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RESEARCH ARTICLE

Isolation and Characterization of Major Phytochemical Constituents from *Bauhinia variegata* Leaf Extract

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ABSTRACT

Bauhinia variegata, a plant with medicinal value in the family Fabaceae, is commonly recognized for its bioactive phytoconstituents that are of therapeutic interest. In this study, the principal phytochemical constituents of *B. variegata* leaf extract were isolated and characterized using different chromatographic and spectroscopic methods. *B. variegata* leaves were harvested, dried, powdered, and subjected to successive solvent extraction with petroleum ether, chloroform, ethyl acetate, and methanol. The ethyl acetate extract with the highest yield (4.5%) was selected for further studies. Preliminary phytochemical screening was conducted to identify different secondary metabolites. Column chromatography, followed by Thin Layer Chromatography (TLC), High-Performance Thin Layer Chromatography (HPTLC), and High-Performance Liquid Chromatography (HPLC), were used to isolate bioactive compounds. Structural elucidation was performed using FT-IR, NMR, and mass spectrometry (MS). Phytochemical screening revealed the presence of carbohydrates, flavonoids, alkaloids, tannins, saponins, glycosides, and phenolic compounds in ethyl acetate extract. TLC analysis revealed three major compounds, with Rf values of 0.72, 0.50, and 0.28. Spectroscopic characterization revealed the presence of phytosterols and triterpenoids. Mass spectrometry revealed a molecular ion peak at m/z 414 with the molecular formula C29H50O. This study isolated and characterized the major bioactive compounds from *B. variegata*, with indications of their chemical structure and possible pharmacological potential. Further research is required to determine the therapeutic utility of these compounds.

Keywords: *Bauhinia variegata*; Phytochemical characterization; Ethyl acetate extract

INTRODUCTION

Bauhinia variegata (*B. variegata*) is a flowering plant belonging to the Fabaceae family. It is widely used in traditional medicine by tribal communities across India and is also popular in indigenous medical systems, such as Ayurveda, Unani, and Homeopathy. *Bauhinia*, a genus of shrubs or trees (rarely climbers), is found in tropical regions worldwide. There are approximately 15 species of this genus in India.[1] *B. variegata* is widely recognized as Kanchnar in Sanskrit and Mountain Ebony in English. The term Kanchnar in Sanskrit translates to "A glowing beautiful lady".[2] Plant bark, roots, leaves, seeds, and flowers are utilized for their medicinal properties. It has been used in treating dyspepsia, bronchitis, leprosy, ulcers, and weight management, as an astringent, tonic, and anthelmintic.

Medicinal plants play a crucial role in promoting health and in treating various ailments. Their therapeutic properties are attributed to bioactive compounds, known as phytochemicals, which exert specific physiological effects on the human body. Alkaloids, tannins, flavonoids, and phenolic compounds are among the significant phytochemicals found in medicinal plants.[3] These compounds contribute to various pharmacological activities, making medicinal plants a valuable source of natural remedies. In particular, *Bauhinia variegata* has been found to exhibit anti-inflammatory properties[4], along with chemoprotective[5] and hepatoprotective[6] effects. The increasing prevalence of adverse effects associated with synthetic drugs has driven interest in plant-derived compounds that offer potential alternatives with reduced toxicity and enhanced therapeutic benefits.[7]

B. variegata has been extensively used in traditional medicine to treat conditions, such as bronchitis, leprosy, and tumors. The stem bark is valued for its astringent, tonic,

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and anthelmintic properties, whereas leaf infusions serve as laxatives and are used in the treatment of piles. Additionally, dried buds are utilized to manage worm infestations, tumors, diarrhea, and piles.[5,8,9] These applications underscore the versatility and medicinal significance of this plant.

The current study aimed to isolate and characterize the major phytochemical constituents of the *B. variegata* leaf extract. By employing advanced analytical techniques, this study sought to elucidate the chemical structures of these bioactive compounds, thereby contributing to a better understanding of their potential therapeutic applications.

METHODOLOGY

Collection and Preparation of Plant Material

Leaves of *B. variegata* were collected from Green Chem Private Limited, Bangalore and identified. The voucher specimen was preserved for future use. The plant material gathered was removed gently from the soil and any attached material and dried at room temperature for 5-6 days to complete dehydration. The dried plant material was powdered with a 60 # sieve and utilized for further research, such as determination of the extractive value.

Extraction of Plant Material

Powdered leaves of *B. variegata* were subjected to successive solvent extraction to separate the active constituents. Petroleum ether, chloroform, ethyl acetate, and methanol were used as solvents. Extraction was performed stepwise in an extractor, with each solvent chosen according to increasing polarity.

Laboratory-scale extraction involved maceration of 200 g of powdered *B. variegata* leaves with various solvents, followed by filtration and concentration. Methanol extraction yielded a 2% extract after three-hour maceration with 200 ml of methanol, which was performed twice. Chloroform extraction using the same procedure yielded 1.2% yield. Ethyl acetate extraction, which gave the highest yield of 4.5%, used 200 ml of ethyl acetate. Petroleum ether extraction was performed using the same procedure to yield a 2.5% extract.

Ethyl acetate, petroleum ether, and methanol were used for pilot-scale extraction because of their higher yields. Petroleum ether extraction involved 3 kg of dried leaves and 6 L of solvent at a temperature of 55-60°C for two hours for three iterations, resulting in 100 g of powder. Methanol extraction under the same conditions at 65-75°C produced 70 g of powder. Ethyl acetate extraction at 80-85°C for two hours twice yielded 200 g of powder, which is the most efficient method. For this reason, the ethyl acetate extract was given higher priority for subsequent analysis because of its better yield.

Preliminary Phytochemical Screening

Phytochemical screening was also carried out to detect secondary metabolites, such as carbohydrates, alkaloids, steroids, glycosides, saponins, flavonoids, triterpenoids, proteins, and amino acids. Various qualitative tests were conducted to ascertain their existence. Carbohydrates were identified through Molisch's, Fehling's, Benedict's, and Barfoed's tests, each of which showed characteristic color changes representing the presence of reducing sugars or monosaccharides. Alkaloids were ascertained by Dragendroff's, Wagner's, Mayer's, and Hager's tests, all of which gave characteristic precipitates. Steroids and sterols were detected by the Libermann-Burchard and Salkowski tests, which indicated color changes as proof of their presence.

Glycosides were detected using Legal's, Baljet, Borntrager's, and Killer-Killani tests, which yielded characteristic color reactions. The foam test established the presence of saponins, whereas flavonoids were detected by Shinoda, lead acetate, and alkaline reagent tests, which yielded red, yellow, and fading yellow colors, respectively. The tin chloride test revealed the presence of triterpenoids via a pink reaction. Proteins and amino acids were verified by the Biuret, Ninhydrin, and Xanthoprotein tests, in which color changes showed the presence of proteins and aromatic amino acids.

Chromatography Techniques

To isolate and identify further bioactive compounds, the ethyl acetate extract was subjected to column chromatography. Silica gel (60-200 mesh) was packed in a glass column and prepped with solvents prior to adsorbing the ethyl acetate extract onto the silica gel. Gradient elution was performed by stepwise increase in polarity using a solvent system containing hexane, benzene, toluene, ethyl acetate, and methanol. The obtained fractions were examined by Thin Layer Chromatography (TLC) to identify the constituents.

The separation and identification of the compounds in the extract were performed using TLC. Various solvents were used in this study. For the ethyl acetate extract, a solvent system of ethyl acetate: acetic acid: water (4:1:5) was used. TLC plates were developed, scanned at 366 nm, and detected using a vanillin-sulfuric acid reagent. High-Performance Thin Layer Chromatography (HPTLC) was performed on a CAMAG instrument using silica gel plates. The mobile phase employed was Benzene: Ethyl acetate: Formic acid (3.0:6.5:0.5). The sample was spotted on plates, which were developed in a saturated tank and scanned under UV light.

The isolated compounds were then subjected to High-Performance Liquid Chromatography (HPLC). The mobile phases used in the gradient system were 0.4% phosphoric acid (A) and acetonitrile (B). A Phenomenex C18 Luna, 5μm, 250 x 4.6 mm column was used, and detection was performed at 325 nm with a flow rate of 1.0 ml/min. The

sample and standard solutions were prepared by dissolving the extracts in methanol and injecting them into the HPLC system for analysis.

Spectral identification of the isolated compounds was performed using FT-IR, NMR spectroscopy, and mass spectrometry (MS). FT-IR spectroscopy was performed using the KBr pellet technique in the range of 4000-400 cm^{-1} to identify functional groups. NMR spectroscopy was employed to establish the chemical structures of the isolated compounds based on their chemical shifts, integration, and multiplicity of the peaks. Furthermore, mass spectrometry was used to identify the mass-to-charge ratio of the isolated compounds, which could contribute to their structural identification. This approach enabled the extraction, isolation, and thorough analysis of bioactive compounds from *B. variegata* leaves, providing useful information regarding their chemical makeup and potential pharmacological activities.

RESULTS

In this study, *B. variegata* of the Fabaceae family was collected and authenticated. Authenticated dried leaves of *B. variegata* were procured from Green Chem Pvt. Ltd. and analyzed for physicochemical evaluation. Identification and estimation of major chemical constituents were performed according to pharmacopoeia standards and literature methods.

The phytoconstituents found in petroleum ether and ethyl acetate extracts of *B. variegata* were compared. Fats and oils, triterpenoids, and phytosterols were present in petroleum ether and ethyl acetate extracts. The petroleum ether extract contained phytosterols, triterpenoids, fats, and oils, while the ethyl acetate extract contained a broad variety of compounds, such as carbohydrates, tannins, phenols, amino acids, saponins, alkaloids, flavonoids, and Vitamin C. This showed that ethyl acetate was more effective than petroleum ether in extracting a variety of phytochemicals from *B. variegata*.

The ethyl acetate extract was subjected to further investigation via column chromatography and thin layer chromatography to test various fractions of EAEBV (Ethyl Acetate Extract of *Bauhinia variegate*). The isolated compounds were analyzed by using a solvent system of Chloroform: Ethanol (9.8:0.2).

Analysis of isolated compounds

Thin-layer chromatography (TLC) showed three violet spots that were visually detected, with an Rf value of 0.72. Sterols were detected by phytochemical tests. The compound was found to have good solubility in ethanol and a melting point range of 144-146° C. No spots or residues were found on TLC in fractions 1-3 to 43-46. All these fractions gave fractions 53-74 showed a brown residue with three violet spots (Figure 1), with Rf values of 0.72, 0.50, and 0.28. Similarly, fractions 54-57 too possessed a brown residue with a solitary spot with an Rf value of 0.72.

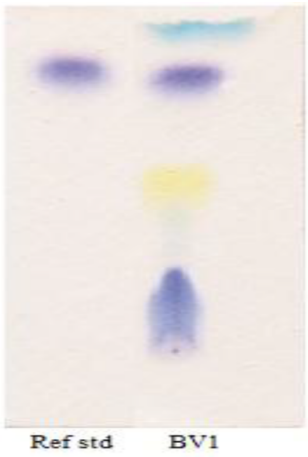

Fig. 1: TLC of Isolated Compound

Figure 2 shows the HP-TLC profile of the *B. variegata* leaf extract, which was detected under UV light at 366 nm and visualized with vanillin sulfuric acid reagent. The chromatographic plate showed three lanes, A, B, and C, representing *Bauhinia* leaf extract, an isolated compound (BLE/RD/01), and a reference standard (BLE/REF/01), respectively. Under UV 366 nm detection, clear bands were observed, indicating the presence of different phytochemical constituents.

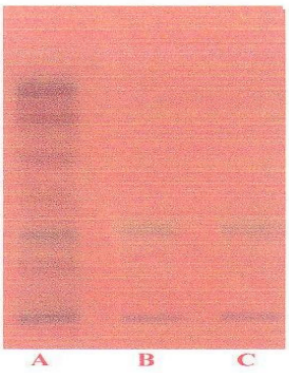

Fig. 2: HP-TLC profile of *Bauhania variegata* leaf extract

Figure 3 depicts the HP-TLC pattern of *B. variegata* leaf extract after treatment with the vanillin sulfuric acid spraying reagent. The chromatography plate showed three lanes, A, B, and C, showing *Bauhinia* leaf extract, an isolated compound (BLE/RD/01), and a reference standard (BLE/REF/01), respectively. Upon the application of reagent spraying, well-defined bands with different shades and intensities were observed, indicating the presence of various phytochemical constituents. The bands in lanes B and

C seemed comparable in position and color, indicative of the existence of the same or structurally homologous compounds. The *Bauhinia* leaf extract sample (lane A) showed multiple bands, consistent with its multi-component phytochemical nature. The reagent-induced color distinction of the compounds facilitated easy comparison among the samples.

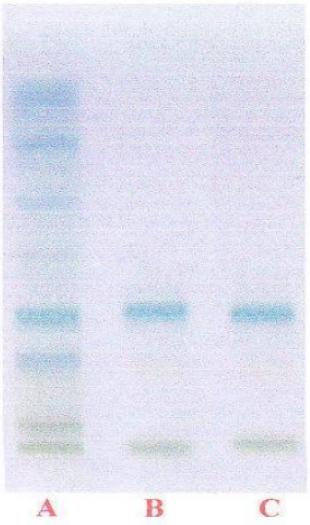

Fig. 3: Vanillin sulphuric acid spraying reagent on *B. variegata* leaf extract

Structural elucidation

The IR absorption spectrum revealed absorption peaks at 3439.6cm^{-1} (O-H stretching.); 2924.7 cm^{-1} and 2867.9 cm^{-1} (aliphatic C-H stretching); 1631.6 cm^{-1} (C=C absorption peak); other absorption peaks include 1410.3 cm^{-1} (CH2); 1043.7 cm^{-1} (cycloalkane) and 881.6 cm^{-1} (Figure 4).

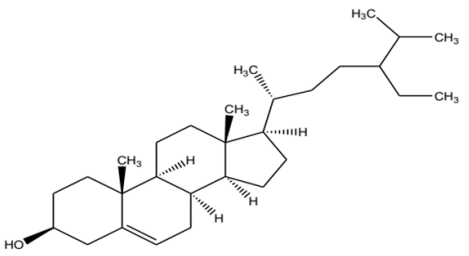

Fig. 4: Structure of isolated compound (IUPAC NAME: (3S,8R,9S,10R,13R,14S,17R)-17-((2R)-5-ethyl-6-methylheptan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-ol)

1 HNMR (CDCl3) of isolated compound: 1HNMR has provided signals at δ3.2 (1H, m, H-3), 5.26 (1H, m, H-6), 5.19(1H, m, H-23), 4.68 (1H, m, H-22), 3.638 (1H, m, H-3), 2.38 (1H, m, H-20), 1.8-2.0 (5H, m) ppm. Other peaks are found at δ 0.76-0.89 (m, 9H), 0.91-1.05 (m, 5H), 1.35-1.42 (m, 4H), 0.69-0.73 (m, 3H), 1.8-2.00 (m, 5H), 1.07-1.13 (m, 3H), 1.35-1.6 (m, 9H) ppm. The mass spectrum of the compound exhibited a molecular ion peak at m/z 414.38, which is equivalent to the molecular formula of C29H52O. Mol. Weight: 414.707. MS indicated molecular ion peaks at 414 equivalents to the molecular formula C29H50O. There were also ion peaks at m/z 367, 271, 255, 229,189, 175, 161, 133, 121, 105, 107, 95, 81, 69, 55, 41. The structure of the isolated compound is shown in Figure 2.

DISCUSSION

The phytochemical compounds in *B. variegata* leaf extract have been widely analyzed, and a rich bioactive compound profile has been identified. Current research supports the existence of phenols, saponins, flavonoids, glycosides, steroids, tannins, and alkaloids that are responsible for their pharmacological activities. This review discusses the importance of these constituents, their functions in plant defense, and their applications in medicine.

Experiments have established the occurrence of flavonoids, saponins, tannins, and alkaloids in *B. variegata*, which is consistent with previous results.[10,11] A preliminary pharmacognostical and phytochemical investigation of *B. variegata* leaves was carried out by Gupta et al., and isolated chief chemical constituents were steroids, saponins, flavonoids, sugars, alkaloids, and tannins.[12] Furthermore, quantitative analysis established a phenolic content of 453 mg GAE/g and flavonoids of 166 mg QE/g, suggesting a high level of occurrence of these constituents.[13]

B. variegata extracts were quite active against bacteria, with an MIC of 3.6 μg/mL against Pseudomonas aeruginosa.[14] *B. variegata* phenolics also play a vital role in protecting plants from UV radiation and certain phytopathogenic microorganisms.[10] In addition, the anti-mutation and anticancer activities of *B. variegata* have been explored by Silva et al. (2007). This study utilized a melanoma and skin cancer tumor model in Swiss albino mice in association with micronucleus and chromosomal aberration tests.[15] Bodakhe et al. also explored the hepatoprotective activity of a *B. variegata* bark extract in rats exposed to CC-induced liver damage. They assessed the effect of the stem bark extract on serum enzymes (AST, ALT, ALP, and GGT) and liver protein and lipid levels, and noted strong liver-protective effects.[6]

To increase the therapeutic potential of *B. variegata*, sophisticated chromatography methods, such as HPLC and column chromatography, have been used to extract and identify individual phytochemicals.[11,16] In 2008, Rao et al. purified new triterpene saponins from the leaves of *B. variegata* and found that one of these showed low toxicity and had significant anti-inflammatory and analgesic properties. Moreover, triterpene saponins show moderate activity against schistosomiasis.[17] *B. variegata* markers

were isolated by column chromatography/HPLC, and the phytochemical markers were identified and characterized by analyzing data from IR and NMR spectroscopy.

CONCLUSION

This study effectively purified and identified the predominant phytochemical compounds in *B. variegata* leaf extracts with varied bioactive compositions. The ethyl acetate extract was more effective at extracting a higher number of compounds than petroleum ether, including alkaloids, flavonoids, tannins, and phenolic compounds. High-performance chromatographic and spectroscopic methods further validated the detection of dominant bioactive molecules, such as phytosterols and triterpenoids, with known pharmacological implications. These results add to the increasing evidence for the medicinal potential of *Bauhinia variegata*, and further studies on its therapeutic uses are warranted.

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