



Research Article

HPTLC analysis of berberine in stem extracts of *Fibraurea darshani*

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Abstract: The present study is a first report on the phytochemical analysis of the plant *Fibraurea darshani* which is endemic to Western Ghats. The plant is a woody dioecious climber belonging to the family Menispermaceae. Preliminary phytochemical screening of methanolic extracts of the stem of *F. darshani* revealed the presence of secondary metabolites like alkaloids, carbohydrates, anthraquinones, terpenoids, flavonoids, phenolics, sterols etc. A simple and reproducible high performance thin layer chromatography was developed to evaluate the presence of berberine in methanol extract of stem of *F. darshani*. This method involves separation of compounds by HPTLC on pre-coated silica gel 60F₂₅₄ plates with a solvent system of Chloroform: Ethyl acetate: Methanol: Formic acid (4:5:4:0.3) and scanned using densitometric scanner in UV reflectance photo mode at 254 and 366nm. The R_f values (0.97) for berberine in the plant sample and the reference standard were found comparable under UV light at 366nm. The HPTLC method developed was simple, accurate and specific.

Keywords: Berberine, *Fibraurea darshani*, HPTLC, Methanolic extract, Phytochemical screening

Introduction

The genus *Fibraurea* Lour. belonging to the family Menispermaceae consists of two species namely *Fibraurea recisa* Pierre. and *Fibraurea tinctoria* Lour. and is mostly restricted to East and South East Asia including Andaman and Nicobar islands (Forman, 1985; Kubitzki *et al.*, 1993; Pramanik, 1993; Mabberley, 2005). *Fibraurea* species have been used in traditional medicines of Thailand and other Asian countries like China (Perry and Metzger, 1980). The stem of *F. recisa* is an effective antifungal herb, and its major active component was reported to be alkaloidal water-soluble berberines (Rao *et al.*, 2009). Berberine (B₁) is an isoquinoline alkaloid reported to have antidiabetic, antipyretic, antimalarial, antiprotozoal (*Leishmania*), antimicrobial and antitumour activities (Patani, 2002). Berberine has been tested and used successfully in experimental and human diabetes and found to lower elevated glucose level as effectively as metformin (Yin *et al.*, 2008). The alkaloids pseudocolumbamine, palmatrubine, magnoflorine and dehydrocorydalmine were isolated and identified by their spectral data from the root and stem bark of *F. tinctoria*. The extracts from the stem of *F. tinctoria* showed antioxidant activity on DPPH radical and also exhibited cytotoxic activity against brine shrimp and human cancer cell line MCF-7 (Keawpradub *et al.*, 2005).

Fibraurea darshani Udayan & Ravikumar is a relatively new species reported from Karnataka and Kerala states of South India. *F. darshani* is a woody dioecious climber found in semi ever green forests.

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Flowering and fruiting is observed in January-April months. *F. darshani* is an endangered plant endemic to Western Ghats (Udayan *et al.*, 2007).

The popularity of herbal drugs in the global health care has increased due to its remarkable efficacy in the treatment of various ailments. Some 120 chemicals extracted in pure form from about 90 species of higher plants are used in medicines throughout the world; an extremely wide range of plant species being used medicinally at a local level (Dar and Farooq, 1997). Specific guiding principles has been developed by the World Health Organization to support the associated countries to instigate nationalized policies on plant based drugs and to study their prospective safety, efficacy and quality, as a prerequisite for global harmonization (Calixto, 2000). The plant extracts are subjected to preliminary phytochemical analysis using standard chemical methods to unfold the diverse classes of chemical constituents present and the identification of different constituents is based on the property of selective reactivity of phytochemicals present in an extract (WHO, 1998).

The major problem of quality assurance of herbal medicine has been solved to a great extent with the help of chromatographic print analysis and is considered as an effective method in discovering bioactive profile of plants of therapeutic importance. High performance thin layer chromatography (HPTLC) is an invaluable separation technique available today as an analytical

quality assessment tool for the rapid evaluation of botanical materials. HPTLC fingerprint profiles are preferred to identify the transparency and potency of herbal formulations due to its accuracy, precision and reproducibility along with its economical mobile phase consumption (Pattanaya *et al.*, 2010). The present study is a first report on the phytochemical analysis of *F. darshani* and a simple, reproducible high performance thin layer chromatography was developed to evaluate the presence of berberine in the methanolic stem extracts of the plant.

Materials and Methods

Collection and identification of plant material

The stem cuttings of *F. darshani* were collected from Vellanipacha forests in Thrissur district. The plant samples were authenticated by taxonomist Dr. P. S. Udayan at Sree Krishna College, Guruvayur, Kerala. Voucher specimens were maintained for further reference.

Preparation of plant extracts

Matured stem cuttings of plants were thoroughly washed in running tap water and then rinsed in distilled water to remove the adherent soil. The stem was then cut into small pieces of 1-1.5 cm and shade dried for 5-6 days and powdered into fine powder. 20 gm of the powdered stem sample of *F. darshani* was taken separately in clean conical flasks and extracted with 200 ml of methanol by placing it in a magnetic stirrer for 12 hours. The extract obtained was filtered through Whatmann filter paper 1 and the process was repeated for three days till exhaustion. The fraction was evaporated to dryness and a semisolid sticky extract was obtained which was stored at 4°C in air-tight bottle for further use.

Chemical and reagents

All the chemicals, were of analytical grade from Merck Pvt. Ltd., Mumbai. Berberine was obtained from Sigma Aldrich, India. The HPTLC was performed on 20cm×20cm plates of silica gel 60F₂₅₄ (Merck KGaA, Darmstadt, Germany).

Preliminary phytochemical screening

The preliminary phytochemical analysis was performed on the methanolic extract of the stem samples following the standard procedures as described by Harborne to identify the active constituents (1973).

Tests for carbohydrates

Molisch's Test: To 2ml of extract 2-3 drops of alpha naphthalene solution in alcohol was added, shaken for 2 min and 1 ml of concentrated sulphuric acid was added slowly from the sides of the test tube. A deep violet colour at the junction of two layers indicates the presence of carbohydrates.

Test for reducing sugars

Fehling's Test: Filtrates were hydrolysed with dil. hydrochloric acid, neutralized with alkali and heated with 1ml each of Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

Benedict's test: Filtrates (2ml) were treated with Benedict's reagent in attest tube and heated gently in boiling water bath for 10min. Changes in colour to yellow, green and orange red precipitate indicates the presence of reducing sugars.

Tests for glycosides

Legal's Test: 2 ml of extract was treated with 1 ml sodium nitropruside in pyridine (1ml) and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

Keller-Killiani test: To the test tubes containing 2 ml of extract, 1 ml of glacial acetic acid, 3 drops 5% W/V ferric chloride and concentrated sulphuric acid were added and observed. Appearance of reddish brown ring at the junction of the liquids indicates the presence of cardiac glycosides.

Test for Alkaloids

Mayer's Test: To 3 ml of the extract, 1ml of Mayer's reagent (potassium mercuric iodide) was added. The appearance of white precipitate indicates the presence of alkaloids.

Wagner's Test: To 3 ml of filtrate, 1ml of Wagner's reagent (iodine in potassium iodide) was added. The appearance of reddish brown precipitate indicates the presence of alkaloids.

Dragendorff's Reagent: To 2 mg of the methanolic extract 5 ml of distilled water was added, 2M Hydrochloric acid was added until an acid reaction occurs. To this 1 ml of Dragendorff's reagent was added. Formation of orange or orange red precipitate indicates the presence of alkaloids.

Test for Saponins

Foam Test: The extract (2g) was shaken vigorously with 20 ml of water and observed for persistent foam, which indicates the presence of saponins.

Test for flavonoids

Shinoda Test: To a small quantity of test residue, 5 ml of ethanol (95% v/v), 5 drops of hydrochloric acid and 0.5g of magnesium turnings were added. Appearance of pink, crimson or magenta colour indicates the presence of flavonoids.

Test for Triterpenoids and Sterols

Liebermann Burchard Test: To the chloroform solution of the extract, 3-4 drops of acetic anhydride was added and mixed well. To this 5 ml of concentrated sulphuric acid was added from the sides of the test tube. A brown reddish ring appears at the junction of two layers. The upper layer showing greenish blue colour indicates the presence of triterpenoids or sterols.

Salkowaski's Test: To the chloroform solution of the extract, few drops of concentrated sulphuric acid was added, shaken and allowed to stand. Appearance of reddish brown colour of the interface indicates the presence of triterpenoids or sterols.

Test for Anthraquinones

Borntrager's Test: Borntrager's test is employed for presences of anthraquinones. The extract is boiled with dilute sulphuric acid, filtered and to the filtrate benzene, or ether or chloroform is added and shaken well. The organic layer is separated to which ammonia is added slowly. The ammoniacal layer shows pink to red color due to presences of anthraquinone glycosides.

Test for Tannins and Phenols

Ferric chloride Test: To 3 ml of extract, 3 ml of 5% w/v ferric chloride solution was added. A dark green or deep blue colour indicates the presence of tannins and phenols.

High Performance Thin layer Chromatography (HPTLC)

Preparation of standard and sample solution

The standard solution was prepared containing known concentration by dissolving 1mg standard of berberine in 10 ml of chromatographic grade methanol (0.1 mg/ml). Concentrated plant extracts were filtered and stock solution containing 10mg of plant extract in 10 ml methanol was prepared (1.0 mg/ml).

Development of the optimum mobile phase

The mobile phase composition for development of chromatographic method was optimized by testing different solvent mixtures of varying polarity in different ratios. The satisfactory resolution was obtained with the solvent system consisting of Chloroform: Ethyl acetate: Methanol: Formic acid 4:5:4:0.3 (v/v/v/v).

Chromatographic conditions

Chromatography was performed on precoated silica gel Merck 60 F₂₅₄ HPTLC plates of 0.2mm thickness (20x20cm). 10µl of methanolic extracts of plant samples and standard compound berberine were applied to the plate positioned at 10 mm from the bottom using a CAMAG automated TLC applicator (automated spray-on applicator equipped with a 100µl syringe operated with a band width 8mm, distance between band 15mm and distance from the side edge of plate 15mm). Plate was eluted in pre-saturated twin trough chamber (CAMAG ADC₂) in ascending mode at room temperature (25 ± 2°C) with the mobile phase Chloroform: Ethyl acetate: Methanol: Formic acid (4:5:4:0.3). The length of the chromatogram run was 80mm. After complete development, the TLC plates were dried on CAMAG TLC plate heater III at 105°C for 5 minutes. After drying the spots were visualized

under CAMAG UV cabinet 254 and 366 nm. Then the plates were scanned in the densitometer by linear scanning using CAMAG TLC scanner III equipped with WINCATS software (CAMAG). The identification of berberine in the methanolic extracts of stem of the plants was confirmed by superimposing the UV spectra of samples and standards within the same retardation factor (R_f) value.

Results and Discussions

The present study is a first report on the phytochemical analysis of the endemic plant *F. darshani*. The preliminary phytochemical screening carried out with the methanolic extract of stem provides evidence that the plant is a potent source of secondary metabolites like alkaloids, terpenoids, sterols, carbohydrates, reducing sugars, anthraquinones, phenols, tannins, terpenoids, sterols and flavonoids which are medicinally important bioactive compounds that can be used for novel drug discovery in the pharmaceutical sector for the treatment of various diseases. Glycosides and saponins were found to be absent in methanol extracts from stem of *F. darshani* (Table 1).

HPTLC fingerprint profiles are important parameters of herbal drug standardization for the proper identification of medicinal plants. Different mobile phase compositions were employed to achieve good separation for optimization of method. The solvent system containing Chloroform: Ethyl acetate: Methanol: Formic acid (4:5:4:0.3) (v/v/v/v) resulted in good resolution of berberine in the presence of other compounds in plant extract of *F. darshani* (Fig. 1). The R_f value (0.97) for berberine in reference standard and plant sample of *F. darshani* was found comparable under UV light at 366 nm (Fig. 2&3). A number of protoberberine alkaloids and an aporphine alkaloid, magnoflorine, were isolated and characterized in previous phytochemical studies of *F. tinctoria* (Siwon et al., 1981). Three protoberberine alkaloids, berberine chloride, berberrubine chloride and thalifendine chloride has been reported to be isolated from the roots of *F. tinctoria* Lour. and was found to show significant cytotoxic activity with one or more human cancer cell-lines (Jin Rui et al., 1993). The compounds palmatine, berberine, jatrorrhizine, palmatrubine, 7, 8-dihydro-8-hydroxyberberine and groenlandicine has been reported from related species *F. recisa* Pierre. (He et al., 2009).

This standardisation method will provide rapid, cost effective and specific method to develop and identify the biomarker berberine in *F. darshani* and assist in the characterisation, selection, multiplication and conservation of genetically

superior planting material of assured uniformity and desired quality.

Table 1. Phytochemical screening in methanolic extracts of stem of *Fibraurea darshani*.

Phytochemical tests	<i>Fibraurea darshani</i>
Test for Carbohydrates	
Molisch's test	+
Test for Reducing Sugar	
Fehling's test	+
Benedict's test	+
Test for Glycosides	
Keller-Killani test	-
Test for Anthraquinones	
Benzene+ammonia	+
Test for Alkaloids	
Mayer's reagent	++
Wagner's test	++
Baeyer's test	++
Test for Saponins	
Froth formation test	-
Test for Sterols and Terpenoids	
Liebermann Burchard test	+
Salkowski's test	++
Test for Tannins and Phenolics	
Ferric chloride test	-
Iodine test	+
Test for Flavonoids	
Alkaline reagent test	++

++Prominently present, + Moderately present, - Absent

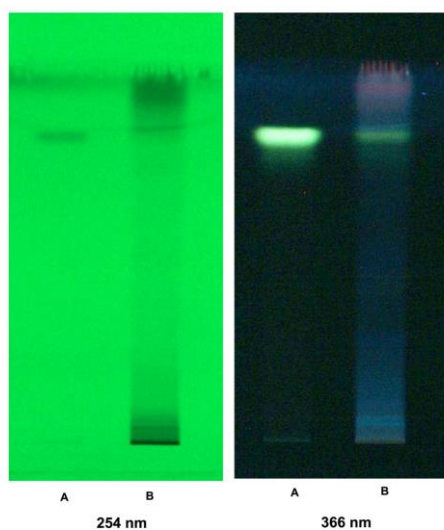


Figure 1. HPTLC profile of standard Berberine (A) and *Fibraurea darshani* (B) at 254 and 366nm

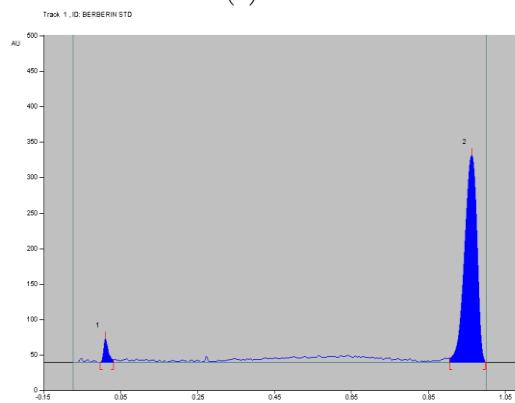


Figure 2. Chromatogram of Berberine at 366nm

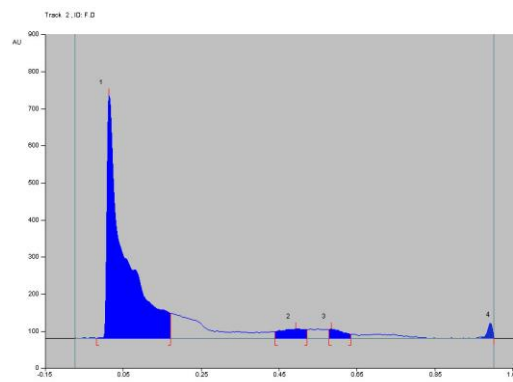


Figure 3. Chromatogram of methanolic extract of stem of *Fibraurea darshani* at 366nm

Conclusion

Various forms of traditional, complementary and alternative medicines are playing progressively more important role in health care globally. Therefore their safety, efficacy, and quality control are important concerns today. In India, medicinal plants are used for various therapeutic purposes in Ayurvedic and folk medicines. Over the years, due to the increased demand and over-exploitation, the much needed medicinal plants have become rare or even depleted from their natural habitats. Moreover, the botanical source of a raw drug is often attributed to one species in most of the Indian systems of medicines. The continuous extraction of a particular species will lead to its depletion as well as the loss of biodiversity of their habitats. Also the herbal industry is facing a major threat in the production of commercial natural products due to adulteration and substitution of medicinal plants. To an extent the use of related species with accurate scientific evaluation can overcome this problem. Hence, the identification of plants and its chemical constituents in the phytochemical industry is an important criteria.

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References

1. Calixto BJ. Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). *Brazilian Journal of Medical and Biological Research* 33(2000): 179-89.
2. Dar GH and Farooq S. How Diverse is Biodiversity! Do we know? *Oriental Science*. 2.1 (1997): 51-69.
3. Forman LL. A revision of tribe *Fibraureae* (Menispermaceae) in Asia. *The Menispermaceae of*

- Malaysia and adjacent areas XIII. *Kew Bulletin* 40 (1985): 539–551.
4. Harborne JB. *Phytochemical Methods*. Chapman and Hall Ltd, London (1973): 49-188.
 5. He R, Ma HF, Yang W, Su ZL and Zhao YF. Study on the determination of alkaloid in *Fibraurea recisa* Pierre with high performance liquid chromatography. *Anhui Nong Ye Ke Xue* 37 (2009): 9980–9981.
 6. Jin Rui D, Heebyung C, John MPA, Douglas K, Soefjan T and Kosasih P. Cytotoxic constituents of the roots of the Indonesian medicinal plant *Fibraurea chloroleuca*. *Phytotherapy Research* 7 (1993): 290-294.
 7. Keawpradub N, Dej-adisai, S and Yuenyongsawad S. Antioxidant and cytotoxic activities of Thai medicinal plants named Khaminkhruea: *Arcangelisia flava*, *Coscinium blumeianum* and *Fibraurea tinctoria*. *Songklanakarin Journal of Science and Technology* 27 (2005): 455-467.
 8. Kubitzki K, Rohwer JG, and Bittrich V. The families and genera of vascular plants, Vol. II. Springer-Verlag, Berlin Heidelberg, Germany (1993).
 9. Mabberle DJ. *Mabberley's plant-book: A portable dictionary of the vascular plants, their classification and uses*. 3rd edition. Cambridge University Press (2008).
 10. Patani A, *Indian Herbal Pharmacopoeia*. Revised New ed. Indian Drug Manufacturers Association, Mumbai (2002).
 11. Perry LM and Metzger J, *Medicinal plants of East and Southeast Asia Attributed Properties and Uses*, Vol. 620, MIT Press, Cambridge, London (1980).
 12. Pramanik A and Gangopadhyay M. *Flora of India*, Vol.1. Botanical Survey of India, Calcutta (1993).
 13. Rao GX, Zhang S, Wang HM, Li ZM, Gao S, Xu GL. Antifungal alkaloids from the fresh rattan stem of *Fibraurea recisa* Pierre. *Journal of Ethnopharmacology* 123.1 (2009):1-5.
 14. Siwon J, Verpoorte R and Baerheim SA. Studies on Indonesian medicinal plants. VI. Further alkaloids from *Fibraurea chloroleuca*. *Planta Medica* 41 (1981): 6568.
 15. Udayan PS, Ravikumar K, Ved DK and Udaiyan K, *Fibraurea darshani*, A new species of Menispermaceae from the Western Ghats, India. *Rheedea* 17 (2007): 9-12.

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