Research Article

FT-IR and GC-MS Analysis of Stem Extract of Ethnomedicinal Plant: *Bridelia montana* (Roxb.) Willd

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ABSTRACT

Human beings have been using plants with medicinal properties since so many years in traditional and folk medicine due to the therapeutic potential they possess. Exploration of the plants with medicinal properties has unearthed numerous novel drugs that are used to treat varied human and animal diseases. *Bridelia montana* (Roxb.) Willd is one of the important ethnomedicinal plants used across the southern part of India. Qualitative analysis of phytochemicals revealed the presence of phenols, glycosides, flavonoids, tannins and carbohydrates. Fourier Transform Infra-Red analysis results showed 5 major peaks and the Gas Chromatography and Mass Spectrometry study revealed the presence of 8 phytocompounds from the stem extract of *Bridelia montana*. The phytocompounds that were present were identified by the comparison of their peak area percentage and the retention time with the help of library sources and by the mass spectra interpretation. Many of these compounds were used in the pharmaceutical industries for their antimicrobial, antitumor and antioxidant properties.

Keywords: *Bridelia montana*, FTIR, GCMS, Medicinal plant, Phytochemical analysis.

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INTRODUCTION

In different countries plants have been used medicinally and are one of the main sources of many powerful drugs that are being used. Plants are an important sources of medicine for humankind for several years, mainly for the folk remedies in the history, they are being used as general traditional medicines.[1]

Plants of higher class that serve as important sources of biologically active phytocompounds play a prominent role in the nurturing of human health. Reports that are available on plants represent an ocean of effective anti-cancer agents, chemo-therapeutics, and anti-microbial agents, these are more systemic and are biologically degradable.[2-4]

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Understanding the chemical components of plants is advantageous not only for the finding of the agents with therapeutic properties, but also for the discovery of new resources of economically important phytochemicals for the production of chemical substances and for finding the importance of traditional remedies.[5] A minute validation of plant drugs hence has become a new branch of science highlighting and prioritizing the standard of the herbal drugs and plant products. Gas chromatography and Mass Spectrometry (GCMS) analysis are usually used for the analysis of phytochemical components present in plant extracts, traditional medicines and medicinal plants. In late years, the application of Gas Chromatography-Mass Spectrometry analysis studies has been increased for the analysis of medicinal plants for the presence of phytochemicals as this particular technique has been proved to be a beneficial method for the analysis of secondary and primary metabolites, polar and non-polar components, volatile and essential oils, fatty acids, lipids[6] and alkaloids.[7]

The species *Bridelia montana* belongs to the Genus Bridelia and the family Phyllanthaceae. It is a large much-branching small tree or shrub, leaves look rather thin and large, and they are thick and small and often yellowish, the main nerves are very oblique usually in 6 to 8 pairs often branched in the margin. The stem is a tree-like in moister or shadier places, they are bushy in open dry lands. This genus consists of 49 accepted species: *Bridelia retusa, B. ferruginea, B. glauca, B. microphylla, B. speciosa, B. tomentosa, B. verrucosa*, etc. *B. montata* is one of the important plants in ethnomedicine.

MATERIALS AND METHODS

Plant Material

Bridelia montana stems were collected from Thoovaipathy, a tribal hamlet of Anaikatti village, Coimbatore, Tamil Nadu, India. The plant was authenticated at the Botanical Survey of India (Southern wing). Herbarium of *Bridelia montana* was prepared and it is preserved in the herbarium collection of the Department of Botany, PSGR Krishnammal College for Women, Coimbatore, Tamil Nadu.

Preparation of Extract

The stem samples were collected from the wild, dried in the shade and crushed to powder using a mixer grinder. The powdered samples were extracted separately by soxhlet apparatus using three different solvents: Hexane, ethanol, and distilled water.

Qualitative Phytochemical Assay

Qualitative screening of the phytochemicals present in the ethanol extract was carried out to analyze the class of organic compounds present. The ethanol extract of *Bridelia montana* stem was analyzed by standard chemical tests as described by Kokate,[8] Harborne[9] and Sofowora[10] to determine tannins, glycosides, alkaloids, flavonoids, phenols and carbohydrates.

FT-IR Analysis

Fourier Transform Infrared Spectrophotometer (FTIR) is a very significant tool in the identification of the chemical bonds and their types and also the presence of functional groups in phytocompounds. The characteristic of the chemical bond is known by the wavelength of light absorbed which could be seen in the FTIR spectra. Interpretation of the infrared absorption spectrum determines the chemical bonds in a molecule. Dried ethanolic extract of *Bridelia montana* stem was used for the FTIR study. The translucent sample disc was prepared by encapsulating 1mg of dried extract in 10mg of Potassium bromide pellet. The powdered sample of the prepared pellet was then loaded in the FTIR Spectroscopy (Shimadzu, Japan) with a scan range from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1} and the results were recorded.

GC-MS Analysis

Gas chromatography has applications in very extensive fields. The main use of GCMS is the separation and analysis of mixtures with multiple components such as plant extracts, essential oils, plant and animal drugs, and solvents. The electron capture detector and flame ionization detector in the gas chromatography quantitatively determine the materials present in the mixture even at very low concentrations. Because of the sensitivity, simplicity, and effectiveness in the separation of compounds present in the mixtures, gas chromatography is considered one of the very useful tools in various fields. It is used extensively for qualitative and quantitative analysis of the mixtures and also for the purification and separation of compounds present in a mixture.[11]

GC-MS analysis of the ethanol stem extracts of *Bridelia montana* was carried out using SHIMADZU GCMS-QP-2010plus model with Rtx-5MS column and length of 30.0 m, the thickness of 0.50µm, the diameter of 0.32 mm. Helium (5.5 lb/in2) was employed as carrier gas at the total flow rate of 41.0 ml/min with an injection temperature of 270°C and a column temperaturof e 45°C. Total GC-MS running time was 0 - 150 min hold. Ion Source with a Temperature 200°C and the interface temperature of 260°C.

Identification of Phytocompounds

Elucidation of GC-MS chromatogram was done using the databases of NIST- National Institute Standard and Technology and the library of WILEY. The chromatogram of the unknown compounds was compared with the spectrum of already known compounds that are in the NIST and WILEY library. The name of the compound, peak area percentage, molecular formula, molecular weight and structure of the components of the sample were determined.

RESULTS

Qualitative Phytochemical Analysis

The qualitative analysis of phytochemicals of ethanol extract of *Bridelia montana* stem was positive for tannins, phenols, glycosides, flavonoids and carbohydrates whereas negative for steroids and terpenoids, proteins, and amino acids (Table 1).

Table 1: Qualitative phytochemical analysis of *Bridelia montana* stem extract.

Sl. no.	Phytochemicals	Methods	Stem extract
1	Alkaloids	Mayer's reagent test	+
		Wagner's reagent test	-
		Dragendroff's reagent test	+
2	Tannins	Ferric chloride test	++
		Lead acetate test	+
3	Terpenoids	Chloroform test	+
		Salkowski's test	-
4	Phenols	Gelatin test	+++
5	Flavonoids	Alkaline reagent test	++
6	Glycosides	Keller Kiliani test	+++
		Molisch's reagent test	+
7	Carbohydrates	Fehling's reagent test	++
		Benedict's reagent test	+
		Molisch's reagent test	+
8	Proteins	Biuret's test	+
		Ninhydrin's test	-

+ - Present ++ - Moderately present +++ - Highly present.

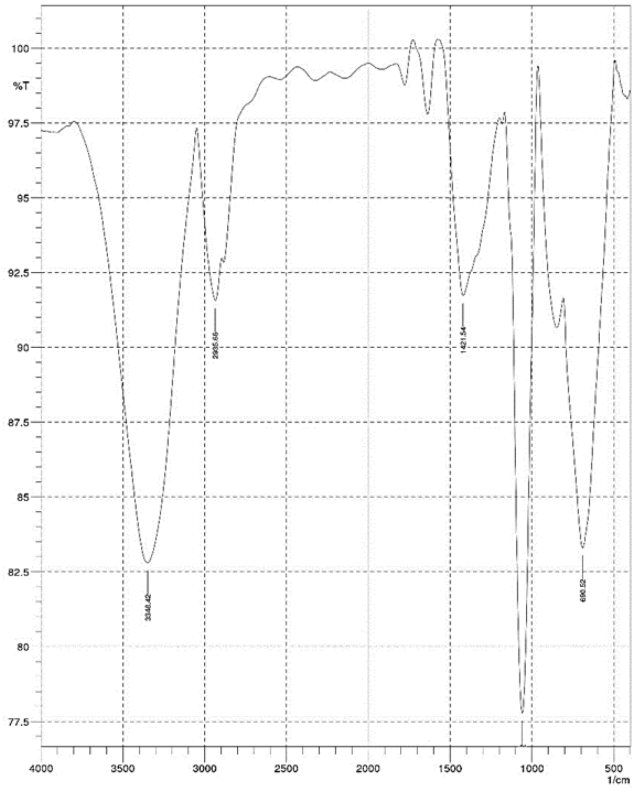

Figure 1: FTIR spectrum of *Bridelia montana* stem extract.

FT-IR Analysis

The FT-IR spectrum of ethanol stem extracts of *Bridelia montana* showed the presence of 5 major peaks (Figure 1).

Table 2: FTIR analysis of *Bridelia montana* stem extract.

Sl. no	Standard (nm)	Bond	Wave no.	Functional group
1	3200-3600	O-H Stretch	3348.42	H bonded alcohol
2	2850-2970	C-H Stretch	2935.66	Alkanes
3	1400-1000	C-F Stretch	1421.54	Alkyl halide
4	1000-1320	C-O Stretch	1058.92	Alcohols, Carboxylic acids, ethers, esters.
5	910-665	N-H Stretch	690.52	Amines

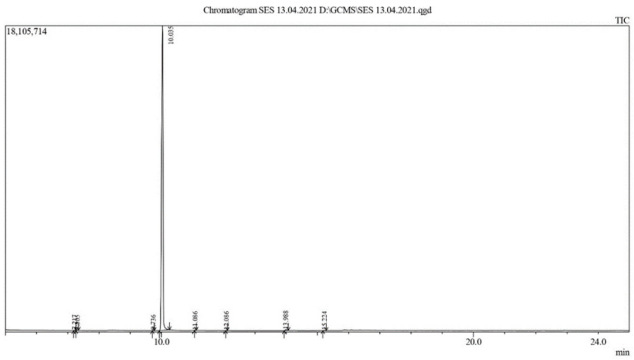

Figure 2: GCMS chromatogram of *Bridelia montana* stem extract.

The spectral absorption at 3348.42 cm^{-1} which represents H- bonded alcohols with O-H stretch, and alkenes at 2935.66 cm^{-1} shows C-H stretch. The results show a peak at the wavelength 1421.54 cm^{-1} which confirms the presence of alkyl halide with C-F stretch. A sharp peak at 1058.92 cm^{-1} has alcohols, carboxylic acids, ethers, and esters with C-O stretch. The lowest wavelength peak is at 690.52cm^{-1} with amines that has N-H stretch (Table 2).

GC-MS Analysis

The analysis of the GC-MS chromatogram of the ethanol stem extract of *Bridelia montana* showed 8 peaks indicating the presence of 8 phytochemical constituents (Figure 2). The identification of the phytochemical compounds was ascertained based on the peak area and the retention time. The biological activities of the compounds with their retention time (RT), molecular formula, molecular weight (MW) and peak area percentage are given out in Table 2. The compound which was identified first with the least retention time (7.217min) was1-Dodecanethiol (CAS) n-Dodecyl mercaptan, followed by an ester compound Nonane, 3,7-dimethyl- 2, 6 - octadecyl - 3 - hydroxyl benzoate with a retention time of 7.305min, 1-Undecene, 9-methyl- (9.736min), Diethyl Phthalate (10.035min), 1,2-benzoldicarbonsaeure, di-(hex-1-en-5-yl-ester) (11.086min), Decanedioic acid, didecyl ester

Table 3: GCMS analysis of *Bridelia montana* stem extract.

Sl.no.	RT	Peak area %	Name of the compound	Molecular formula	Molecular weight	Biological activity
1	7.217	0.07	1-Dodecanethiol (CAS) n-Dodecyl mercaptan	$C_{12}H_{26}S$	202.40	-
2	7.305	0.05	Nonane, 3,7-dimethyl-2, 6 - octadienyl - 3 - hydroxyl benzoate	$C_{11}H_{24}$	156.30	Antitumoral, antimicrobial, antioxidative
3	9.736	0.07	1-Undecene, 9-methyl-	$C_{12}H_{24}$	168.31	Antivirulent, antimicrobial
4	10.035	99.20	Diethyl Phthalate	$C_{12}H_{14}O_4$	222.24	Plasticizer
5	11.086	0.04	1,2-Benzoldicarbonsaeure, Di-(Hex-1-en-5-yl-ester)	$C_{20}H_{26}O_4$	330	Antimicrobial, anticancer, antifouling
6	12.086	0.06	Decanedioic acid, didecyl ester	$C_{30}H_{58}O_4$	482.77	Antiseptic, antimicrobial
7	13.988	0.39	Hexadecanoic acid (CAS) Palmitic acid	$C_{16}H_{32}O_2$	256.42	Antibacterial
8	15.224	0.12	1-Undecanol (CAS) n-Undecanol	$C_{11}H_{24}O$	172.31	Antibacterial

(12.086min), Hexadecanoic acid (CAS) Palmitic acid (13.988min), whereas 1-Undecanol (CAS) n-Undecanol was the last compound with the retention time of 15.224min (Table 3).

DISCUSSION

The significance of plants in different aspects is known to us very well. The Kingdom of plants is a treasure trove of drug materials and in the past few years the awareness about the significance of plants with medicinal properties is increasing rapidly. Plants are being used for medicinal purposes for several thousand years. They constitute the most valuable choice for examining the current hunt for new drugs with possible therapeutic values such as anti-diabetic, anti-cancer, anti-tumoral and hepatoprotective properties. Drugs from the plants as sources are cost-effective, easily available, safe, efficient and rarely or have no possible side effects. The stem of *Bridelia montana* was extracted using ethanol as a solvent using soxhlet apparatus. The conventional method of Soxhlet extraction has some fair advantages. In this method of extraction, the sample is repeatedly brought in contact with fresh portions of extractant, which facilitates the extraction of more phytocompounds from the plant samples.[12]

The preliminary phytochemical analysis by Vinatha *et al.* (2017) on ethanol leaf extract of *Bridelia montana* showed the presence of different phytocompounds like alkaloid, tannin, glycoside, resins and phenolic compounds.[13]

The extracted samples were tested for the presence of alkaloids, tannins, terpenoids, phenols, flavonoids, glycosides, carbohydrates, and proteins, the results revealed the presence of phenols, glycosides, flavonoids, tannins and carbohydrates. These phytochemicals are known to possess various medicinal properties which could treat diseases and ailments with anti-inflammatory, anti-oxidant, anti-microbial, anti-pyretic, antiseptic, hepatoprotective, and anti-diabetic properties.[14]

The FT-IR analysis showed the presence of H bonded alcohol, alkanes which is a component of plant wax,[15] alkyl halide,[16] alcohols, carboxylic acids, ethers, esters, and amines which are the reason for possible medicinal properties of this plant,[17] these components were also present in *Mentha spicata*[18] and *Micrococca mercurialis*.[19] GC-MS chromatogram of the sample showed the presence of various phytocompounds like 1-Dodecanethiol, Nonane, 3,7-dimethyl-2, 6 - octadecyl - 3 - hydroxyl benzoate which has anti-tumoral, anti-microbial, anti-oxidative properties which are also reported in *Guiera senegalensis,*[20] 1-Undecene, 9-methyl with anti-virulent and anti-microbial properties has been reported in *Alstonia boonei,*[21] Diethyl Phthalate which is a plasticizer,[22] 1,2-Benzoldicarbonsaeure, Di-(Hex-1-en-5-yl-ester) which is also reported in *Annona squamosa* has anti-microbial, anti-cancer and anti-fouling properties,[23] Decanedioic acid, didecyl ester that has anti-septic and anti-microbial properties,[24] Hexadecanoic acid which is also called as Palmitic acid which is also present in walnut and coconut oil[25] has anti-bacterial property[26] and 1-Undecanol or n-Undecanol which also has anti-bacterial property[27] and is also present in essential oils of *Senecio belgaumensis* flowers.[28] All these phytocompounds possess one or more biological activity and thus indicate the medicinal properties present in the plant extract.

CONCLUSION

Bridelia montana is used in various traditional and folk systems of medicine to treat different ailments such as dysentery, diarrhea, etc. Preliminary phytochemical analysis showed the presence of phenols, glycosides,

flavonoids, tannins and carbohydrates. FTIR showed 5 major peaks which indicated the presence of alkanes, alcohols, alkyl halides, esters, and amines. GC-MS analysis of ethanol stem extract of *Bridelia montana* showed the presence of 8 compounds which include organosulphur compounds, alkane hydrocarbons, phthalic acid, esters, palmitic aci,d and fatty alcohols. These compounds exhibit anti-tumoral, anti-cancer, anti-microbial, anti-fungal, anti-inflammatory, and neuroprotective properties. The presence of these compound sanalyzed through GC-MS analysis has opened an avenue for new research.

CONFLICT OF INTEREST

The authors declare no conflict of interst.

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