

# Extraction, Isolation, and Characterization of *Bauhinia variegata* Flower

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## ABSTRACT

**Background:** *Bauhinia variegata* Linn. is a native plant of Asia and China. *B. variegata* is found in tropical regions of the world. It belongs to family Leguminosae. It is used for diarrhea, hemorrhoids, constipation, piles, edema, leprosy, wounds, tumors, etc. The objective of the present study was to perform extraction of *B. variegata* flower and isolation of active constituents from the extract.

**Materials and Methods:** The ethanolic extraction of *B. variegata* flower was performed using the Soxhlet apparatus. The isolation of active constituents from the extract was performed using chromatographic techniques. In column chromatographic studies, n-hexane-dichloromethane (DCM) (2:8) was used as an eluting system and further purified through thin layer chromatography (TLC). Compound A and B were isolated through chromatographic techniques, then the molecular formula and characterization of these compounds were carried out with mass and infrared (IR) spectral analysis.

**Results:** The percentage yield of *B. variegata* ethanolic extract (BVE) was found to be 20.8% w/w. The different fractions were F1 having 12.5 grams with n-hexane, F2 (17.1 grams) with CH<sub>2</sub>Cl<sub>2</sub>, F3 (21.2 grams) with EtOAc, and F4 (13.4 grams) with EtOH. Compound A and B were isolated from the solvent fractions of n-hexane-DCM (2:8) and EtOAc-DCM (1:9), respectively. The compound A was characterized as 3-hydroxy-6-methoxy-2-phenyl-4H-chromen-4-one. The compound B was characterized as 3-hydroxy-6-methyl-2-phenyl-4H-chromen-4-one.

**Conclusion:** Thus, *B. variegata* flowers possess active components that need to identify their biological activities.

**Keywords:** *Bauhinia variegata*, Column chromatography, Fourier transform infrared (FTIR), Mass spectroscopy.

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## INTRODUCTION

Herbal medicines are the oldest known form of medicine and have been used over 2,000 years.<sup>[1]</sup> It is still the major form of medicine for over 75% of the world's population. The use of herbal medicines and phytonutrients or nutraceuticals continues to expand rapidly across the world, with many people now resorting to these products for the treatment of various health challenges in different national healthcare settings.<sup>[2]</sup> It is estimated that up to four billion people (representing 80% of the world's population) living in the developing world rely on herbal medicinal products as a primary source of healthcare and traditional medical practice, which involves the use of herbs, is viewed as an integral part of the culture in those communities.<sup>[3]</sup> Plant secondary metabolites, such as, alkaloids, flavonoids, terpenoids, etc., have been used for different medicinal purposes.<sup>[4]</sup>

*B. variegata* Linn. is a native plant of Asia and China. *B. variegata* is found in tropical regions of the world. It belongs to the family Leguminosae.<sup>[5]</sup> It is generally planted in garden, park, and roadsides as an ornamental plant in many warm temperate and subtropical regions. The whole plant parts have been used since ancient times in the treatment of several diseases, such as, diarrhea, hemorrhoids, constipation, piles, edema, leprosy, wounds, tumors, etc.<sup>[6]</sup> The flowers are edible, and find their place in various cuisines, and are widely used as a pickle in north India. The dried buds are used in the treatment of piles and worms. The juice of the flowers is used to treat diarrhea, dysentery, and other

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stomach disorders. An infusion from its bark is used as an astringent, tonic, and useful in scrofula, skin diseases, and ulcers.<sup>[7]</sup> The root is carminative, and its decoction is used in dyspepsia, flatulence, prevention of obesity, and as an antidote to snake poison.<sup>[8]</sup> The secondary metabolites of plants are flavonol glycoside, flavones, phenanthraquinone, glycosides, steroids, flavonoids, and triterpene. It possesses several biological activities like anticancer, hepatoprotective, and anti-inflammatory activities.<sup>[9]</sup>

## MATERIALS AND METHODS

### Extraction of *B. variegata* Flower

The extraction was performed by collecting flowers from the local garden of Allahabad, Uttar Pradesh. It was identified by the local healers and authenticated by the pharmacognosy department of the institute. It was washed, dried, and powdered using a mixer. The flowers (1.2 kg) were extracted with petroleum ether to remove fatty substances. The mark was further extracted with 95% ethanol by hot percolation method. The extract was filtered and concentrated under vacuum at 40°C and stored in a desiccator. The percentage yield of ethanolic extract (BVE) was found to be 20.8% w/w. This unrefined extract was put in ethanol, and refined through water to get different fractions comprising F1 having 12.5 grams with n-hexane, F2 (17.1 grams) with CH<sub>2</sub>Cl<sub>2</sub>, F3 (21.2 grams) with EtOAc, and F4 (13.4 grams) with EtOH.<sup>[10]</sup>

### Isolation of Compound from Extract of *B. variegata*

The CH<sub>2</sub>Cl<sub>2</sub> fraction F2 (17.1 grams) was transferred to the silica gel (70–230 mesh) column, and elution was carried out with n-hexane-DCM mixture in increasing order of polarity. Considering this column chromatographic studies, at n-hexane-DCM (2:8) eluting system, and further purification through TLC analysis, we obtained an unknown compound as yellow amorphous solid. Different spectroscopic techniques, i.e., IR and mass spectrometry, were applied and detected a new compound as compound A. Additionally, at 100% DCM indicated one prominent spot along with some minor spots on the TLC card. This portion was further repurified through a silica gel column with eluting system EtOAc-DCM (1:9), which resulted in another compound that was identified as compound B after structural elucidation, by applying different spectroscopic techniques.<sup>[11]</sup>

### Characterization of Isolated Compounds

#### Fourier Transform Infrared (FTIR) Spectroscopy

Infrared (IR) spectroscopy is a technique based on the vibrations of the atoms of a molecule, combined with chemometrics for simple analysis of flavonoid in the medicinal plant extract. It is a nondestructive analytical technique, requires a relatively small amount of sample, is fast, and is accurate. An IR spectrum of selected medicinal plant extract was correlated with flavonoid content using chemometrics.<sup>[12]</sup>

**Instrumentation:** FTIR spectrometer (Perkin Elmer V 10:03:06) was equipped with triglycine sulfate as a detector and germanium as a beam splitter. The spectroscope was interfaced to the computer and connected to the software of the OPUS operating system (version 7.0 Bruker optic) for FTIR spectra acquisition. Samples were placed in contact with attenuated total reflectance (ATR) plate. The FTIR spectra were collected at 4,000 to 650 cm<sup>-1</sup> frequency region by co-adding 32 scans, and at the resolution of 4 cm<sup>-1</sup>. The ATR plate was carefully cleaned by scrubbing with isopropyl 70%

twice, followed by drying with soft tissue before being filled in with the next sample. These spectra were recorded as absorbance values at each data point and replicated twice.

#### Mass Spectroscopy

Liquid chromatography-electrospray ionization-quadrupole-time of flight-mass spectrometry (LC-ESI-Q-TOF-MS) involved the use of ultra-performance liquid chromatography (UPLC) with an electrospray ionization Synapt HDMS G1 hybrid quadrupole time-of-flight mass spectrometer [Agilent 6522 (Q-TOF) positive mode ESI hrMS]. The Synapt HDMS G1 was operated in positive ionization mode with electrospray capillary voltage 3 kV. The cone and nitrogen desolvation gas flows were 50 and 500 L/hr, respectively. Desolvation temperature was 250°C. Mass data acquisitions were set at m/z 200 to 400 for spectra analysis, in centroid data mode with scan time 0.2 seconds, and tolerance widow 0.02 dalton. The precursor isolation window was 3 thomson, and the collision energy of the MS/MS analysis was set at 50 eV. A UPLC Ethylene bridged hybrid (BEH) Hydrophilic interaction chromatography (HILIC) column (1.7 µm, 2.1 × 100 mm, Waters) was used at 25°C with elution gradients of the mobile phase, which consisted of 0.1% FA in 2% ACN (buffer A) and 0.1% FA in 100% ACN (buffer B). The gradient conditions were buffer A, 5 to 5%, 0 to 1-minute; 5 to 50%, 1 to 2 minutes; 50 to 60%, 2 to 4 minutes; 60 to 60%, 4 to 5 minutes; 60 to 5%, 5 to 5.1 minutes; and 5 to 5%, 5.1 to 7 minutes. The flow rate was 0.4 mL.min<sup>-1</sup>. Data were analyzed with the use of MassLynx 4.1 software.<sup>[13]</sup>

**Identification of the compound:** Interpretation of mass spectrum of MS was done using the NIST/EPA/NIH mass spectral database (NIST11), with NIST MS search program v.2.0g [National Institute Standard and Technology (NIST), Scientific Instrument Services, Inc., NJ, USA]. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight, and structure of the components of the test materials were ascertained.

## RESULT AND DISCUSSION

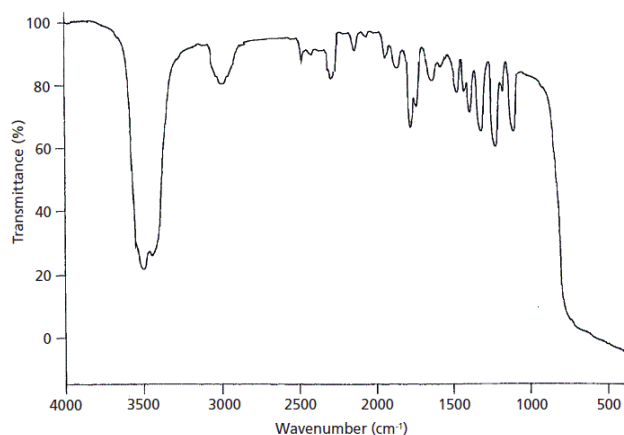
### Characterization of Compound A isolated from Extract of *B. variegata*

#### Infrared Spectroscopy

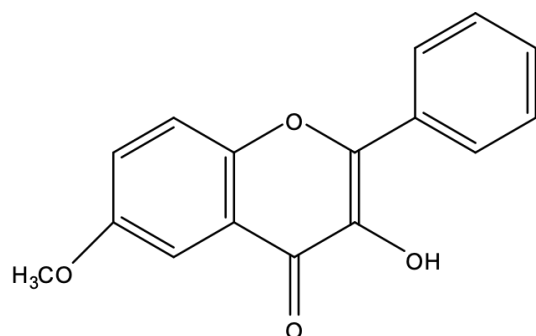
The IR spectrum of Compound A1 showed absorption bands at 3,350 to 3,235 cm<sup>-1</sup> (O-H stretching), 2,932 to 3,060 cm<sup>-1</sup> (CH<sub>4</sub> stretching of aromatic ring), 1,640 to 1,652 cm<sup>-1</sup> (CO stretch), 1,464 to 1,498 cm<sup>-1</sup> (CH bending), 1,407 cm<sup>-1</sup> (OH bending), and 757 cm<sup>-1</sup> (CH bending). Figure 1 shows the IR spectrum for isolated compound from *B. variegata*.

#### Mass Spectroscopy (MS)

MS can be used to determine the molecular weight and confirm the structure of the isolated compounds or natural products. The mass data which showed m/z = 268.07 (100%),



**Figure 1:** IR spectra of compound A isolated from extract of *B. variegata*



**Figure 3:** 3-hydroxy-6-methoxy-2-phenyl-4H-chromen-4-one

269.08 (17.6%), and 270.08 (2.3%). Figure 2 shows the mass spectra of compounds isolated from extract of *B. variegata*. The structure was identified as isoflavonoids on the basis of extensive spectroscopic data analysis and by comparison of their spectral data with those reported in the literature. The compound A was characterized as 3-hydroxy-6-methoxy-2-phenyl-4H-chromen-4-one. The structure of the isolated compound from extract *B. variegata* is shown in Figure 3.

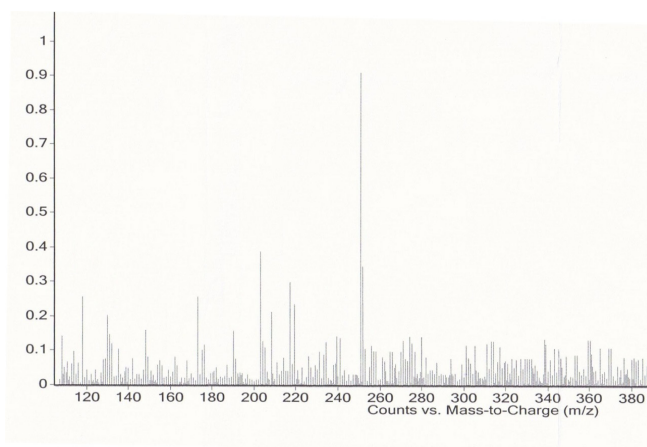
### Characterization of Compound B isolated from Extract of *B. variegata*

#### Infrared Spectroscopy

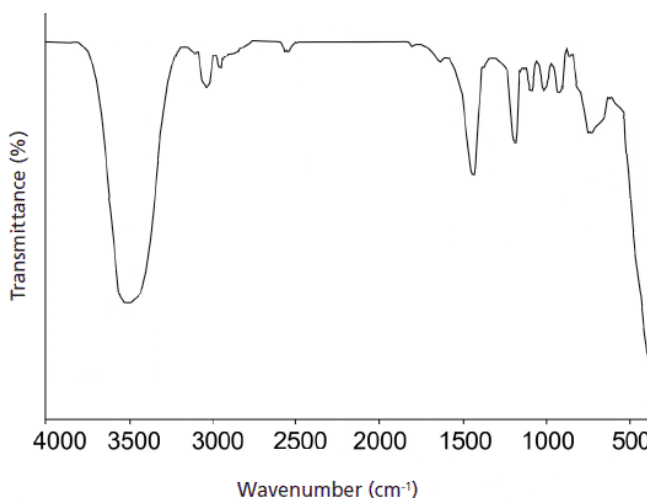
The IR spectrum of compound B1 showed absorption bands at 3,495 to 3,435  $\text{cm}^{-1}$  (O-H stretching), 2,945 to 3,076  $\text{cm}^{-1}$  ( $\text{CH}_4$  stretching of aromatic ring), 1,622 to 1,655  $\text{cm}^{-1}$  (CO stretch), 1,478 to 1,495  $\text{cm}^{-1}$  (CH bending), 1,390  $\text{cm}^{-1}$  (OH bending), and 765  $\text{cm}^{-1}$  (CH bending). Figure 4 shows the IR spectrum for isolated compound from *B. variegata*.

#### Mass Spectroscopy (MS)

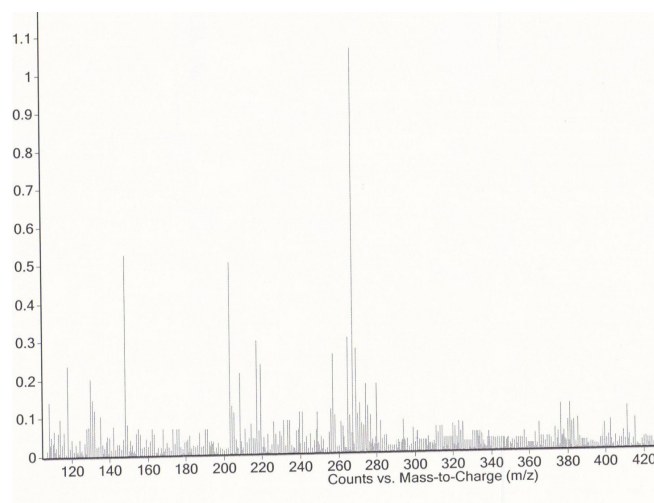
MS can be used to determine the molecular weight and confirm the structure of the isolated compounds or natural products. The mass data which showed  $m/z = 252.08$  (100%), 253.08 (17.6%), and 254.09 (1.4%). Figure 5 shows the mass spectra of compounds isolated from the extract of *B. variegata*. The structure was identified as isoflavonoids



**Figure 2:** Mass spectra of compound A isolated from extract of *B. variegata*

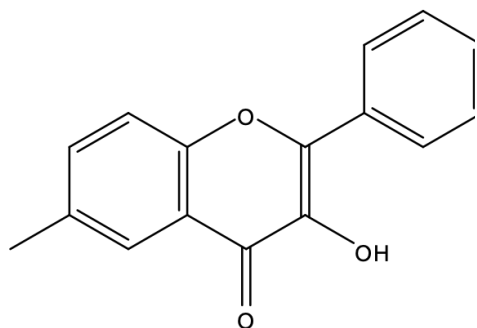


**Figure 4:** IR spectra of compound B isolated from extract of *B. variegata*



**Figure 5:** Mass spectra of compound B isolated from extract of *B. variegata*

on the basis of extensive spectroscopic data analysis and by comparison of their spectral data with those reported in the literature. The compound B was characterized as 3-hydroxy-



**Figure 6:** 3-hydroxy-6-methyl-2-phenyl-4H-chromen-4-one

6-methyl-2-phenyl-4H-chromen-4-one. The structure of the isolated compound from extract *B. variegata* is shown in Figure 6.

## CONCLUSION

The ethanolic extract of the flowers of *B. variegata* belonging to the family Leguminosae was successfully carried out. The chemical constituents isolated from this extract must be accounted for the biological activities exhibited by the crude ethanolic extract of the plant. Therefore, it is now the turn of the pharmacologists/biologists to explore the plant more systematically by carrying out individual bioactivity of the isolated chemical constituents. Therefore, the present work will boost the scientific communities to do more research work on this important medicinal plant to explore it in the drug development program going on around the world.

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