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Morphological, Phytochemical Screening and HPLC Profiling of *Fibraurea darshani* (Menispermaceae), An Endemic Species to Western Ghats

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A B S T R A C T

The genus *Fibraurea* which belongs to the family Menispermaceae consist of three species *Fibraurea recia* Pierre., *Fibraurea tinctoria* Lour. and *Fibraurea darshani* Udayan and Ravikumar and they are mostly found in South East Asia. Among these *Fibraurea darshani* is a relatively new species reported from Kerala and Karnataka and also it is an endangered plant which is endemic to Western Ghats. The present study deals with the morphological, phytochemical analysis and HPLC profiling of the plant *Fibraurea darshani*. *F. darshani* is a dioecious woody climber found mostly in semi evergreen forest. The leaves are coriaceous with truncate base and umbellate racemes inflorescences with short peduncles and pedicels. Preliminary phytochemical screening of methanolic and chloroform extracts revealed the presence of secondary metabolites such as alkaloids, terpenoids, flavonoids, phenolics, steroids. Quantitative phytochemical analysis showed that species contained more phenolic compounds followed by alkaloids and terpenoids. The HPLC study showed that methanol leaf extracts contained berberine compounds. This standardization method identifies the compound berberine in *F. darshani* and helps in characterization and conservation of plant. This justifies the use of these plants as drugs to treat various ailments.

***Key words*:** Morphological, Phyochemical, HPLC, Berberine, Menispermaceae

The Family Menispermaceae comprises of 71 genera with 450 species [1]. Menispermaceae are mostly dioecious climbling plants. The main feature used to define the family is the curved seed found in many of the genera, hence the name moonseed family. The genus *Fibraurea* consist of three species *Fibraurea recisa* Pierre., *Fibraurea tinctoria* Lour. and *Fibraurea darshani* Udayan and Ravikumar and are mostly found in South East Asia [2-3].

Among these, *Fibraurea darshani* is a relatively new species reported from Kerala and Karnataka and also it is an endangered plant which is endemic to Western Ghats [4]. The stem of *Fibraurea recisa* Pierre. contain active alkaloidal component that can act as an effective antifungal agent. Bioassay tests revealed that the water-soluble berberines are the most important antifungal substances [5]. The stem of *Fibraurea tinctoria* are used in the treatment of syphilitic ulcers, wounds as antiseptic, hepatitis, dysentery, gastritis and the plant produce a yellow sap, which is used to dye mattings or clothes [6]. *F. tinctoria* stem extract showed significant antioxidant activity and cytotoxic activity against brine shrimp and human cancer cell line MCF-7 [7]. Morphological and epidermal characters are also given importance to identify and classify different plant species. Anatomical character provides many evidences that shows inter relationships and affinities to uncertain taxonomic status.

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MATERIALS AND METHODS

Morphological studies

Aerial parts (stem and leaves) of *Fibraurea darshani* were collected from Western Ghats. The healthy plants were selected for the morphological investigation. The specimens were identified with the help of floras, Revisions and Monographs and also referred to experts for accurate identification.

Phytochemical studies

Preparation of plant extract

The collected plant parts were cleaned, shade dried and powdered by a mechanical grinder. 10 g of the sample was

extracted in a Soxhlet apparatus using 150 ml of chloroform and methanol as the solvent, and the Soxhlet was run overnight until the sample loaded became colorless. After the Soxhlet extraction is over, extracts were put inside rotor evaporator until it gave the solidified residues. The sample extracts were collected, weighed and stored in a freezer.

Qualitative and quantitative analysis of secondary metabolites

The preliminary phytochemical analysis was performed using the standard procedures by Harborne to identify the active constituents [8]. Quantitative phytochemical study was done by standard procedure. The alkaloids estimation was performed by spectrophotometric method of Dragendroffs reagent as it was described by Sreevidya and Mehrotra [9]. The total terpenoid content was determined by spectrophotometric method. The phenolic estimation was performed by spectrophotometric method of Folin Cio-calteau reagent [10].

HPLC analysis

The analysis was carried out with Agilent 1260 series HPLC system (Agilent Technologies, Palo Alto, CA, USA) comprising a quaternary pump (Agilent Technologies 1260), a vacuum degasser, a variable wavelength detector and a 20 µl sample injector. The separation was performed on Zorbax Eclipse plus C18 column. The detection wavelength was set at 360 nm. The column thermostat was maintained at 30 ± 1°C.

Standard and sample preparations

1 mg of the standard was accurately weighed and dissolved in 1ml of mobile phase/methanol and different concentrations of standard were prepared by serial dilution. Methanolic extract was accurately weighed and dissolved with 1ml of mobile phase/ methanol.

RESULTS AND DISCUSSION

Morphological studies

Fibraurea darshani is a large climbing lianas and the wood is cream coloured and they produce latex when they cut. Leaves are simple, alternate and ovate-oblong with acuminate apex and trunicate base. Leaves are dark green, coriaceous and shining above, 3-5 veined at the base and 2-4 lateral veins on either side of the midrib (Fig 1). Petioles are long, slender and swollen near the ends. Inflorenscence are axillary, panicle and the inflorescence arise from the leafless older stem. Flowers are unisexual with shorter pedicle. In Male flower, tepals are 6-9, elliptic, concave with 6 stamens and the filaments are thick, collar like near the base with elongated anther (Fig 2). Female flowers are staminodes, ellipsoid ovary with one ovule. Fruits drupes, curved, short ellipsoid and glabrous. The species differs from its closely allied species, *F. tinctoria* Lour., in having non-tendrilliform young shoots, cream-coloured stem with watery sap [4].

Fig 1 Habit of *Fibraurea darshani*, Leaf morphology

Fig 2 Inflorescence and fruits

Phytochemical analysis

Qualitative and quantitative analysis of secondary metabolites

Phytochemical investigation of *Fibraurea darshani* in two different solvent systems revealed that chloroform extract shows more secondary metabolites than methanolic extracts (Table 2). The preliminary phytochemical investigation showed the presence of alkaloids, phenols, terpenoids, flavanoids, and steroids and similar observations were documented [7].

The study showed that the plant contains various bioactive molecules in different concentration in two different solvent system. The plant species contained more phenolic compounds (425 ± 0.79) followed by alkaloids (389.96 ± 0.25) and terpenoids (230.76 ± 0.80). The amount of phytochemical contents was more in chlorofomic extract than in methanolic extract (Table 3, Fig 1). A simple and reproducible high performance thin layer chromatography was developed to

evaluate the presence of berberine in methanol extract of stem of *Fibraurea darshani* [7].

Table 2 Preliminary phytochemical screening

Name of the test	Chloroform	Methanol
Tannin and phenolic compound	+	+
Terpenoid	+	+
Steroid	+	+
Glycosides	-	-
Quinone	-	-
Anthraquinone	-	+
Saponin	-	-
Alkaloid	+	+
Coumarin	-	-
Flavanoid	+	-
Resin	-	-

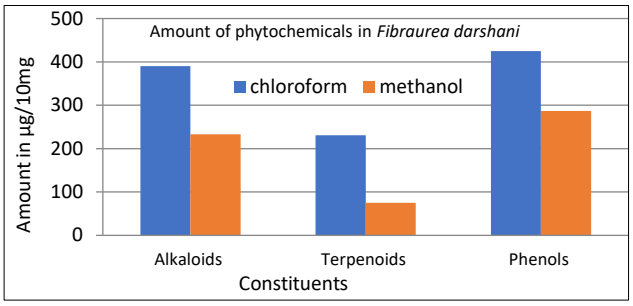

Fig 1 Graph showing the amount of phytochemicals

HPLC chromatogram analysis

The HPLC analysis of methanolic extract of leaf showed the presence of berberine (retention time-1.087, Concentration-43.0678) and the chromatogram were shown in fig 8. Mobile phase consisted of solvent A: Acetonitrile, Solvent B: 0.04 M Potassium dihydrogen phosphate buffer at a flow rate of 1.0 mL/min. A HPTLC was developed to evaluate the presence of berberine in methanol extract of stem of *Fibraurea darshani* using solvent system of Chloroform: Ethyl acetate: Methanol: Formic acid at 254 and 366nm [11]. Berberine chloride, berberrubine chloride and thalifendine chloride has been reported to be isolated from the roots of *Fibraurea darshani* Lour. and was found to show significant cytotoxic activity with human cancer cell-lines [12].

CONCLUSION

Fibraurea darshani is a dioecious woody climber found mostly in semi evergreen forest. Phytochemical study was also useful to isolate the pharmacologically active principles present in the drug. This justifies the use of these plants as drugs to treat various ailments.

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Table 3 Quantitative analysis of phytochemical compounds

Name of the plant	Solvent used	Amount of phytochemical contents (µg/10mg)		
		Alkaloids	Terpenoids	Phenols
Fibraurea darshani	Chloroform	389.96 ± 0.25	230.76 ± 0.80	425 ± 0.79
	Methanol	232.96 ± 0.81	75.26 ± 0.32	286.73 ± 0.94

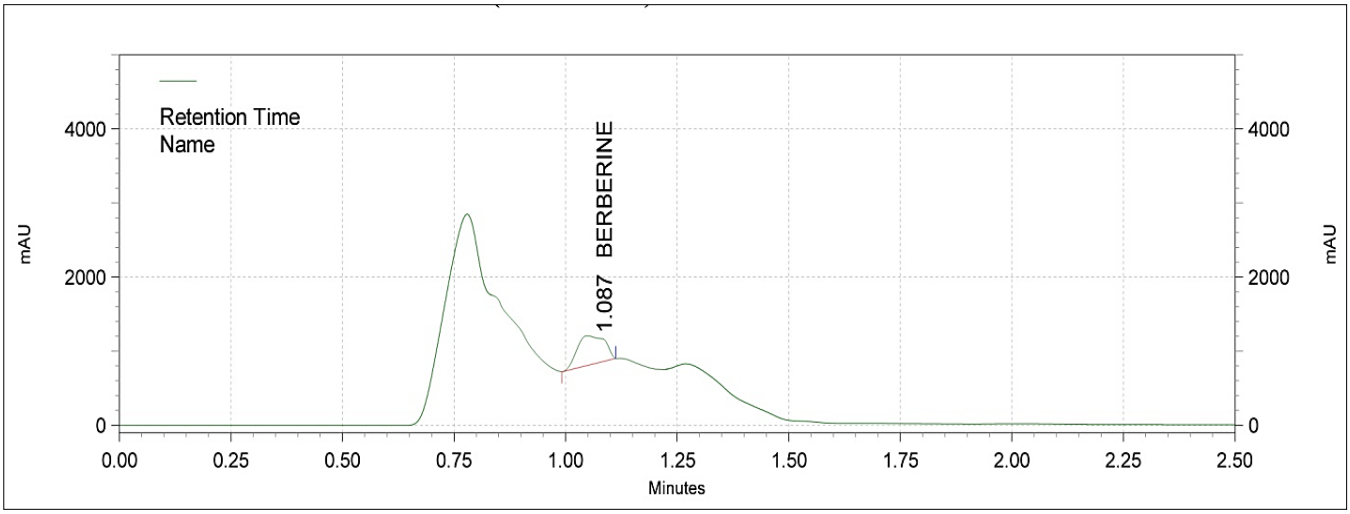

Fig 2 HPLC chromatogram of *Fibraurea darshani* in methanolic extract

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