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Research Article

Phytochemical Screening, HPTLC Fingerprint Analysis of Leaf Extract of *Bridelia montana* (Roxb) Willd

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ABSTRACT
This research analyzed the major chemical constituents present in the ethanolic extract of the leaves of *Bridelia montana (ROXB) WILLD*. The leaves of *Bridelia montana* were collected, powdered, de-fatted with n-hexane and then extracted with 90%ethyl alcohol. Preliminary phytochemical screening of the ethanolic leaf extract of *Bridelia montana* showed the presence of chemical constituents like alkaloids, glycosides, phenolic compounds, tannins and resins. HPTLC fingerprint analysis of the extract was carried out using CAMAG HPTLC system equipped with Linomat 5 sample applicator, UV cabinet and WIN-CATS-4 software. The results from HPTLC fingerprint analysis of the ethanolic leaf extract of *Bridelia montana* revealed the presence of 6 polyvalent constituents.

Keywords: *Bridelia montana* (ROXB) WILLD. HPTLC, CAMAG, WIN CATS-4.

INTRODUCTION

Phytotherapeutic agents or phytomedicines are standardized herbal preparations consisting of complex mixtures of one or more plants which are used in most countries for the management of various diseases. According to the WHO definition, herbal drugs contain active ingredients of plant parts or plant materials in the crude or processed state plus certain excipients, i.e., solvents, diluents or preservatives[1]. Plants have always been a common source of medicaments, either in the form of traditional preparations or as pure active principles[2]. Chromatographic fingerprinting techniques are most significant methods which can be used for the routine herbal drug analysis and for quality assurance. HPTLC offers better resolution and estimation of active constituents can be done with reasonable accuracy in a shorter time[3].

The genus *Bridelia* belonging to the family Phyllanthaceae is found to be rich in tannins and phenolic compounds which are associated with various pharmacological activities including the hepatoprotective, anti-oxidant. *Bridelia montana (ROXB) WILLD* is a large shrub or small tree usually growing up to 10 meters tall but occasionally up to 20 meters, quite common in forests and open land, reported from dry evergreen or deciduous forests; sandy-loamy soil, granite or basalt derived sandy soil, and limestone, at elevations from 50 - 600 meters, occasionally to 1,400 meters. The bark is used for tanning, as a liniment with Gingelly oil in treating rheumatism, for the removal of urinary concretions (Ayurveda), astringent. The leaves are used as cattle-fodder[4]. As the scientific research is less on the leaves of the species *montana* of genus *Bridelia*, an attempt is made to identify the chemical constituents and HPTLC finger print analysis of the ethanolic leaf extract of *Bridelia montana*.

MATERIALS AND METHODS

Collection of the plant material

The plant specimens for the proposed study were collected from Tirumala hills, Tirupati. The plant was authenticated by Dr. Madhava Chetty, Department of Botany, Sri Venkateshwara University, Tirupati, India, and specimen was preserved at University herbarium.

Preparation and extraction of the plant material

The 300 grams of the coarsely powdered plant material of leaf of *Bridelia montana* was defatted with n-hexane and extracted with 90% ethanol using Soxhlet apparatus. The extraction was carried out until the extractive becomes colorless. The extract was filtered through a cotton plug, followed by Whatmann filter paper (no.1). The extract was evaporated under reduced pressure using Rotavac evaporator.

Preliminary Phytochemical screening

The plants may be considered as biosynthetic laboratory for multitude of compounds like alkaloids, glycosides, volatile oils, tannins, Saponins, flavonoids etc. The phytochemical investigation of the ethanolic leaf extracts of *Bridelia montana* was carried out with standard protocol[5]. The results were presented in Table 1.

HPTLC profile (High Performance Thin Layer Chromatography)

HPTLC studies were carried out following the method of Harborne[6] and Wagner et al[7].

Sample Preparation

The ethanolic extract obtained were evaporated under reduced pressure using Rotavac evaporator.

Development of the solvent system

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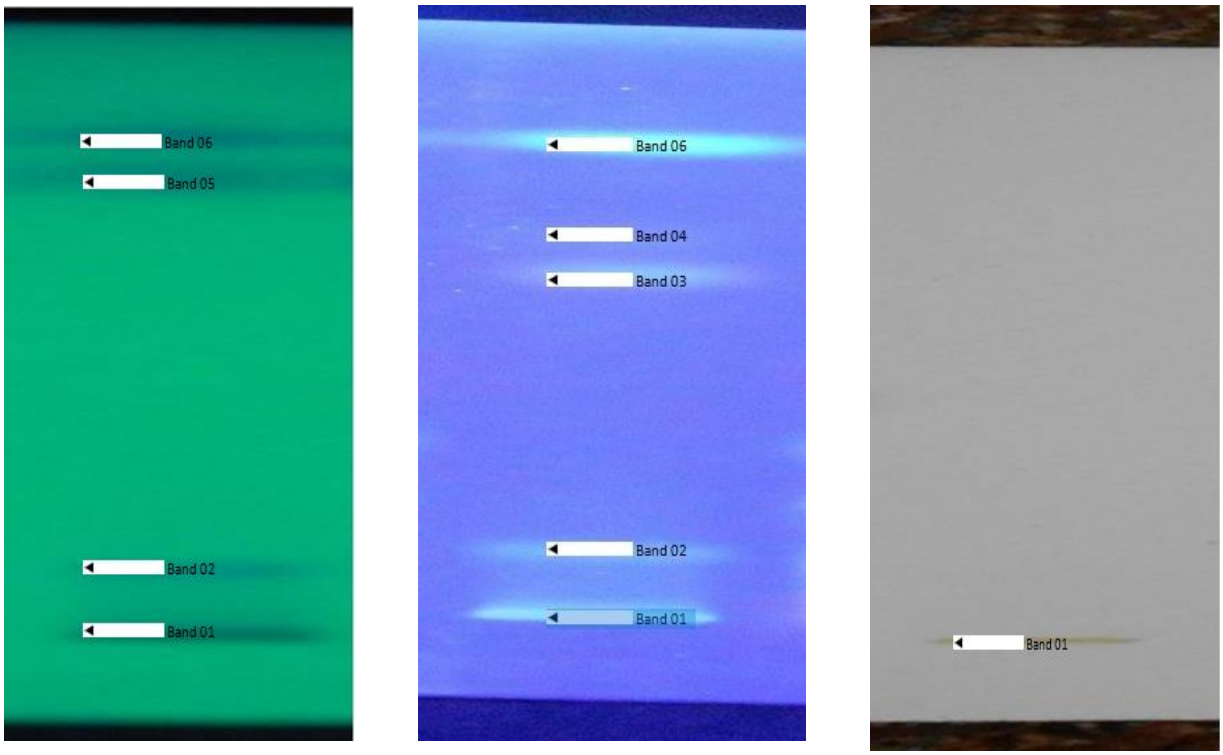
Table 1: The Phytochemical test on ethanolic leaf extract of *Bridelia montana.*

S. No.	Constituents	Test Performed	Ethanolic leaf extract
1.	Alkaloids	Mayer's Reagent	+
		Dragendroff's Reagent	+
		Hager's Reagent	-
		Wagner's Test	+
		Molisch's Test	-
2.	Sugars And Carbohydrates	Barfoed's Test	-
		Fehling's Test	-
		Benedict's Test	-
3.	Glycosides	Legal's Test	+
		Borntrager's Test	-
4.	Steroids	Salkowski Test	-
		Liebermann's Test	-
5.	Tannins	Lead Acetate Test	+
		Bromine Water	+
		Ferric Chloride Test	+
6.	Proteins	Xanthoprotein Test	-
		Millon's Test	-
7.	Terpenoids	Noller's Test	-
8.	Flavonoids	Shinoda Test	-
9.	Anthocyanins	Sodium Hydroxide Test	-
10.	Quinone	Sodium Hydroxide Test	-
11.	Saponins	Foam Test	-
12.	Phenolic Compounds	Ferric Chloride Test	+
		Lead Acetate Test	+
		Gelatin Solution	-
13.	Fixed Oils and Fats	Spot Test	-
14.	Resins	Turbidity Test	+
		HCl Test	-

+: Present; -: Absent



Under 254 nm Under 366 nm In visible light

Figure1: HPTLC fingerprint profiles of ethanolic leaf extract of *Bridelia montana*.

A number of solvent systems were tried for extract, but the satisfactory resolution was obtained in the solvent n-hexane:ethyl acetate (2:8) for the ethanolic leaf extract.

Instrumentation

HPTLC system of CAMAG, Muttenz, Switzerland, Anchrom Enterprises (I) Pvt. Ltd, Mumbai, consisting of sample applicator (Linomat 5), Twin trough chamber with lid {10×10 cm, CAMAG, Muttenz, Switzerland}, UV cabinet (Aetron, Mumbai) with dual wavelength (254/366 nm) and the HPTLC photo documentation (Aetron, Mumbai) was used for study.

Sample Application

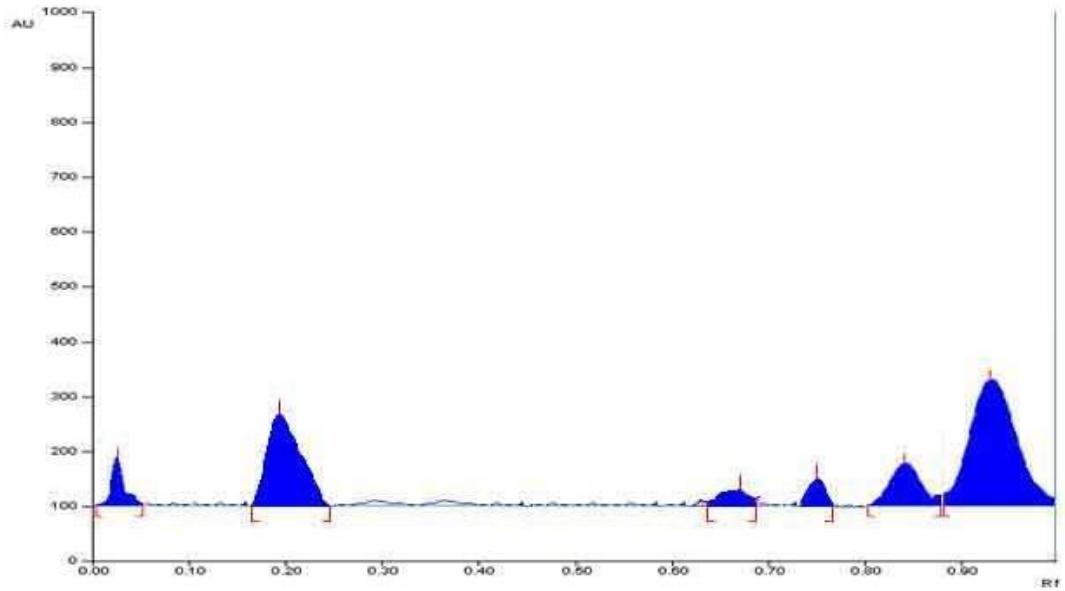
10 µL of sample (Ethanolic Extract after filtration) was applied in the form of band of width 6 mm with a 100 µL sample syringe (Hamilton, Bonaduz, Switzerland) on precoated silica gel aluminium plate 60 F254 (5 × 10) with 250 µm thickness (E. MERCK, Darmstadt, Germany) using a CAMAG Linomat 5 sample applicator (Switzerland). The plate was prewashed with methanol and activated at 110 0C for 5 minute, prior to chromatography.

Development of chromatogram

After the application of sample, the chromatogram was developed in Twin trough glass chamber 10 x 10 cm saturated with solvent n-hexane: ethyl acetate (2:8) for 15

Table 2: R_f Values of Peaks in HPTLC Fingerprint of Ethanolic Extract of *Bridelia montana*.

PEAK	START POSITION	START HEIGHT	MAX POSITION	MAX HEIGHT	MAX %	END POSITION	END HEIGHT	AREA	% AREA
1.	0.01 Rf	6.3 AU	0.02 Rf	21.6 AU	12.61 %	0.03 Rf	4.3 AU	270.5 AU	4.98 %
2.	0.16 Rf	9.5 AU	0.20 Rf	34.6 AU	34.6 %	0.22 Rf	30.2 AU	1437.3 AU	26.47 %
3	0.65 Rf	4.8 AU	0.67 Rf	26.2 AU	26.2 %	0.69 Rf	3.6 AU	547.4 AU	7.67 %
4.	0.73 Rf	3.0 AU	0.75 Rf	12.5 AU	12.5 %	0.79 Rf	7.5 AU	416.7 AU	10.08 %
5.	0.86 Rf	28.5 AU	0.87 Rf	30.8 AU	30.8 %	0.88 Rf	3.3 AU	951.0 AU	17.52 %
6.	0.91 Rf	0.2 AU	0.95 Rf	45.7 AU	45.7 %	1.00 Rf	0.2 AU	1806.4 AU	33.27 %



Peak Table (Data obtained at Scan of plate at 300 nm)

Figure 2: Chromatogram of ethanolic leaf extract of *Bridelia montana.*

min. The length of chromatogram run was 8 cm. HPTLC plate was dried in a current of air with the help of an air dryer. The slit dimensions of 5 × 0.45 mm and scanning speed of 20 mm/sec were employed in analysis.

Detection of Spots

The chromatograms were scanned by densitometer Plate was observed in the daylight, under UV light (254 and 366 nm). (Figure1) After each observation the central points of spots appeared on chromatogram were marked with needle. The R_f values and finger print data were recorded by WIN CATS software.

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening

The phytochemical test on ethanolic extract of *Bridelia montana* leaves showed the presence of various phytoconstituents like alkaloid, glycoside, tannin, resins and phenolic compounds. (Table. 1).

High Performance Thin Layer Chromatography

The HPTLC fingerprinting analysis was performed according to the aforesaid procedure and the bands were observed on the HPTLC plates and the Rf values were calculated. (Table. 2)

The results from HPTLC finger print scanned at wavelength 300nm for alcoholic extract of *Bridelia montana* leaf. There are six polyvalent phytoconstituents and corresponding ascending order of R_f values start from 0.01 to 1.00 in which highest concentration of phytoconstituents was found to be 26.75% and its corresponding R_f value was found to be 0.95 respectively.

CONCLUSION

The standard preliminary phytochemical investigation of the Ethanolic extract of *Bridelia montana* leaves showed the presence of some secondary metabolites like Alkaloids, Tannins, Phenolic Compounds, Resins, and Cardiac Glycosides. The HPTLC analysis of the Ethanolic extract of *Bridelia montana* leaves showed six spots. These chromatographic profiles can be used for the identification and evaluation of the quality of the plant. Phytochemical Studies, HPTLC finger print profile has been useful for fixing standardization for this plant. As a preliminary basic work in this area we have presented herein all the above mentioned data's. More work is required for the identification of exact core components, their formulas and chemical structure to establish the docking ability. Finally, for the development of better therapeutic agents for clinical assessment, detailed pharmacology and toxicology, including genotoxicity and reproductive toxicology studies need to be performed in order to generate data on the potential short and long term toxicities as well as affirmed pharmacological action.

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