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COMPARATIVE PHYTOCHEMICAL STUDY OF STEM BARK VERSUS SMALL BRANCHES OF FICUS RELIGIOSA LINN USING HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHIC ULTRA VIOLET DETECTION METHOD

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ABSTRACT

Ficus religiosa Linn, family Moraceae is an important medicinal plant and immensely used in the Indian System of Medicine to cure human diseases. Stem bark of *F. religiosa* has been reported for various medicinal properties such as an astringent, aphrodisiac, anti-inflammatory, antiseptic, antidote, antibacterial against *Staphylococcus aureus* and *Escherichia coli*, and in bleeding, bone fracture, burns, cooling, diabetes, diarrhoea, dysentery, gonorrhea, gastrohelcosis, haemorrhoids and paralysis and many more. *F. religiosa* is commonly known as Udamber in India. Chemo-profiling screening of two parts of *F. religiosa* plants revealed variations in phytochemicals within stem bark and small branches. The unique properties of the chromatographic fingerprint were validated by analyzing stem bark and small branches of *F. religiosa*. Our results revealed that the chromatographic Finger print combined with similarity measurement could efficiently identify and distinguish *F. religiosa* from the other investigated *Ficus* species. In this paper a new, simple method is proposed in which the HPTLC-UV pattern of the extracts of stem bark and small branches of *F. religiosa* content is used for comparison of phytochemical present in both drug. The method can also be used for identification of different *F. religiosa* species. The proposed method uses cold- extraction then clean-up by solid-phase extraction before chromatographic analysis. The results revealed that the retention factor (R_f) of *F. religiosa* stem bark and small branches furnished a specific HPTLC chromatogram fingerprint which might be helpful for quality assurance and detection of adulteration of crude extracts.

KEYWORDS: *F. religiosa* 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 religiosa Linn belongs to the Moraceae family and commonly known as 'Peepal tree' and 'Bodhi tree'. It is a fast growing, perennial, deciduous tree with large broad branches. The branches are covered with leathery, heart-shaped leaves having long slender petioles. The purple fruits growing in pairs and hidden with the figs. The fig bearing the flowers just below the leaves seems like the berries. The bark is flat or slightly curved, varying from 5 to 8 mm in thickness, outer surface is grey [1-2]. It shed its leaves in the month of March and April [3]. The tree is regarded as a sacred tree to both Hindus and Buddhists. It has got mythological, religious and medicinal importance [4]. The tree grows throughout India but mainly grown in state of Bihar, Haryana, Kerala, Madhya Pradesh and Tamil Nadu [3]. It is found up to 170 m altitude in the Himalayas [5]. It is also widely cultivated in south-east Asia especially in vicinity of temples [6]. It commonly exists around the water streams and is also cultivated [7]. It is largely planted as an avenue and roadside tree especially near temples [8].

Figure 1: ***Ficus religiosa* Linn. Plant.**

Botanical Classification [9]
Kingdom: Plantae
Division : Magnoliophyta
Class: Magnoliopsida
Order : Rosales
Family: Moraceae
Genus : *Ficus*
Species : *F. religiosa*
Common Name: Peepal

Figure 2: **Stem Bark.**

Figure 2: **Small Branches.**

Ayurvedic Formulation
1. Panchavalkaladi Kwatha(gel) [10-11]
2. Herbal powder for female sterility [12] Panchavalkala Kwatha (Ayurvedic Hand Sanitizers) [13]
3. Marma gutika, Mutrasangrahniya kashay churna, Nalpamaradi taila Nyagrodhadi churna [14]

F. religiosa is reported to have numerous therapeutic uses in folk medicine. In traditional system of medicine, various parts such as stem bark, root bark, aerial roots, vegetative buds, leaves, seeds, fruits and latex are used for different medical purposes [1, 15]. *F. religiosa* is achieved large consideration because it has many compounds which are beneficial in treatment of many diseases [16]. The stem bark is used as an astringent, aphrodisiac, anti-inflammatory, antiseptic, antidote, antibacterial against *Staphylococcus aureus* and *Escherichia coli*, in bleeding, bone fracture, burns, cooling, diabetes, diarrhoea, dysentery, gonorrhea, gastrohelcosis, haemorrhoids and paralysis [1,17]. The bark decoction is used in cooling, gonorrhoea, hiccup, skin diseases, scabies and vomiting [1]. The young leaves are pink in color, changes to copper and finally to green at maturity [16].The leaves have been reported to have the hypoglycemic, anticonvulsant, wound healing, anti-ulcer, anti-oxidant and immunomodulatory activity. Leaf juice has been used for the treatment of asthma, cough, diarrhoea, ear-ache, eye troubles, gastric problems, migraine, haematuria, sexual disorders, scabies and toothache; leaf decoction has been used as an analgesic for toothache [1, 5, 17]. When fruits are raw, they are green in colour during summer but after ripening they turn black through rainy season [16]. Fruits are used for the treatment of asthma, laxative, digestive, other respiratory disorders and scabies. [1,17]. Unripe fruits are used as an astringent, carminative, digestive, stomachic. Fresh whole fruits, used as a source of dietary fibre, exhibited more hypocholesterolemic activity than pure cellulose [18]. Seeds are used as refrigerant, laxative and the latex of the plant is used in Neuralgia, inflammations, haemorrhages [1]. According to the Ayurveda, it possesses anticonvulsant activity, shows its action on CNS, also having acetyl cholinesterase inhibitory activity and anti anxiety activity. In India and Arab countries, it is used for the treatment of abdominal pain, congestive heart failure, diabetes, dyspepsia, enlargement of spleen, inflammation, jaundice, and stress [6,17,19].The fruit extracts had reduced convulsions resulting from the electrical shocks and chemicals. The extracts were also helpful in inducing deep sleep on the subjects [3]. The methanol extract of figs of *F. religiosa* had anticonvulsant activity [17].

Preliminary phytochemical screening of *F. religiosa* barks, showed the presence cardiac glycosides, flavonoids, saponins, steroids, tannins, terpenoids, bergapten, bergaptol, lanosterol, β-sitosterol, stigmasterol, lupen-3-one, β-sitosterol-d-glucoside (phytosterolin), vitamin K, wax, leucocyanidin-3-0-β-D-glucopyrancoside, leucopelargonidin- 3-0-β-D glucopyranoside, leucopelargonidin-3-0-α-L- rhamnopyranoside, lupeol, ceryl behenate, lupeol acetate, α-amyrin acetate, leucoanthocyanidin and leucoanthocyanin. Leaves have

been reported having carbohydrate, protein, lipid, lupeol, campestrol, stigmasterol, and tannic acid [5,17]. Campestrol, stigmasterol, isofucosterol, α-amyrin, lupeol, arginine, serine, aspartic acid, glycine, threonine, alanine, proline, tryptophan, tryosine, methionine, valine, isoleucine, leucine, nnonacosane, n-hentricontanen, hexa-cosanol and n-octacosan [6]. Figs (fruits) of this plant contain numerous amino acids like asparaginase and tyrosine in fruit edible part. Alanine, threonine, tyrosine and valine proteins were found in seeds [17, 19].

MATERIALS AND METHODS

Plant Materials and Chemicals

Plant materials (stem barks and small branches of stem) of *F. religiosa* 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).

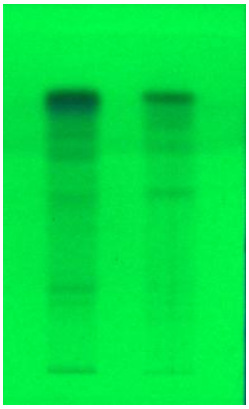
Sample Preparation

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. religiosa* 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. religiosa* (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

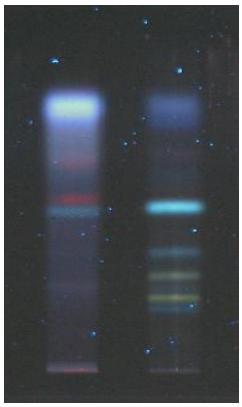
High Performance Thin Layer Chromatography was performed on 10 cm × 10 cm TLC plates pre-coated with 0.25 µm thin layers of silica gel 60 F_{254} (E. Merck). Both samples (stem bark and small branches) were applied on the plates as bands 10 mm wide by use of a Linomat-IV applicator (CAMAG, Switzerland) 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*: *8: 2 (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 1 – 2. Further, the same TLC plate was derivatized with anisaldehyde-sulphuric acid reagent and visualized in white light obtained fingerprints were as evident in Figures 3 using CAMAG Reprostar and Win CATs software (V 1.4.2; CAMAG). HPTLC of ethyl acetate extract and alcoholic extract of both drugs was performed same procedure with the mobile phases of *Toluene: Ethyl acetate 7: 3 (v/v)* and *Toluene: Ethyl acetate: Acetic acid 6:4:0.1 (v/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 4-9.



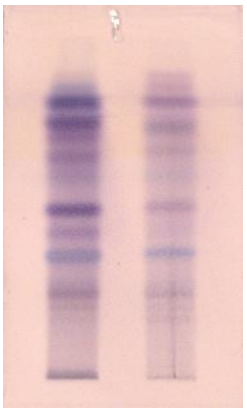
1 2
254 nm

Figure 1



1 2
366 nm

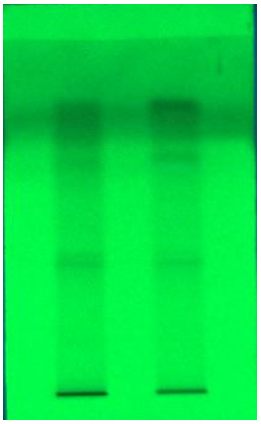
Figure 2



1 2
After derivatization with anisaldehyde sulphuric acid reagent

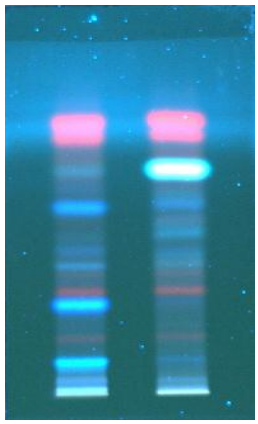
Figure 3

Figure 1-3: TLC fingerprint of *n*- hexane extract of *F. religiosa* (1= St. Bk.; 2= Sm. Br.)



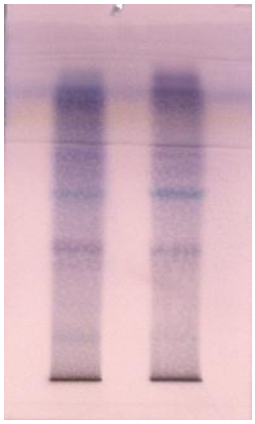
1 2
254 nm

Figure 4



1 2
366 nm

Figure 5



1 2
After derivatization with Anisaldehyde sulphuric acid reagent

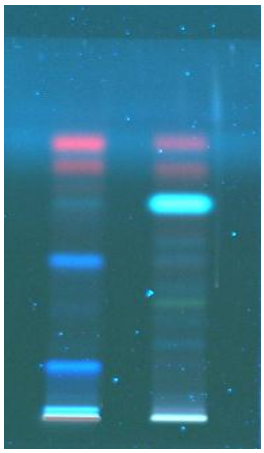
Figures 6

Figure 4-6: TLC fingerprint of ethyl acetate extract of *F. religiosa* (1=St. Bk.; 2= Sm. Br).

1 2

254 nm

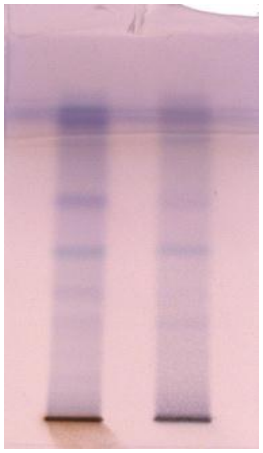
Figure 7



1 2

366 nm

Figure 8



1 2

After derivatization with Anisaldehyde sulphuric acid reagent

Figures 9

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

Table 1: R_f value of phytochemicals present in *n*-hexane, ethyl acetate and ethanol extract of *F. religiosa* (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	0.25, 0.53, 0.65, 0.71, 0.82	0.53, 0.65, 0.71, 0.82	0.36, 0.39, 0.66, 0.81	0.36, 0.66, 0.81	0.80	0.80
366	0.49, 0.53, 0.63, 0.75, 0.81	0.19, 0.23, 0.29, 0.36, 0.49, 0.75, 0.81	0.10, 0.17, 0.26, 0.30, 0.34, 0.36, 0.40, 0.53, 0.58, 0.63, 0.74, 0.78	0.10, 0.17, 0.26, 0.30, 0.34, 0.36, 0.40, 0.46, 0.53, 0.55, 0.63, 0.74, 0.78	0.14, 0.28, 0.41, 0.56, 0.65, 0.71	0.20, 0.26, 0.30, 0.35, 0.43, 0.46, 0.56, 0.65, 0.71
Visible light after derivatization	0.21, 0.26, 0.37, 0.43, 0.51, 0.59, 0.66, 0.74, 0.82, 0.86	0.21, 0.26, 0.37, 0.51, 0.59, 0.66, 0.74, 0.82, 0.86, 0.89	0.12, 0.37, 0.52, 0.63, 0.78, 0.80, 0.85	0.12, 0.37, 0.52, 0.63, 0.78, 0.80, 0.85	0.26, 0.34, 0.49, 0.57, 0.72, 0.80	0.26, 0.49, 0.57, 0.72, 0.80

RESULTS AND DISCUSSION

No such study was found in literature for comparative phytochemical study of stem bark versus small branches of *F. religiosa* 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. religiosa* revealed that many similarities in phytochemical

Finger prints were found and evident in Table-1 and Fig. 1-9. Phytochemical fingerprints of *n*-hexane extract of stem bark and small branches showed five and four bands respectively, out of which four bands were found similar at R_f 0.53, 0.65, 0.71 and 0.82 (all are black) under UV detection at 254 nm. Under 366 nm UV detection, stem bark and small branches showed five and seven bands respectively, out of which three bands at R_f 0.49, 0.75 and 0.81 (all are blue) were found similar. After TLC plate derivatized with Anisaldehyde sulphuric acid reagent and visualized under white light, stem bark and small branches both were showed ten bands, out of which nine bands at R_f 0.21(brown), 0.26 (brown), 0.37 (blue), 0.51 (violet), 0.59 (blue), 0.66 violet), 0.74 (violet), 0.82 (violet), 0.86 (violet) were found similar as represented in Table 1 and Fig. 1-3.

Phytochemical fingerprints of ethyl acetate extract of stem bark and small branches under 254 nm represented four and three bands respectively. Out of which, three bands were similar at R_f 0.36, 0.66 and 0.81 (all black). Under 366 nm UV detection, stem bark and small branches showed twelve and thirteen bands respectively, out of which eleven bands at R_f 0.10 (blue) ,0.17 (red) ,0.30 (red),0.34 (red),0.36 (blue),0.40 (blue),0.46 (blue) ,0.53 (blue), 0.63 (blue), 0.74 (red) and 0.78 (red) were found similar. After derivatized with Anisaldehyde sulphuric acid reagent and visualized under white light total seven bands were visible in both the parts and all seven bands at R_f 0.12 (light brown), 0.37 (violet), 0.52 (dark blue), 0.63 (blue), 0.78 (blue) 0.80 (violet), 0.85 (violet) were found similar as showed in Table 1 and Fig. 4-6. Phytochemical fingerprints of ethanol extract of stem bark and small branches showed only one band in both stem bark and small branches under UV detection at 254 nm which is common at R_f 0.80 (black). While under 366 nm UV detection, stem bark and small branches showed six and ten bands respectively, out of which three bands at R_f 0.56 (blue), 0.65 (red), 0.71 (red) were found similar. After TLC plate derivatized with Anisaldehyde sulphuric acid reagent and visualized under white light, stem bark and small branches showed six and five bands respectively, out of which four bands at R_f 0.49 (blue), 057 (violet), 0.72 (violet), 0.80 (violet) were found similar in both parts (St. Bk. and Sm. Br.) as evident in Table 1 and Fig.7-9.

CONCLUSION

Phytochemical fingerprint profiling of various parts of *F. religiosa* indicated that different types of phytoconstituents present in each part but many similarities in fingerprinting were found in stem bark and small branches. The phytochemical fingerprint profiling of small

branches of *F. religiosa* were slightly similar with stem bark as a official part of *F. religiosa* plant, therefore small branches may not be used in place of stem bark and vice-versa. The R_f helped in evaluation of phytochemical diversity in different parts of *F. religiosa*. The phytochemical diversity was found more in stem bark followed by small branches at one geographical region. TLC phytochemical fingerprint profiling of *n*-hexane, ethyl acetate, ethanolic extracts of stem bark and small branches of *F. religiosa* 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.

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Authors have no conflict of interest.

REFERENCES

1. Makhija KI, Sharma IP, Khamar D. Phytochemistry and Pharmacological properties of *Ficus religiosa*: an overview. Annals of Biological Research, 2010; 1(4): 171-180.
2. Babu K, Shankar SG, Rai S. Comparative pharmacognostic studies on the barks of four Ficus species. Turk J Bot, 2010; 34: 215-224.
3. Saravanan P, Chandramohan G, Mariajancyrani J, Shanmugasundaram P. GC-MS Analysis of Phytochemical Constituents In Ethanolic Bark Extract of *Ficus Religiosa* Linn. Int J Pharm Sci, 2014; 6(1): 457-460.
4. Manimozhi DM, Sankaranarayanan S, Kumar GS. Effect of different extracts of stem bark of ficus sp. on multidrug resistant pathogenic bacteria. Int J Pharma Sci Res, 2012; 3(7): 2122-2129.
5. Kapoor M, Jasani N, Acharya N, Acharya S, Kumar V. Phytopharmacological evaluation and anti-asthmatic activity of *Ficus religiosa* leaves. Asi Pac J Tro Med, 2011; 642-644.
6. Paliwal D, Murti K, Sangwan Y, Kaushik M, Kiran D. Preliminary and pharmacological profile of *Ficus religiosa* L.: an overview. Pharmacol online, 2011; 3: 387-395.
7. Joseph Baby, Raj SJ. Phytopharmacological and Phytochemical Properties of Three Ficus Species - An Overview. Int J Pharma Bio Sci, 2010; 1(4).

8. The Ayurvedic Pharmacopoeia of India, Part- I, Vol – I, New Delhi. Government of India, Ministry of Health and Family Welfare, Department of Indian Systems of Medicine and Homopathy, 1999; 17.
9. http://www.ecoindia.com site assessed on 13.10.2014.
10. Sakhitha KS, Dattatray D, Santhosh B, Parimi S. Formulation, anti bacterial activity and wound healing property of panchavalkaladi ointment. Int Ayur Med J, 2013; 1(4).
11. Vyas P, Prajapati PK, Shukla VJ. An herbal wound healing gel prepared with pachavalkala kwatha, Nimba kwatha and kumari, swarasa with their physicochemical parameters. 2013; 3(2): 2278–5701.
12. http://www.biznet1.com/ayurveda/ site assessed on 13.10.2014.
13. Vyas P, Galib, Patgiri BJ, Prajapati PK. Int J Pharm Bio Arc, 2011; 2(2): 762-766.
14. http://ayurvedacart.in/index.php/ayurvedic-herbs/herbs-start-with-a/ashwath.html site assessed on 13.10.2014.
15. Salem MZM, Salem AZM, Camacho LM, Ali HM. Antimicrobial activities and phytochemical composition of extracts of *Ficus* species: An over view. Afr J Microbiol Res, 2013; 7(33): 4207-4219.
16. Gautam S, Meshram A, Bhagyawant SS, Srivastava N. *Ficus Religiosa*– Potential Role in Pharmaceuticals. Int J Pharm Sci Res, 2014; 5(5): 1616-1623.
17. Sirisha N, Sreenivasulu M, Sangeeta K, Chetty CM. Antioxidant Properties of *Ficus* Species – A Review. Int J Pharm Tech Res, 2010; 2(4): 2174-2182.
18. Khare CP. Indian medicinal plants An Illustrated Dictionary, Springer Science + Business Media, LLC. 2007; 268.
19. Gulecha VS, Sivakumar T, Mahajan MS, Khandare RA, Upasani CD. *Ficus Religiosa* and *Ficus Glomerata* Extracts Exhibits Anti-inflammatory Activity in the Rat. Pharmacol online, 2010; (1): 324-331.