RESEARCH PAPER

Extraction, Qualitative and Quantitative analysis of Bauhinia variegata and Grevillea robusta

Yogesh Tiwari [1], Tarun Parashar [2*], Sayantan Mukhopadhyay [3]

[1]Research Scholar, School of Pharmacy and Research Dev Bhoomi Uttarakhand University, Dehradun, Uttarakhand, India 248007

[2] School of Pharmacy and Research Dev Bhoomi Uttarakhand University, Dehradun, Uttarakhand, India 248007

3College of Pharmacy, Shivalik Campus, Dehradun, Uttarakhand, India 248197

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ABSTRACT

Background:B. variegata is an indigenous species of China, Asia, and the tropical areas of the world. It belongs to the Leguminosae family. G. Robusta is a native of Eastern Australia, commonly known as the Australian silver oak. It is used to treat wounds, tumors, hemorrhoids, diarrhea, constipation, piles, edema, leprosy, and shows strong antioxidant effects. The main objective of this study was to perform leaf extraction of B. variegata and G. robusta and to perform phytochemical screening of the active constituents in the extracts.

Materials and Methods:The hydroalcoholic, ethanolic extraction of B. variegata and G. robusta leaves was performed using a Soxhlet apparatus. Active constituents in the extract were identified using phytochemical screening (qualitative and quantitative). Characterization of these extracts was carried out using UV spectroscopy and FTIR spectra.

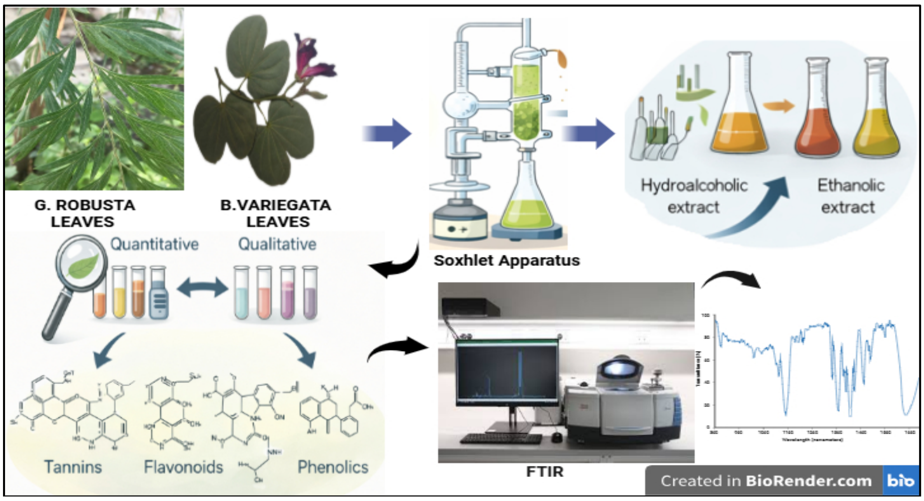
Results :The % yield of B. variegata ethanolic-based extract was found to be 1.22% w/w, and hydroalcoholic-based extract 0.27% w/w. G. robusta percent yield 0.67 % w/w and 0.27% w/w, respectively. B. variegata has a larger quantity of bioactive compounds, such as TPC 85.4 mg/g and TFC 64.2 mg/g. G. robusta have greater amount of tannin, 32.4 mg/g. FTIR peaks show O-H, C-H, C-O, and C=O.

Discussion:The biological activities of the active components found in B. variegata and G. robusta leaves must be identified.

Major Findings:This study showed that both plants produced bioactive compounds when extracted effectively. The secondary metabolites, including alkaloids, saponins, flavonoids, and terpenoids, were confirmed by phytochemical screening. Spectroscopy revealed functional groups consistent with pharmacologically significant phytochemicals...

Keywords: B. variegata, G. robusta, Phytochemical Screening, TPC, TFC, and FTIR

Graphical Abstract



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INTRODUCTION

Herbal remedies have been utilized for more than 2,000 years, making them the most traditional form of medicine [1]. More than 80% of people worldwide continue to use it as the most common form of medicine [2]. Herbal remedies and phytonutrients, often known as nutraceuticals, are being used by rising numbers of individuals worldwide to manage a variety of diseases in national healthcare systems [3]. Nearly 4 billion people rely on herbal medicines as their main

*Author for Correspondence: Parashar89@gmail.com

source of treatment in developing nations [4]. Traditional medicine is seen as an integral component of those community cultures [5]. Alkaloids, flavonoids, terpenoids, and other secondary metabolites have been used for a variety of therapeutic applications. *Bauhinia variegata* is also known as "Kachnar" or black rocks, a natural plant of China and Asia [6]. Tropical areas of the world are home to *B. variegata*. It belongs to the Leguminosae family [2]. In many mild temperate and subtropical countries, it is typically grown as an attractive plant in gardens and parks. Since ancient times, a variety of illnesses, including diarrhea, hemorrhoids, constipation, piles, edema, leprosy, wounds, diabetes, and tumors, etc., have been treated using the whole plant. *Grevillea robusta* is also known as silver oak, a species of flowering plant that belongs to the Proteaceae family [7]. The health of people totally depends on medicinal plants. The medicinal properties are determined by their specific physiological effects on the human body[8]. These are a few methods for medicinal plants in the phytochemical extraction process. Ethanol and hydroalcoholic solvents are polar substances that have been proposed for Soxhlet. Some Bioactive compounds like flavonoids, tannins, saponins, and alkaloids, etc. contribute to the pharmacological characteristics of these plants. Flavonoids can contribute to treating diseases caused by oxidative stress because of their antioxidant and anti-inflammatory effects [9]. The scavenging of free radicals by an increase in tannin and phenolics boosted *B. variegata* and G. *robusta* antioxidant capacity [10]. Ancient literature contains extensive documentation of the concept of polyherbal formulations. Polyherbal formulation offers more and longer-lasting medicinal potential than a single plant [11].

2. Materials and Methods:

Collected fresh leaves of *Bauhinia variegata* and *Grevillea robusta* from the Dev Bhoomi Uttarakhand University campus. The Botanical Survey of India, Dehradun, verified the authenticity of the plants. The material was washed with double-distilled water, extraneous matter was removed, and allowed to dry at room temperature in the shade. The above material was then crushed and converted into a fine powder using a mechanical grinder. The extract was obtained using a Soxhlet apparatus with ethanol and hydroalcoholic solutions as solvents.

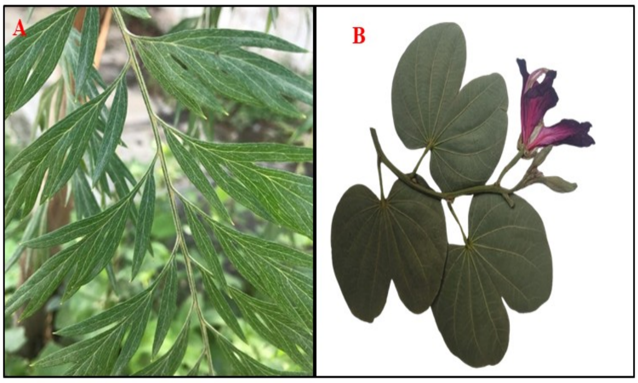

Figure 1. (a) *Grevillea robusta* (b) *Bauhinia variegata*

Figure 2. Extraction process using Soxhlet

2.1 Extract yield

Extraction of *B. variegata and G. robusta* leaves was performed using two solvents with different polarities, such as ethanol and a hydroalcoholic solution. The efficacy of the active ingredients in *B. variegata* and *G. robusta* leaves, responsible for their biological activity, and it was measured by the yields of the extracts. It was crucified using a Soxhlet extraction process. The amount of extracts recovered in grams after drying and then placed in vials is displayed in Tables 1 and 2. Extraction yield of ethanol provided the highest yield for both plants, 1.22% and 0.67%, respectively. The hydroalcoholic yields are 0.27% and 0.28%, respectively. These variations are due to the polarity of the solvents, since ethanol and hydroalcoholic solutions are more effective at dissolving various phytochemicals. The higher yield of *B. variegata* aligns with its known phytochemical profile richness.

Table 1. Yield of *B. variegata* and *G. robusta* extract

Plant name	Plant parts	Method extraction	Solvents	Color and consistency	Weight of extract (g)	% yield
B. variegata	Leaves	Soxhlet Extraction	Ethanol	Sticky greenish brown	10 g	1.22
			Hydroalcoholic	Slight green	2.2 g	0.276
G. robusta	Leaves	Soxhlet Extraction	Ethanol	Yellow to light brown	5.4 g	0.675
			Hydroalcoholic	Yellowish brown to amber	2.5 g	0.289

2.2 Chemical test for Phytochemicals Screening

2.3 Qualitative analysis of phytochemical constituents

The chemical test was carried out on the extract of two different solvents, *B. variegata* and *G. robusta*, to detect the phytochemical components. The qualitative results are expressed as (-) absent, (±) trace present, (+) present, (++) abundant.

2.3.1 Test for Alkaloids

All four extracts were exposed to a few drops of Mayer's test reagent. Brown color formation indicated the alkaloids were present [12].

2.3.2 Test for Flavonoids

Few drops of very diluted $FeCl_3$ solution were added to 1 mL of each extract. The presence of flavonoids is indicated by the changing of color to reddish-brown [13].

2.3.3 Test for saponins

1 mL of diluted alcohol and 20 mL of distilled water were added to each extract, and they were thoroughly shaken for fifteen minutes. The presence of saponins is indicated by the development of a foam layer [14].

2.3.4 Test for Carbohydrates

2.3.4.1 Molisch's Test

A small quantity of extract was taken, 2 drops of alcoholic α-naphthol solution were added to 2mL of filtrate, the mixture was agitated well, and then one mL of concentrated sulfuric acid was progressively added to the test tube and allowed to stand. Carbohydrates are confirmed by the development of a reddish color [15].

2.3.5 Test for Tannins

1 mL of lead acetate solution was mixed with 5 mL of extract solution. The presence of tannins is confirmed by the production of a flocculent brown precipitate [16].

2.3.6 Test for Terpenoids

All four extracts were treated with 2 ml of $CHCl_3$. 3 ml of conc. Sulfuric acid was added. After a few minutes, a reddish-brown layer formed. The two layers formed indicated the presence of terpenoids [17].

2.3.7 Test for Protein and Amino Acid

2.3.7.1 Biuret test

1 drop of 2% copper sulphate solution was added to 2 mL of filtrate. Pellets of potassium hydroxide were then added, after which 1 mL of 90% ethanol. The presence of proteins was revealed by the ethanol layer's pink hue[18].

2.3.7.2 Ninhydrin test

1mL of the aqueous filtrate was mixed with 2 drops of ninhydrin solution, producing a distinctive purple hue that suggested the presence of amino acids. [19].

2.3.7.3 Test for fixed Oils and Fats

Treat all four extracts separately with 0.2 N potassium hydroxide and a few drops of phenolphthalein, then heat over a water bath for 2 hours. Formation of partial neutralization indicated the presence of fixed oils and fats [20].

Table 2. Phytochemical screening analysis of the extract of leaves of *B. variegata*

S. No.	Phytochemical Test	Ethanol	Hydro alcoholic
1.	Alkaloids Test	±	+
2.	Flavonoid Test	+	++
3.	Saponin Test	-	+
4.	Carbohydrate Test		
	Molisch Test	+	++
5.	Tannins Test	+	++
6.	Terpenoids Test	+	++
7.	Protein and Amino acid		
	Biuret test	-	±
	Ninhydrin Test	±	+
8.	Fixed Oils and Fats	-	-

Table 3. Phytochemical screening analysis of the extract of leaves of *G. robusta*

S. No.	Phytochemical Test	Ethanol	Hydro alcoholic
1.	Alkaloids Test	+	+
2.	Flavonoid Test	+	++
3.	Saponin Test	-	+
4.	Carbohydrate Test		
	Molisch Test	±	+
5.	Tannins Test	+	++
6.	Terpenoids Test	+	++
7.	Protein and Amino acid		
	Biuret test	-	±
	Ninhydrin Test	-	±
8.	Fixed Oils and Fats	±	±

2.4 Quantitative analysis of phytoconstituents

2.4.1 Estimation of Total Phenolic Content (TPC)

The phenolic content of all the extracts was determined using a spectrophotometer. The extract was over-heated with 50 mL of $(CH_3CH_2)_2O$ for 15 minutes. 5 mL of the sample was transferred to a 50 mL flask, and 10 mL of double-distilled water was added to the flask. 2 mL of ammonium hydroxide solution, 5 mL of conc. $CH_3(CH_2)_3CH_2OH$ was added. The absorbance was measured at 760 nm. Gallic acid equivalents (GAE) milligrams per gram of extract were used to express the results [21].

2.4.2 Estimation of Total Flavonoid Content (TFC)

The colorimetric test using aluminum chloride was used to determine the TFC. After combining 1 mL of extract with 4 mL of double-distilled water in a test tube, a 5% sodium nitrate solution was added and carefully mixed. 10 percent aluminum chloride was added after five minutes to start the complicated creation process. The liquid was then diluted to 10 mL with double-distilled water, 2 mL of 1 M NaOH solution was added, and the color change was allowed to proceed for 15 minutes. At 415 nm, the absorbance was measured [22].

2.4.3 Estimation of Total Alkaloid Content

In a beaker, 2.5 g of extract was dissolved in 100 mL of 10% CH_3CO_2H. After being covered, the mixture was allowed to stand for three hours. After filtration, the extract was concentrated in a water bath until it was only one-fifth of its initial volume. Gradually, concentrated NH_4OH was added until the precipitation was completed. The precipitate was then gathered, cleaned with diluted NH_4OH, and filtered through Whatman paper once the mixture had settled. The residue was then dried and weighed [23,24].

2.4.4 Estimation of Tannin Content

UV spectroscopy was used to measure the amounts of tannin. 0.25 g of the sample was weighed and placed in a 25 mL plastic container. After adding 25 mL of double-distilled water, the mixture was swirled for half an hour. After filtering the sample, fill a 25 mL volumetric flask to the brim. After that, pipette 2.5 mL of the filtered solution into a test tube and combine it with 0.008 M $K_4Fe(CN)_6 \cdot 3H_2O$ and 1 mL of 0.1 M $FeCl_3$ in 0.1 M HCl. Within ten minutes, the absorbance at 430 nm was measured [25].

Table 4. Quantitative analysis of Phytochemical constituents

Compound	*B. variegata* (mg/g extract)	*G. robusta* (mg/g extract)
Total Phenolic Content (TPC)	85.4 ± 2.3	72.1 ± 1.8
Total Flavonoid Content (TFC)	64.2 ± 1.5	48.7 ± 1.2
Total Alkaloid Content	20.5 ± 0.8	18.3 ± 0.7
Total Tannin Content	25.7 ± 1.0	32.4 ± 1.1

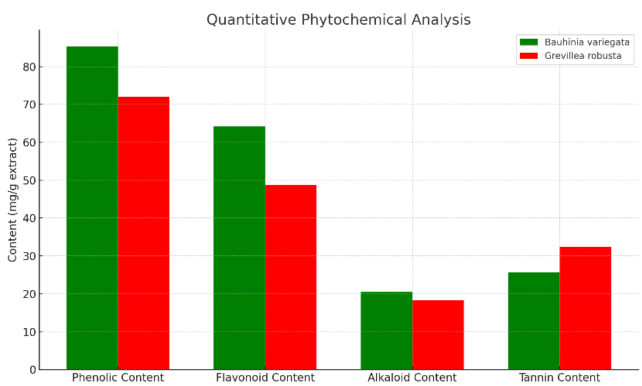

Figure 3: Quantitative analysis of Phytochemicals.

3. Uv-Vis Spectroscopy

For the identification of various phytoconstituents present in ethanolic and hydroalcoholic extracts of *B. variegata* and *G. robusta,* UV-Vis spectroscopy was done. The qualitative Uv-vis spectrum of *B. variegata* was obtained over the wavelength range 200-400 nm. The show peaks at 228, 255, 268, 345, and 367 nm, and the absorbance is 0.078, 0.172, 0.231, 0.154, and 0.093, respectively. *G. robusta* showed peaks at 215, 224, 245, 289, and 387 nm, and absorbance of 0.107, 0.109, 0.365, 0.253, and 0.90, respectively [26]. Absorbance bands observed in the *B. variegata* and *G. robusta* plant leaf extracts are displayed in Tables 5 and 6.

Table 5: Uv-vis peak values of the extract of *B. variegata*

S.NO.	Peaks	Absorbance
1.	228	0.078
2.	255	0.172
3.	268	0.231
4.	345	0.154
5.	367	0.093

Table 6: Uv-vis peak values of the extract of *G. robusta*

S.NO.	Peaks	Absorbance
1.	215	0.107
2.	224	0.109
3.	245	0.365
4.	289	0.253
5.	387	0.900

4. FTIR Analysis

FTIR analysis was conducted for the identification of the functional groups of the active components based on peak intensities in the IR region. The FTIR spectrum of the ethanolic-based extract of *B. variegata* is shown in Figure 3, and the hydroalcoholic-based extract of *B. variegata* is shown in Figure 4. The FTIR absorption spectra of the extracts were measured over 4000-400 cm^{-1}. The characteristic peaks of ethanolic-based extract were assigned at 2920, 1640, 1384, 1061, and 519 cm^{-1}. The peaks have revealed the O-H (stretch., water absorbed), C-H (bend., hydrocarbon chain), C-O (strech.,), and C-I (strech.,), etc. bonds. For hydroalcoholic-based extracts, peaks were assigned at 1636, 1064, and 617 cm^{-1}. These peaks revealed C=C (strech.,), C-O (strech.,) and C-Br (strech.,), etc. FTIR spectrum of the ethanolic-based extract of *G. robusta* is shown in Figure 4, and the hydroalcoholic-based extract of *G. robusta* is shown in Figure 5. *G. robusta ethanolic-based* extracts peaks were assigned at 1624,1442, 1059, and 611 cm^{-1}. The peaks have divulged the presence of C-H, O-H (bend.), C-O, C=C, and others. For the hydroalcoholic extract, peaks are observed at 1652, 1443, 1067, and 667 cm^{-1}.[27] These peaks indicated the C=C, O-H, C-O, and other functional groups.

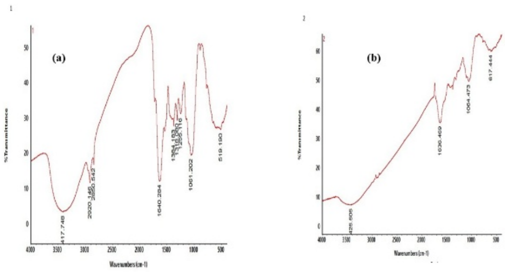

Figure 4. (a) FTIR spectrum of ethanolic-based extract of *B. variegata*, (b) hydroalcoholic-based extract of *B. variegata*

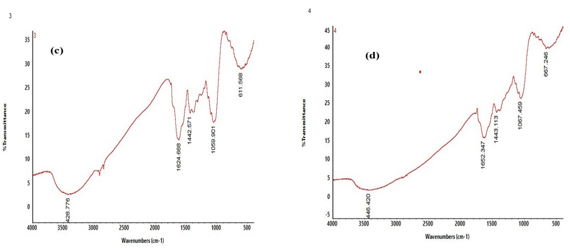

Figure 5. (c) FTIR spectrum of ethanolic-based extract of *G. robusta*, (d) hydroalcoholic-based extract of *G. robusta*

5. CONCLUSION

This research effectively highlighted the rich phytochemical content of *B. variegata* and *G. robusta*. Phytochemical analysis plays a vital role in assessing a plant's medicinal properties and pinpointing the active compounds responsible for its biological activities. Extraction-based testing verifies the presence of specific phytochemicals in the extracts. The efficiency of extracting phytochemicals from plant leaves largely depends on the solvents employed, such as ethanol and hydroalcoholic solutions. Consequently, conducting multiple tests is advisable to ensure more reliable results. These results suggest their potential use in pharmaceutical formulations aimed at treating diseases related to oxidative stress and bacterial infections. Beyond medicine, these phytochemicals could find applications in various industries, including natural antioxidants, antimicrobial agents, and cosmetic products. The strong antioxidant activity of *B. variegata* makes it suitable for nutraceuticals, while *G. robusta*, rich in tannins, could act as a natural preservative in food and beverages.

6. ACKNOWLEDGMENTS

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7. AUTHOR CONTRIBUTION

Mr. Yogesh Tiwari prepared this manuscript, including laboratory work, drafting, and editing. Prof. Tarun Parashar contributed to the manuscript's structure and provided supervision. Prof. Sayantan Mukhopadhyay was critical of the revision and approved the final version for publication.

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