphorbinol, isoeuphorbol, palmitic acid, taraxerol, tinyatoxin, tirucallol, trimethyl ellagic acid and an unusual thermo stable aspartic protease was also isolated.[5, 8]

F. racemosa is considered as an Ayurvedic medicine to treat several diseases.[4] In the traditional system of medicine, the plant is used for various health problems and diseases.[3] *F. racemosa* possesses various biological effects such as hepatoprotective,[9] chemopreventive,[10] anti-diabetic.[11] anti-inflammatory.[12] antipyretic.[13] and antidiuretic,[14] The plant is used in dysentery, pectoral complaints, applied in mumps, other inflammatory glandular enlargements and hydrophobia.[1] Stem bark of the plant posse's hypoglycemic activity.[1] The bark has also been evaluated for cytotoxic effects using 1BR3, Hep G2, HL-60 cell lines and found to be safe and less toxic than aspirin, a commonly consumed anti-inflammatory drug.[15] Reports indicate that stem bark of the tree is used in the indigenous systems of medicine for a variety of purposes, including coughs and colds. The bark is astringent, antiseptic, antipyretic, and vermicidal. An infusion of the bark is employed as a mouthwash for spongy gum condition, and the decoction is used for treating various skin diseases and ulcers. It is used as a poultice in inflammatory swellings and boils. It is also reported to be effective in the treatment of piles, dysentery, asthma, gonorrhea, gleet, menorrhagia, leucorrhea and hemoptysis, and urinary diseases.[16] The Kwath douche of its stem bark can be used for the treatment of leucorrhoea and vaginitis. Gluanol acetate isolated from bark is useful in bilious affection.[17]

The other plant parts such as bark, root, leaf, fruits and latex are used as astringent, carminative, vermifuge and anti-dysentery. It is believed to be a good remedy for visceral obstructions and extract of the fruit is used in leprosy, diarrhea, circulatory and respiratory disorders and menorrhagia[18, 19]

Scientific Classification[2]

Kingdom	**Plantae**
Division	Magnoliophyta
Class	Magnoliopsida
Order	Rosales
Family	Moraceae
Genus	*Ficus*
Species	*F. racemosa*
Synonyms	*Ficus glomerata* Roxb

Figure 1: *Ficus racemosa* Linn. Plant.

Figure 2: Small Branches

Figure 3: Stem bark

MATERIALS AND METHODS

Plant Materials and Chemicals

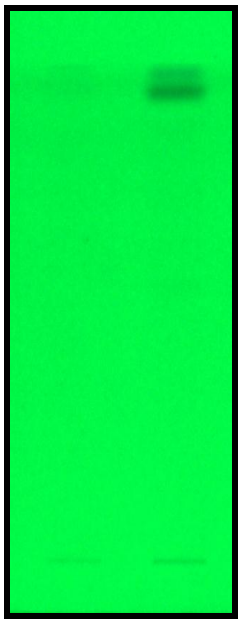
Plant materials i.e. small branches of stem (Fig.2) and stem barks (Fig. 3) of *F. racemosa* were collected in December 2013 and authenticated by Dr. R. K. Tiwari, Research Officer, Pharmacognosy, National Veterinary Research Institute & Hospital, Lucknow. All chemicals (AR grade) and TLC plates were purchased from E. Merck Pvt. Ltd. (Mumbai, India).

Sample preparation

The plant parts were dried under a gentle stream of air in the laboratory till no loss in weight (temperature 30± 2^0C and relative humidity 50 ± 5%) and powdered in an electric grinder. Conventional extraction of stem bark and small branches of stem of *F .racemosa* were performed at room temperature ($28^o \pm 3^o$C) with a variety of solvents ranging from non-polar to polar ones, i.e. *n*-hexane, ethyl acetate and ethanol. Dried and powdered parts of *F. racemosa* (10 g each) were extracted three times (3 × 50 mL) for 18 h of each extraction with each of the above-mentioned solvents separately. Each extract was filtered by using Whatman filter paper no.1 and the solvents were removed under vacuum at 50°C, separately and concentrated up to 10 mL to get the sample solution of 100 mg mL^{-1}. 5 µL of each sample was applied separately to TLC plate for the development of fingerprints.

HPTLC-UV detection Method

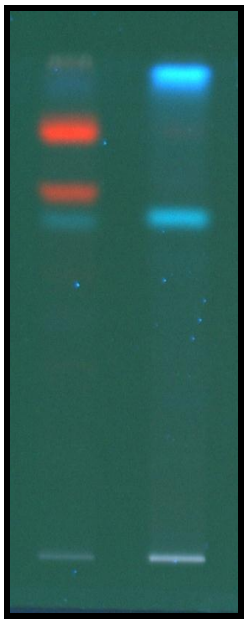
High Performance Thin Layer Chromatography was performed on 10 cm × 10 cm TLC plates pre-coated with 0.25 µm thin layers of silica gel 60 F_{254} (E. Merck). Both samples (stem bark and small branches) were applied on the plates as bands 10 mm wide by use of a Linomat-IV applicator (CAMAG, Switzerland) fitted with a 100 µL syringe (Hamilton, Switzerland). The application positions X and Y were both 10 mm, to avoid edge effects. Linear ascending development to a distance of 80 mm with *Toluene: Ethyl acetate 7.5:2.5 (v/v/)* and as mobile phase for both *n*-hexane extract was performed in a twin-trough glass chamber (20 cm × 10 cm) previously saturated with vapours of mobile phase for 20 min. The plates were dried in air and visualized under λ 254 nm and λ 366 nm for ultra violet detection and taken the fingerprints as evident in Figures 4-5. Further, the same TLC plate was derivatized with anisaldehyde-sulphuric acid reagent and visualized in white light obtained fingerprints were as evident in Figures 6 using CAMAG Reprostar and WinCATs software (V1.4.2; CAMAG). HPTLC of ethyl acetate extract and alcoholic extract of both drugs was performed same procedure with the mobile phases of *Toluene: Ethyl acetate 8:2 (v/v)* and *Toluene: Ethyl acetate: 7:3 (v/v)* respectively and then visualized in λ 254 nm, λ 366 nm and white light using CAMAG Reprostar and WinCATs software as shown in Figure 7-12.



1 2

254 nm

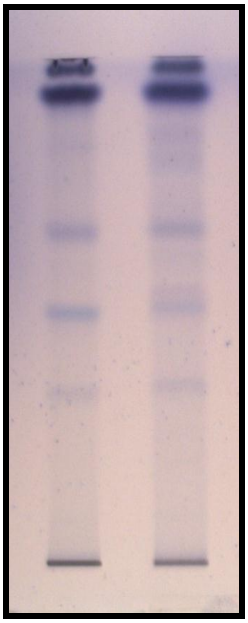
Figure 4



1 2

366 nm

Figure 5

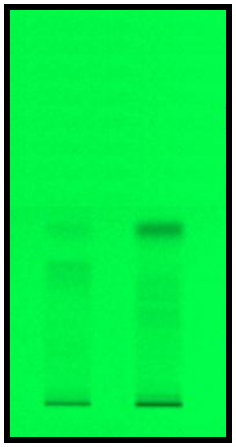


1 2

After derivatization

Figure 6

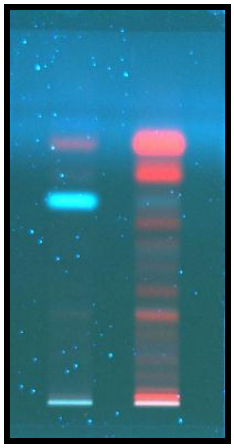
Figure 4-6: TLC fingerprint of *n*- hexane extract of *F. racemosa* (1= St. Bk.; 2= Sm. Br.)



1 2

254 nm

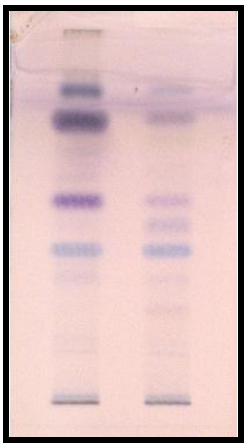
Figure 7



1 2

366 nm

Figure 8

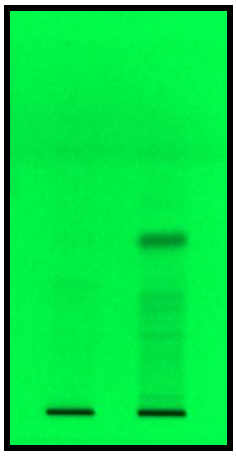


1 2

After derivatization

Figures 9

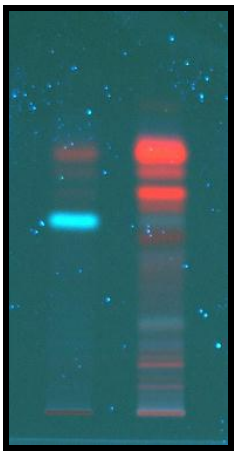
Figure 7-9: TLC fingerprint of ethyl acetate extract of *F. racemosa* (1= St. Bk.; 2= Sm. Br.)



1 2

254 nm

Figure 10



1 2

366 nm

Figure 11

1 2

After derivatization

Figures 12

Figure 10-12: TLC fingerprint of ethanol extract of *F. racemosa* (1= St. Bk.; 2= Sm. Br.)

Table 1: R_f value of phytochemicals present in *n*-hexane, ethyl acetate and ethanol extract of *F. racemosa* (St. Bk. and Sm. Br.) at different wave-lengths.

Wave-length	*n*- Hexane extract		Ethyl acetate extract		Ethanol extract	
	Stem bark	Small branches	Stem bark	Small branches	Stem bark	Small branches
254	No band	0.87,0.91	0.38,0.49	0.26,0.31,0.49	No band	0.22,0.28,0.33,0.49
366	0.57,0.66, 0.78,0.91	0.57,0.91	0.56,0.71	0.13,0.20,0.24, 0.32,0.42,0.45, 0.50,0.56,0.62, 0.66,0.71	0.46,0.65 ,0.70	0.08,0.13,0.19,0.25,0.46,0.51,0.55,0.58,0.65,0.70
Visible light after derivatization	0.27,0.46, 0.58,0.86, 0.91	0.27,0.46,0.58,0.77,0.86 ,0.91	0.15,0.34, 0.42,0.48, 0.55,0.77, 0.84	0.15,0.25,0.34, 0.42,0.48,0.55, 0.77,0.84	0.05,0.41 ,0.52,0.77,0.85	0.05,0.41,0.46,0.52,0.77,0.85

RESULTS AND DISCUSSION

No such study was found in literature for comparative phytochemical study of stem bark versus small branches of *F. racemosa* Linn by using High Performance Thin Layer Chromatographic-Ultra Violet detection Method. Comparative study of TLC fingerprints of stem bark and small branches of *F. racemosa* revealed that many similarities in phytochemical fingerprints were found and evident in Table-1 and Fig. 4-12.

Phytochemical fingerprints of n-hexane extract of stem bark and small branches showed no band in stem bark and two bands in small branches, thus no band was found similar under

UV detection at 254 nm. Under 366 nm UV detection, stem bark and small branches showed four and two bands respectively, out of which two bands at R_f 0.57 and 0.91 (both are blue) were found similar. After TLC plate derivatized with Anisaldehyde sulphuric acid reagent and visualized under white light, stem bark and small branches showed five and six bands, out of which five bands at R_f 0.27(blue),0.46 (blue),0.58 (blue),0.86 (violet) and 0.91(violet) were found similar.

Phytochemical fingerprints of ethyl acetate extract of stem bark and small branches under 254 nm represented two and three bands respectively. Out of which, one band was similar at R_f 0.49 (black).Under 366 nm UV detection, stem bark and small branches showed two and eleven bands respectively, out of which two bands at R_f 0.56(blue) and 0.71(red) were found similar. After derivatized with Anisaldehyde sulphuric acid reagent and visualized under white light, seven and eight bands were visible in stem bark and small branches respectively, out of which seven bands at R_f 0.15 (blue), 0.34(blue), 0.42(blue), 0.48 (blue), 0.55 (violet), 0.77(violet) and 0.84(violet) were found similar.

Phytochemical fingerprints of ethanol extract of stem bark and small branches showed no band in stem bark and four bands in small branches under UV detection at 254 nm, thus no band was found similar. While under 366 nm UV detection, stem bark and small branches showed three and ten bands respectively, out of which three bands at R_f 0.46(blue), 0.65(red) and 0.70 (red) were found similar. After TLC plate derivatized with Anisaldehyde sulphuric acid reagent and visualized under white light, stem bark and small branches showed five and six bands respectively, out of which five bands at R_f 0.05(blue),0.41(blue),0.52(blue),0.77 (violet), 0.85(violet) were found similar in both parts (St. Bk. and Sm. Br.).

CONCLUSION

The phytochemical fingerprint profiling of small branches of *F. racemosa* were found similar with stem bark as an official part of *F. racemosa* plant, therefore small branches may be used in place of stem bark and vice-versa. The R_f helped in evaluation of phytochemical diversity in different parts of *F. racemosa*. TLC phytochemical fingerprint profiling of *n*-hexane, ethyl acetate, ethanolic extracts of stem bark and small branches of *F. racemosa* have been given an idea about the presence of various phytochemicals in their reported parts. The TLC spots provided valuable clue regarding presence or absence of various phytochemicals or metabolites of the plants.

ACKNOWLEDGMENTS

Authors are thankful to Director General, Central Council for Research in Ayurvedic Sciences, New Delhi to provide the financial support under IMR scheme for this research work. Authors are grateful to Dr. R. K. Tiwari, NVRI&H Lucknow for providing the genuine plant materials.

REFERENCES

1. Jagzap RK, Nirmal SA, Kadam SK. "Potential of *Ficus Racemosa* Bark: An immunomodulatory agent", Indian J. basic App Med Res., 2012; 2(1): 120-127.
2. Rawat P, Rawat P, Kumar P. Extraction and Isolation of A -Amyrin Acetate From The Fruits of *Ficus Racemosa*, J Drug Discovery and Therapeutics, 2013, 1(1): 15-18.
3. Shiksharthi AR, Mittal S. *Ficus Racemosa*: Phytochemistry, Traditional Uses and Pharmacological Properties: A Review, Int J Recent Adv Pharm Res, 2011; 4: 6-15.
4. Sophia D, Manoharan S, Hypolipidemic Activities of *Ficus Racemosa* Linn. Bark in Alloxan Induced Diabetic Rats, Afr J Trad CAM, 2007; 4(3): 279 – 288.
5. Joseph B, Raj SJ, Phytopharmacological Properties of *Ficus Racemosa* Linn - An Overview, Int J Pharm Sci Rev Res, 2010; 3(2): 134-138.
6. Mohammed R, Mohammed U, Patil RS, Medicinal Uses of *Ficus Racemosa* Linn, Int J Pharm Arc, 2013; 2(3): 33-42.
7. Shaikh T, Rub R, Bhise K, Pimprikar RB, Sufiyan A. Antibacterial activity of *Ficus racemosa* Linn. Leaves on Actinomyces viscosus, J Pharm Sci Res, 2010; 2(1): 41-44.
8. Bhalerao SA, Verma DR, Teli NC, Didwana VKS, Thakur SS. *Ficus racemosa* Linn. : A Comprehensive Review, J Appl Chem, 2014; 3(4): 1423-1431.
9. Mandal SC, Tapan K, Maity J, Das M, Pal M, Saha BP. Hepatoprotective activity of *Ficus racemosa* leaf extract on liver damage caused by carbon tetrachloride in rats. Phytother Res, 2003; 13: 430-32.
10. Khan N, Sultana S. Chemomodulatory effect of *Ficus racemosa* extract against chemically induced renal carcinogenesis and oxidative damage response in Wistar rats, Life Sci, 2005; 29: 1194– 1210.
11. Rao BR, Murugesan T, Sinha S, Saha BP, Pal M, Mandal SC. Glucose lowering efficacy of *Ficus racemosa* bark extract in normal and alloxan diabetic rats, Phytother Res, 2002; 16: 590-592.
12. Mandal SC, Maity TK, Das J, Saha BP, Pal M. Anti-inflammatory evaluation of *Ficus racemosa* Linn. leaf extract, J Ethnopharmacol, 2000; 72: 87-92.

13. Rao BR, Anupama K, Swaroop KR, Murugesan T, Pal M, Mandal SC. Evaluation of anti-pyretic potential of *Ficus racemosa* bark. Phytomedicine, 2002; 9: 731–33.
14. Ratnasooriya WD, Jayakody JR, Nadarajah T. Antidiuretic activity of aqueous bark extract of Sri Lankan *Ficus racemosa* in rats. Acta Bio Hung, 2003; 54(3-4): 357-63.
15. Li RW, Leach DN, Myers SP, Lin GD, Leach GJ, Waterman PG. A new anti-inflammatory glucoside from *Ficus racemosa* L. Planta Med, 2004; 70: 421-426.
16. Ahmed F, Urooj A, Glucose-lowering, Hepatoprotective and Hypolipidemic Activities of Stem Bark of *Ficus racemosa* in Streptozotocin-Induced Diabetic Rats, J Young Pharm, 2009; 1(2): 160-164.
17. Goyal PK, Antimicrobial Activity of Ethanolic Root Extract of *Ficus racemosa* Linn, Int J Chem Tech Res, 2012; 4(4): 1765-1769.
18. Joseph B, Raj SJ. Phytopharmacological and phytochemical properties of three *Ficus* species - an overview, Int J Pharma Bio Sci, 2010; 1: 246-253.
19. Sharma SK, Gupta VK. In vitro antioxidant studies of *Ficus racemosa* Linn. root. Pharmacognosy Magazine, 2008; 4: 70-74.