Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage:www.elsevier.com/locate/apjtd

Document heading

Comparative Botanical and Phytochemical Evaluation of Medicinally Important Stem Bark of *Ficus* species

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ARTICLE INFO

Article history:
Received 15 June 2012
Received in revised form 27 June 2012
Accepted 18 October 2012
Available online 28 October 2012

Keywords:
Ficus species
HPTLC
Quantification
Pharmacognosy
Antioxidant

ABSTRACT

Objective: To evaluate the pharmacognostical comparison (Botanical study, physicochemical parameters, HPTLC analysis) in stem barks of four *Ficus* species (family-Moraceae) viz. *F. religiosa* L, *F. glomerata* Roxb, *F. retusa* auct. & *F. carica*. **Methods:** Estimation of Phytochemical markers viz: β-sitosterol and lupeol was quantified by HPTLC method and antioxidant studies by carried by DPPH method. **Results:** HPTLC method showed considerable amount of variation with two reference standard viz: β-sitosterol and lupeol content in stem bark of *F. religiosa*, F. glomerata, *F. retusa* & *F. carica* and it were found 0.084, 0.041, 0.059 & 0.131 and 0.020, 0.043, 0.069 & 0.049 respectively. The antioxidant potential of ethanolic extract of stem bark of *F. religiosa*, *F. retusa*, *F. glomerata* & *F. carica* were found 46.86%, 42.56%, 31.25% & 25.63% at 0.1mg/ mL concentration. **Conclusion:** The present work was taken up with a view to lay down standards which will contribute significantly to quality control of these medicinally useful *Ficus* species. It also provides suitable criteria to differentiate the stem barks of four *Ficus* species.

1. Introduction

Stem bark of four *Ficus* species (family-Moraceae) viz. *F. religiosa* L, *F. glomerata* Roxb, *F. retusa* auct. non L. Syn. *Ficus* microcarpa L. and *F. carica* L. commonly known as 'Pipala', 'Gular', 'Chinese banyan' and 'Anjir' respectively has been most widely used in traditional medicinal system of all over world including India [1-2]. *Ficus* species are native to India, Nepal, Sri Lanka, Southwest China and Indochina also found throughout the plains of India upto 170 m altitude in the Himalayan region [3]. Bark of *F. glomerata* Roxb., antidiabetic, refrigerant and useful as a wash for wounds, highly efficacious in threatened abortions and also recommended in uropathy [4]. *F. retusa* bark extract is used in liver complaints, astringent, refrigerant, acrid and stomachic. *F. religiosa* is useful in wounds, ulcers, flatulent colic, hepatopathy, diarrhea, Aphrodisiacs, Antiinflammatory, analgesic activity and psychopathy [5-8]. Stem bark of *F. carica* is used as anti-inflammatory agent ~~in head~~ wounds [9].

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Although the stem bark of these species are important but very less studies has been reported so far on pharmacognostic and phytochemical parameters. Hence this study was undertaken to develop comparative quality standards and antioxidant studies of stem bark of these *Ficus* species and their evaluation. This may be useful to pharmaceutical industries for authentication of commercial sample and also to explore the possibility of using other species as complementary to each other.

2. Material & methods

2.1. Collection of plant material

In the present study plant materials were collected from the botanical garden of National Botanical Research Institute (NBRI), Lucknow, India in the month of August and were authenticated by Dr. A.K.S. Rawat, Head Pharmacognosy & Ethnopharmacology Division, National Botanical Research Institute Lucknow (India), herbarium specimens were prepared and deposited (Field voucher no. 262520, 262521, 262522 and 262523) to the National Herbarium

of institute.

2.2. Pharmacognostic evaluation

The fresh materials were preserved in 70% alcohol for microscopic evaluation. Microtome sections were cut and double stained with safranin and fast green, photographed with Olympus CX 31 camera. Histochemical, powder and maceration studies were carried out by taking free hand sections by standard method. Physico-chemical parameters were calculated from shade dried powdered material according to the recommended procedures [10]. The antioxidant activity was achieved by DPPH method for four medicinally important *ficus* species [11].

HPTLC studies

A densitometry HPTLC analysis was also performed for the development of characteristics fingerprint profile, which may be used as marker for quality evaluation and standardization of the drug.

2.3. Extraction of plant samples

Accurately weighed 2.0 gm of the coarse powder of four *Ficus* species were extracted separately with methanol (4 × 25 mL) under reflux (30 min each time) on water bath. The combined extracts were filtered, mixed & concentrated on rotavapour. The syrupy compounds evaporated to dryness on freeze drier and prepare 10 mg / mL solution with analytical grade methanol.

2.4. Chromatographic conditions

Plates were developed to a distance of 80 mm, with toluene: ethyl acetate (80: 20 v/v) as mobile phase in a Camag twin-trough chamber (20 cm × 10 cm) previously saturated with mobile phase vapour for 10 min: Room temperature was 28 ± 2 °C. After removal of plates from chamber completely dried with dryer and then sprayed with anisaldehyde-sulphuric acid solution, followed by heating at 110 ℃ for 15 min and β- Sitosterol and Lupeol was simultaneously quantified by using CAMAG TLC Scanner model-3 equipped with winCATS [version 3.2.1] Software (Figure 1 & 2).

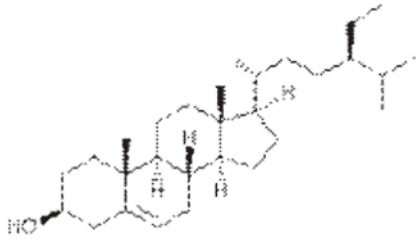

Figure 1. Structure of β-Sitosterol

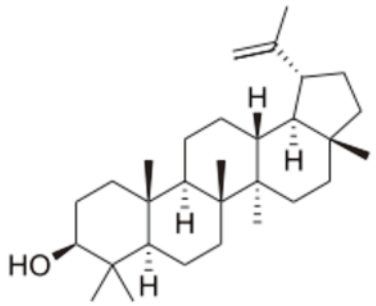

Figure 2. Structure of Lupeol

The following scan condition were applied slit width- 4 × 0.45 nm, wavelength (λ max-600 nm), Absorption -Reflection scan mode.

2.5.Validation of the HPTLC method

2.6.1. Linearity, LOD and LOQ

The linearity of the detector response for the prepared standards was assessed by means of linear regression regarding the amounts of each reference standard, measured in ng, and the area of the corresponding peak on the chromatogram. After chromatographic separation, the peak areas obtained were plotted against the extract concentrations by linear regression. LOD and LOQ were determined by calculation of the signal-to noise ratio. Signal-to-noise ratios of approximately 3: 1 and 10: 1 were used for estimating the detection limit and quantification limit, respectively, of used method [12].

2.6.2. Accuracy

Recovery studies were carried out to check accuracy of the method. Recovery experiments were performed by adding three different amounts of standards i.e. 25%, 50% and 75% in the each *ficus* species, and the result was analysed (*n*=5)

2.6.3. Precision

The intra-day precision was evaluated by analysing β - sitosterol & Lupeol repeatedly at a concentration range of 5-50 ng per spot (n=5). The inter-day precision was evaluated by analysing β - sitosterol & Lupeol at a concentration range of 5-50 ng per spot over a period of 5 days (*n*=5).

2.6. Determination of in vitro anti oxidant activity

The effects of free radical scavenging activity by ethanolic extracts of *ficus* bark species processed by DPPH method [15]. To evaluate antioxidant activity, solution of 0.135mM DPPH (2, 2-Diphenyl-2-picryllhydrazyl) in methanol was prepared and 1.0ml of this solution was mixed with 1.0ml of extract in methanol containing 0.02-0.1mg of the extract. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30min. The absorbance of the mixture was measured at 517nm using Double beam UV-Vis Spectrophotometer (Thermo Electron Corporation, Cambridge, England). Ascorbic acid, Rutin and Quercetin

were used as reference standard. The ability to scavenge DPPH radical was calculated by the following equation.

DPPH radical scavenging activity (%) = [(Abs control − Abs sample) / (Abs control)] × 100

Where Abs control is the absorbance of DPPH radical + methanol; Abs sample is the absorbance of DPPH radical + sample extract/standard.

3. Result

The present study was focused at the pharmacognostical evaluation viz. Botanical study, physicochemical parameters and HPTLC analysis and antioxidant studies to make a comparison among stem barks of above four *Ficus* species. Determinations of various physicochemical constants were carried out according to the methods provided in Ayurvedic Pharmacopoeia of India (API). The macro and microscopical character of these *Ficus* species also shows moderate variation (Figure 3−10).The detailed physico− chemical analysis is given in figure 11 & figure 12. Tannin content was found to be maximum in *F. religiosa* (6.36%) and minimum in F. carica (0.14%) (Figure11). This result was supported by microscopical studies which showed the presence of numerous dark brown cell contents in case of *F. religiosa* (Figure 7). Total phenolic content was also found to be maximum in *F. religiosa* (20.57%) and minimum in *F. carica* (3.04%) (Figure11). Sugar content was found to be maximum in *F. religiosa* (2.04%) and minimum in *Ficus* glomerata (1.36%) (Figure11). Starch content was found to be maximum in *F. carica* (6.44%) and minimum in *F. glomerata* (1.49%) (Figure11).

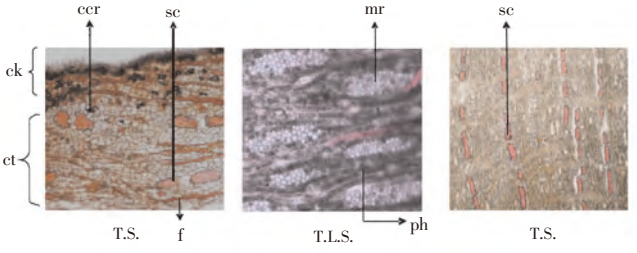

Figure 3. Microscopy of *Ficus* religiosa stem bark. ck, cork ; ct, cortex; sc, stone cell; mr, medullary rays; ccr, calcum oxalate crystal; ph, phloem; f, phloem fibre.

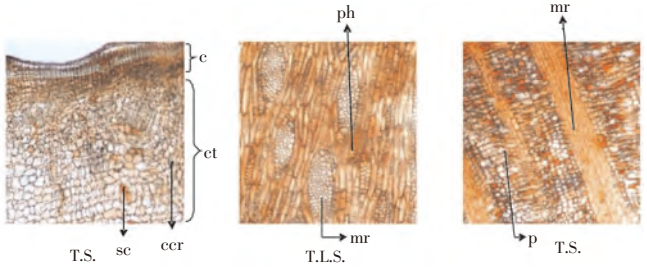

Figure 4. Microscopy of *Ficus* glomerata stem bark. ck, cork ; ct, cortex; sc, stone cell; mr, medullary rays; ph, phloem; ccr, calcium oxalate crystal

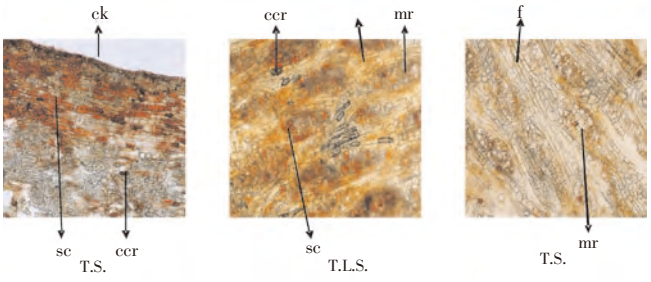

Figure 5. Microscopy of *Ficus retusa* stem bark. ck, cork ; ccr, calcium oxalate crystal; sc, stone cell; mr, medullary rays; ph, phloem; f, phloem fibre.

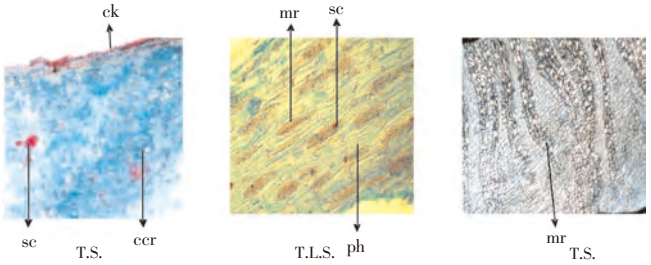

Figure 6. Microscopy of *Ficus carica* stem bark. ck, cork ; sc, stone cell; mr, medullary rays; ph, phloem; ccr, calcium oxalate crystal

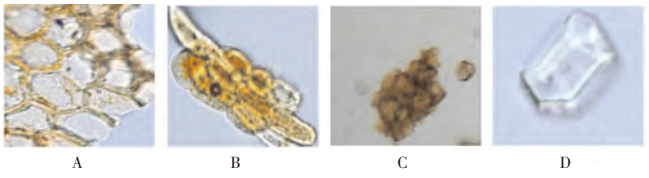

Figure 7. Powder microscopy of *Ficus* religiosa stem bark. A, cork in surface view; B, stone cell; C, cells with brownish content; D, prismatic crystals of calcium oxalate.

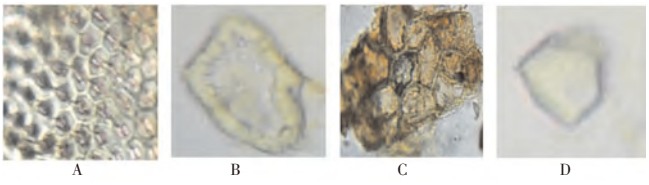

Figure 8. Powder microscopy of *Ficus* glomerata stem bark. A, cork in surface view (Polygonal cells); B, stone cell; C, cells with brownish content; D, prismatic crystals of calcium oxalate.

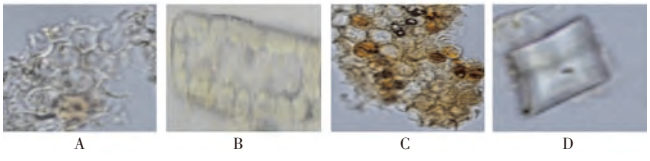

Figure 9. Powder microscopy of *Ficus* retusa stem bark. A, parenchymatous cells in surface view; B, stone cell; C cells (with crystals of calcium oxalate and brownish content); D, rhomboidal crystals of calcium oxalate

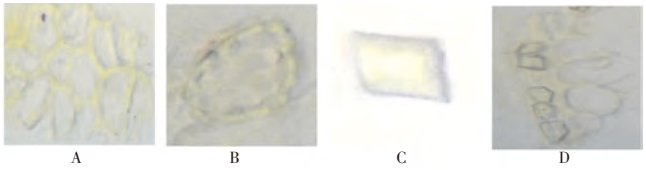

Figure 10. Powder microscopy of *Ficus* carica stem bark. A, cork in surface view; B, stone cell; C, rhomboidal crystals of calcium oxalate; D, cells embedded with crystals of calcium oxalate.

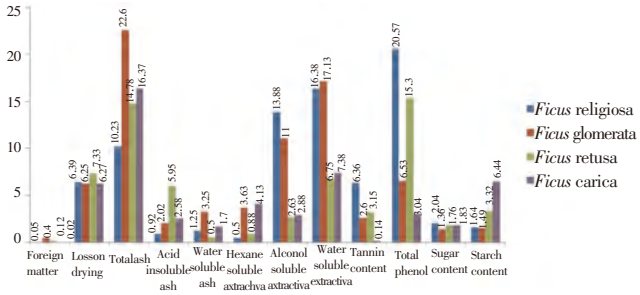

Figure 11. Comparative quantitative Physico-chemical parameter for four *Ficus* species

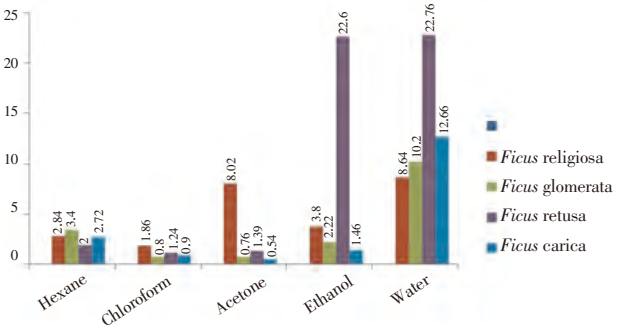

Figure 12. Comparative quantitative successive solvent extractive values of four *Ficus* species

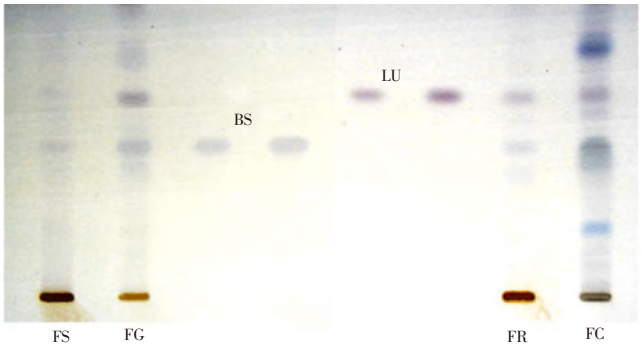

Figure 13. HPTLC fingerprinting profile of Samples along with reference compounds
FS: *Ficus religiosa*, FG: *Ficus glomerata*, FR: *Ficus retusa*, FC: *Ficus carica* BS: β -sitosterol, LU: Lupeol

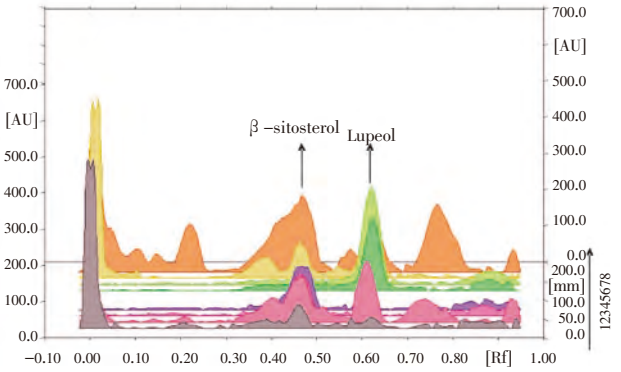

Figure 14. HPTLC Chromatogram of samples FS, FG, FR & FC (1, 2, 7 & 8) with reference compounds β -sitosterol & lupeol (3, 4, 5 & 6)

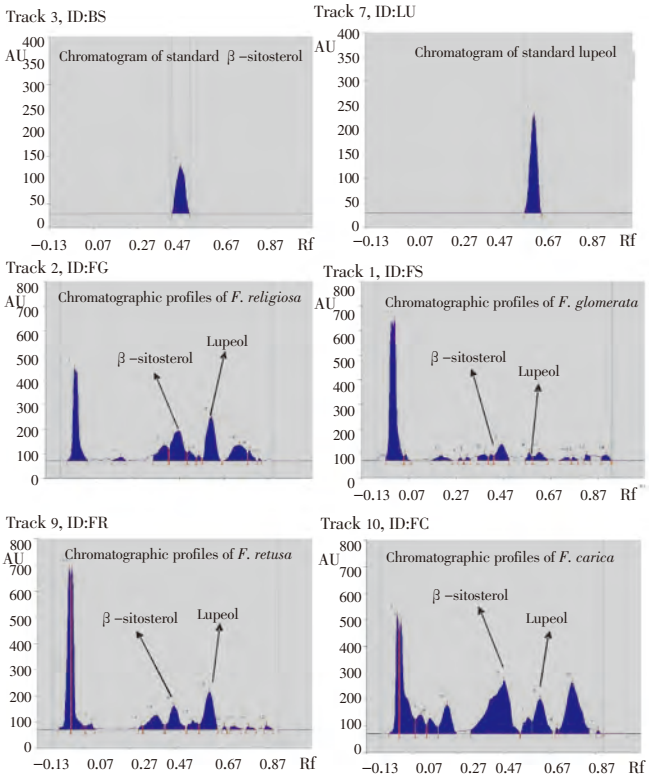

Figure 15. HPTLC densitometric scan (at 600nm) of reference compounds and samples

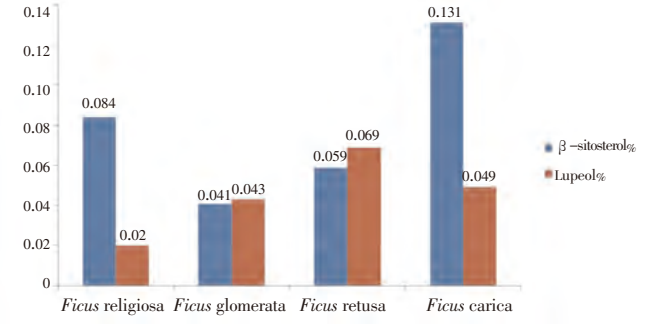

Figure16. Quantitative analysis of reference compounds in four medicinally important *Ficus* species

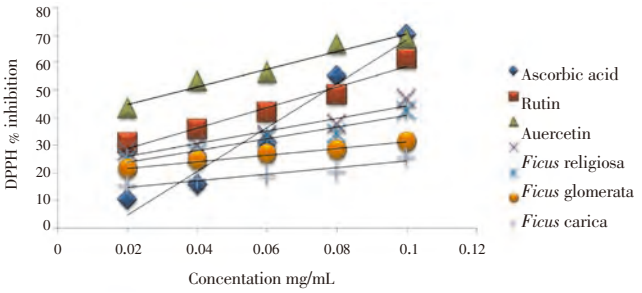

Figure 17. Comparative DPPH % inhibition of four medicinally important *Ficus* species at different concentration with reference compounds

HPTLC analysis showed the presence of β-sitosterol and lupeol in the ethanolic extract of bark of all the four *Ficus* species (Figure 13). The Rf values of β-sitosterol and lupeol was found to be 0.46 and 0.62 respectively which is clearly visualized in HPTLC chromatogram (Figure 14) and in densitometric chromatogram (Figure 15). Concentration of β-sitosterol was found maximum in *F. carica* (0.131%) & minimum in *F. glomerata* (0.041%) (Figure 16) and conc. of lupeol was found to be maximum in *F. retusa* (0.069%) & minimum in *F. religiosa* (0.020%) (Figure 16).

The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability. By DPPH method free radical scavenging abilities of the extracts were significantly less than those of ascorbic acid (70.06), rutin (61.48) and quercetin (68.60). The study showed that the extracts have the proton donating ability and could serve as free radical inhibitor or scavengers, acting possibly as primary antioxidants. The *Ficus* religiosa plant extract showed maximum 46.86% radical scavenging ability and *Ficus* carica showed minimum activity 25.63% at 0.1mg/ml concentration (Figure 17). Polyphenols are the major plant compounds with antioxidant activity. This activity is believed to be mainly due to their redox properties, which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides.

4. Discussion

Till date, there is no comparative phytochemical, anatomical and antioxidant literature available of this traditionally valuable drug; the present work was taken up with a view to lay down standards which will contribute significantly to quality control of medicinally useful *Ficus* species. Biomarkers β-sitosterol & lupeol were estimated through HPTLC method in all species and it was found maximum in *F. carica* & *F. retusa* respectively. Bark of *Ficus* religiosa shows strong antioxidant activity (DPPH assay), it suggests secondary metabolites present in *Ficus* religiosa having antioxidant effects, which indicate their effectiveness in diseases caused by overproduction of free radicals. Over all, this comparative data provide a suitable criteria to differentiate the stem barks of above four *Ficus* species and open a new vista for exploitation of these species for the development of herbal formulations.

Conflict of interest Statement

We declare that we have no conflict of interest.

Acknowledgements

The authors are thankful to Director, NBRI for providing all the facilities (under OLP-0010/DU-05) to conduct this research work, authors are also thankful to CSIR for financial grant under CSIR-In house (OLP-0010/DU-05).

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