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Comparative physicochemical, phytochemical and HPTLC study of stem bark versus small branches of *Flacourtia indica*

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Abstract

Flacourtia indica commonly called sruvavrksa is a medicinal plant widely used in Ayurveda. As per the Ayurvedic literature, stem bark of this plant is used in raktavikara, sopha, dusta, vrana. Removal of stem bark from trunk of this tree may make this plant weak and susceptible to damage by insects and natural elements. Due to which availability of this plant may be difficult in near future for use in Indian system of medicine. Present study is carried out in *Flacourtia indica* to evaluate the possibilities of using small branches in place of stem bark which will help sustainable utilization. Stem bark and small branches of *Flacourtia indica* are compared on the basis of physicochemical analysis, phytochemical analysis and high performance thin layer chromatography (HPTLC). On phytochemical analysis, both stem bark and small branches showed presence of almost similar phytochemicals in different extracts tested. Total phenolic contents of stem bark and small branches in terms of gallic acid equivalent were 39.62 ± 0.93 and 17.41 ± 0.94 mg/g, respectively and total flavonoid contents in terms of querecetin equivalent were 94.57 ± 2.81 and 43.68 ± 1.11 mg/g, respectively. HPTLC profile of n-hexane, ethyl acetate and ethanol extracts of stem bark and small branches also showed almost similar phytochemical profile. Almost similar results of phytochemical analysis and HPTLC profiles suggest that small branches may be used in place of stem bark and vice-versa after comparison and confirmation of same for pharmacological activities.

Keywords: *Flacourtia indica*, physicochemical analysis, phytochemical analysis, HPTLC profile.

INTRODUCTION

Medicinal plants as potential source of therapeutics aids are playing a significant role in providing health care all over the world for both humans and animals not only in the diseased condition but also as potential material for maintaining proper health. There are many medicinal plants which are slow-growing forest trees, and stem bark of which is the part mainly utilized. It is difficult to get huge amount of stem bark from the big tree because removal of the stem bark from the trunk of the tree makes the plant weak and susceptible to damage by insects and natural elements. The usages of stem barks of the trunk are therefore forbidden with an aim to conserve and protect the medicinal plants from extinction and make them available for future generation. Because of this manufacturers and dealers of Ayurveda, Siddha and Unani drugs face the difficulty in getting the regular supply of stem bark of big trees. Therefore, the management of traditional medicinal plant resources has become a matter of urgency. An approach which would satisfy the necessities of sustainable harvesting, yet simultaneously provide for health care needs, would be the substitution of bark or underground parts with aerial part of the same plant.

Flacourtia indica (Family: Flacourtiaceae) commonly called sruvavrksa is a medicinal plant widely used in Ayurveda. As per the Ayurvedic literature, stem bark of this plant is used in raktavikara, sopha, dusta, vrana [1]. The bark is also reported for antioxidant[2] and antimalarial activity [3]. The stem bark mainly contains glycoside flacourtin [4]. Removal of stem bark from trunk of this tree may make this plant weak and susceptible to damage by insects and natural elements. Due to which availability of this plant may be difficult in near future for use in Indian system of medicine. Present study is carried out in *F. indica* to evaluate the possibilities of using small branches in place of stem bark which will help sustainable utilization.

MATERIAL AND METHODS

Plant material

The stem bark and small branches of *F. indica* were collected from Gwalior, identified and authenticated by the botanist of NRIASHRD, Gwalior.

Instrumentation

A CAMAG HPTLC system (Muttenz, Switzerland) equipped with a semi automatic TLC applicator Linomat IV, twin trough plate development chamber, Win CATS software version 1.4.2. and Hamilton (Reno, Nevada, USA) Syringe (100 µl).

Material and reagents

All chemicals, reagents and solvents used during the experimentation were of analytical grade and HPTLC plates were purchased from E. Merck Pvt. Ltd. (Mumbai, India).

Physicochemical parameters

Stem bark and small branches were studied for various physicochemical standards like foreign matter, loss on drying at 105°C, total ash, acid-insoluble ash, alcohol soluble extractive, water-soluble extractive and p^H 10% solution using standard methods [5,6].

Preliminary phytochemical screening

n-Hexane, ethyl acetate and ethanol extract of both stem bark and small branches were screened for the presence of phenol, tannins, carbohydrates, saponins, alkaloids, proteins, flavonoids, phytosterol, furanoids, coumarin and quinone by the methods described by Harborne[5] and Kokate *et al* [6].

Estimation of total phenolic and flavonoid content

Five grams of each of the shade-dried plant material was pulverized into coarse powder and subjected to ethanolic extraction using soxhlet apparatus. The extracts were concentrated to dryness. The dried residues were then dissolved in 100 ml of 95% ethanol. The extracts were used for total phenolic and flavonoid assay.

The total phenolics content was determined by using the Folin-Ciocalteu assay [7]. An aliquot (1 ml) of extracts or standard solution of gallic acid (20, 40, 60, 80 and 100 µg/ml) was added to a 25 ml volumetric flask, containing 9 ml of distilled water. A reagent blank was prepared using distilled water. One millilitre of Folin-Ciocalteu phenol reagent was added to the mixture and shaken. After 5 min, 10 ml of 7% Na_2CO_3 solution was added to the mixture. The volume was then made up to the mark. After incubation for 90 min at room temperature, the absorbance against the reagent blank was determined at 550 nm with an UV/Vis spectrophotometer. Total phenolics content was expressed as mg gallic acid equivalents (GAE).

Total flavonoid content was measured by the aluminum chloride colorimetric assay [8]. An aliquot (1 ml) of extracts or standard solutions of quercetin (20, 40, 60, 80 and 100 µg/ml) was added to a 10 ml volumetric flask containing 4 ml of distilled water. To the flask, 0.30 ml of 5% $NaNO_2$ was added and after 5 min, 0.3 ml of 10% $AlCl_3$ was added. After 5 min, 2 ml of 1M NaOH was added and the volume was made up to 10 ml with distilled water. The solution was mixed and absorbance was measured against the blank at 510 nm. The total flavonoid content was expressed as mg quercetin equivalents (QE).

HPTLC profiles

HPTLC studies were carried out following the method of Sethi [9], Stahl[10] and Wagner *et al* [11]. The stem bark and small branches were powdered coarsely. Ten gram powdered samples of each of stem bark and small branches were accurately weighed and exhaustively extracted by *n*-hexane, ethyl acetate and ethanol (each 100 ml) separately using soxhlet apparatus. The extracts were filtered and concentrated under reduced pressure and made up to10 ml in standard flasks separately.

The mobile phase used for developing the *n*-hexane, ethyl acetate and ethanol extracts of stem bark and small branches was toluene: ethyl acetate 8:2 (v/v).

The samples were spotted in the form of bands of width 10 mm with a 100 µl Hamilton syringe on aluminum TLC plates pre-coated with Silica gel 60 F_{254} of 0.2 mm thickness with the help of TLC semi-automatic applicator Linomat IV attached to CAMAG HPTLC system, which was programmed through Win CATS software version 1.4.2. 10 µl of each extracts of stem bark and small branches were applied in two tracks as 10 mm bands at a spraying rate of 10 seconds/µl. Track 1 was stem bark and track 2 was small branches for each of the extracts applied.

Development of the plate up to a migration distance of 80 mm was performed at 27 ± 2°C with mobile phase for each extracts in a CAMAG HPTLC chamber previously saturated for 30 min. After development the plate was dried at 60°C in an oven for 5 min and visualized under wavelength 254 nm and 366 nm for ultra violet detection. The developed plate was then dipped in anisaldehyde sulphuric acid reagent for derivatization and dried at 105°C in hot air oven till the colour of the band appears and visualized under white light. Images were captured by keeping the plates in photodocumentation chamber and R_f values were recorded by Win CATS software [12].

RESULTS AND DISCUSSION

Physicochemical parameters like foreign matter, loss on drying at 105º C, ash values, acid insoluble ash, extractive values and p^H values are given in Table 1. These data can be used for identification of the drug. Both the parts of *F. indica* were found to possess little moisture and hence can be stored at room temperature without fear of spoilage. Approximately same value for alcohol soluble and water soluble extractives for both stem bark and small branches indicates the presence of approximately same amount of polar and non polar extractable compounds in stem bark and small branches.

The results of phytochemical analysis of different extracts of stem bark and small branches are shown in Table 2. Proteins were found to be present in hexane extract of both stem bark and small branches. Similarly in ethyl acetate extract tannins, carbohydrates, proteins, flavanoids, quinone and furanoids were found present in both stem bark and small branches. In ethanol extract phenols, tannins, carbohydrates, proteins, flavanoids, coumarin, quinone and furanoids were found present in both stem bark and small branches while saponins were found present only in stem bark. Alkaloids and steroids were found to be absent in all the extracts tested.

Total amount of phenolics and flavonoids content of ethanoilc extract of stem bark and small branches of *F. indica* are summarized in Table 3. Results indicate that in comparison to small branches, stem bark had the high total phenolic and flavonoid content.

Comparative HPTLC profile of *n*-hexane, ethyl acetate and ethanol extracts of stem bark and small branches of *F. indica* were recorded to reveal the chemical pattern of each extract.

Table 1: Physicochemical parameters of stem bark and small branches of *F. indica*

S. No.	Parameters	Results	
		Stem bark	Small branches
1.	Foreign matter (% w/w)	Nil	Nil
2.	Loss on drying (% w/w)	6.30	6.36
3.	Total ash (% w/w)	12.01	4.83
4.	Acid insoluble ash (% w/w)	0.61	0.15
5.	Alcohol soluble extractive value (% w/w)	7.41	5.98
6.	Water soluble extractive value (% w/w)	11.03	8.21
7.	pH of 10 % aqueous solution	5.52	5.00

Table 2: Phytochemical analysis of extracts of stem bark and small branches of *F. indica*

Phytochemicals	Stem bark			Small branches		
	n-Hexane	Ethyl acetate	Ethanol	*n*-Hexane	Ethyl acetate	Ethanol
Phenols	-ve	-ve	+ve	-ve	-ve	+ve
Tannins	-ve	+ve	+ve	-ve	+ve	+ve
Alkaloids	-ve	-ve	-ve	-ve	-ve	-ve
Carbohydrates	-ve	+ve	+ve	-ve	+ve	+ve
Saponins	-ve	-ve	+ve	-ve	-ve	-ve
Proteins	+ve	+ve	+ve	+ve	+ve	+ve
Steroids	-ve	-ve	-ve	-ve	-ve	-ve
Flavanoids	-ve	+ve	+ve	-ve	+ve	+ve
Coumarin	-ve	-ve	+ve	-ve	-ve	+ve
Quinone	-ve	+ve	+ve	-ve	+ve	+ve
Furanoids	-ve	+ve	+ve	-ve	+ve	+ve

Table 3: Total phenolic and total flavonoid content of ethanol extracts of stem bark and small branches of *F. indica*

S. No.	Plant parts	Total phenolics mg of GAE/g dry weight*	Total flavonoids mg of QUE/g dry weight*
1.	Stem bark	39.62 ± 0.93	94.57 ± 2.81
2.	Small branches	17.41 ± 0.94	43.68 ± 1.11

*Values are expressed as Mean ± SD

The HPTLC profile of *n*-hexane extract of both stem bark and small branches (Table 4 and Figure 1) showed no band when visualized under UV at 254 nm. At UV 366, both stem bark and small branches showed four bands at R_f 0.52 (florescent blue), 0.55 (red), 0.59 (florescent blue), 0.65 (red). Visualization under white light after derivatization with anisaldehyde sulphuric acid reagent, both stem bark and small branches showed five bands at R_f 0.30 (blue), 0.37 (blue), 0.45 (blue), 0.56 (blue), 0.86 (blue). Similar HPTLC profile under UV at 254 nm, 366 nm and after derivatization with anisaldehyde sulphuric acid reagent indicates the presence of almost similar compounds in hexane extract of stem bark and small branches.

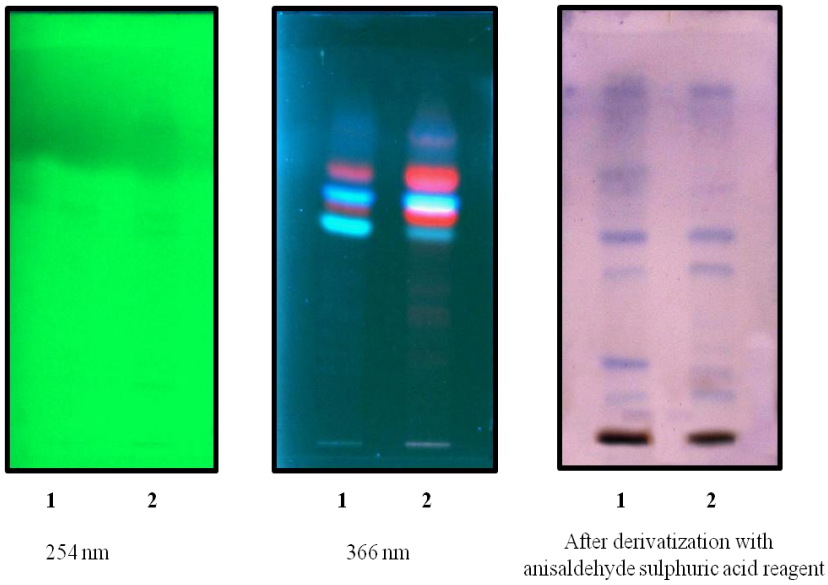

Figure 1: HPTLC profile of n-hexane extracts of stem bark and small branches of *F. indica*. (track 1: stem bark, track 2: small branches)

Table 4: Rf value of n-hexane extract of *F. indica*

S. No.	Wavelength	Stem bark	Small branches
1.	254 nm	No band	No band
2.	366 nm	0.52, 0.55, 0.59, 0.65	0.52, 0.55, 0.59, 0.65
3.	Visible light after derivatization	0.30, 0.37, 0.45, 0.56, 0.86	0.30, 0.37, 0.45, 0.56, 0.86

The HPTLC profile of ethyl acetate extract of stem bark and small branches (Table 5 and Figure 2) also showed no band when visualized under UV at 254 nm. At UV 366 both stem bark and small branches showed four bands at R_f 0.25 (red), 0.57 (florescent blue), 0.61 (red), 0.70 (red). Visualization under white light after derivatization with anisaldehyde sulphuric acid reagent, both stem bark and small branches showed five bands at R_f 0.15 (blue), 0.34 (blue), 0.42 (blue), 0.55 (blue), 0.85 (blue). Again similar HPTLC profile of ethyl acetate extract under UV 254 nm, 366 nm and after derivatization with anisaldehyde sulphuric acid reagent indicates the presence of almost similar compounds in ethyl acetate extract of stem bark and small branches.

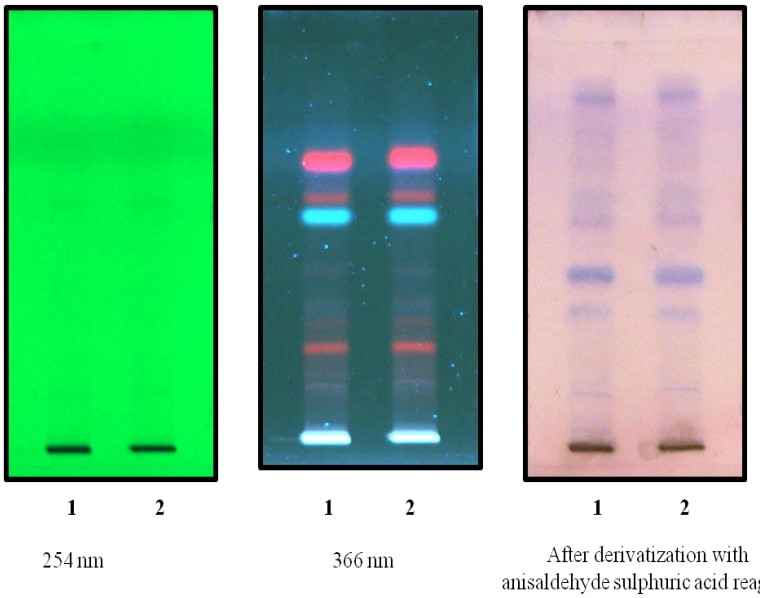

Figure 2: HPTLC profile of ethyl acetate extracts of stem bark and small branches of *F. indica*. (track 1: stem bark, track 2: small branches)

Table 5: R_f value of ethyl acetate extract of *F. indica*

S. No.	Wave-length	Stem bark	Small branches
1.	254 nm	No band	No band
2.	366 nm	0.25 , 0.57 , 0.61 , 0.70	0.25 , 0.57 , 0.61 , 0.70
3.	Visible light after derivatization	0.15 , 0.34, 0.42 , 0.55, 0.85	0.15, 0.34, 0.42, 0.55, 0.85

HPTLC profile of ethanol extract of stem bark and small branches (Table 6 and Figure 3) also showed no band in both stem bark and small branches when visualized under UV at 254 nm. At UV 366 both stem bark and small branches showed five bands at R_f 0.44 (red), 0.62 (florescent blue), 0.71 (blue), 0.74 (red), 0.77 (red). Visualization under white light after derivatization with anisaldehyde sulphuric acid reagent, stem bark and small branches both showed five and four bands respectively, out of which four bands at R_f 0.37 (Blue), 0.44 (Blue), 0.52 (Blue), 0.88 (Blue) were found similar indicating the presence of at least four similar compounds in ethanol extract of stem bark and small branches.

Table 6: R_f value of ethanol extract of *F. indica*

S. No.	Wave-length	Stem bark	Small branches
1.	254 nm	No band	No band
2.	366 nm	0.44, 0.62, 0.71, 0.74, 0.77	0.44, 0.62, 0.71, 0.74, 0.77
3.	Visible light after derivatization	0.37, 0.44, 0.52, 0.76, 0.88	0.37, 0.44, 0.52, 0.88

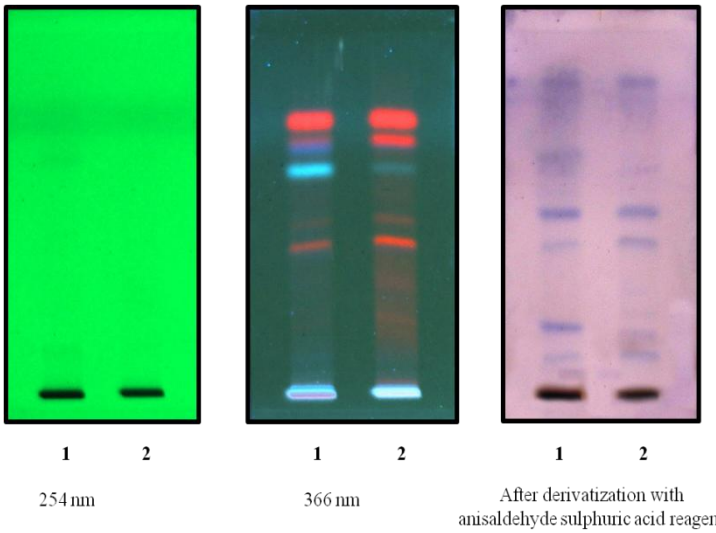

Figure 3: HPTLC profile of ethanol extracts of stem bark and small branches of *F. indica*. (track 1: stem bark, track 2: small branches)

CONCLUSION

The present study carried out in *F. indica* to evaluate the possibilities of using small branches in place of stem bark will help sustainable utilization. Almost similar results for phytochemical analysis and HPTLC profiles of stem bark and small branches of this plant indicates the presence of almost similar compounds in both the parts of this plant. Therefore small branches may be used in place of stem bark and vice-versa after comparison and confirmation of same pharmacological activities. The results of qualitative evaluation of HPTLC profile will also be helpful in the identification and quality control of the drug and can provide standard HPTLC profiles with selected solvent system. The HPTLC profile can also be used as a reference for the proper identification/ authentication of the drug.

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