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PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF BOENNINGHAUSENIA ALBIFLORA: A COMPARATIVE EVALUATION OF AQUEOUS AND METHANOL EXTRACTS

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Phytochemical Screening And Antimicrobial Activity Of *Boenninghausenia Albiflora*: A Comparative Evaluation Of Aqueous And Methanol Extracts

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Abstract

This study presents a comparative evaluation of the phytochemical constituents and antimicrobial activity of *Boenninghausenia albiflora,* a traditionally valued medicinal plant known for its wide range of therapeutic applications, by using its aqueous and methanol extracts. Phytochemical screening confirmed the presence of alkaloids, flavonoids, glycosides, phenolic compounds, saponins, terpenoids, and steroids in both extracts, with methanol showing great extraction efficiency. Whereas antimicrobial test against *Lactobacillus* and *Pseudomonas* species through disc diffusion method, revealed that both extracts inhibit the growth of these species, aqueous extract being more effective against *Lactobacillus,* while methanol extract shows slightly more potency against *Pseudomonas.* The study emphasizes the critical influence of solvent polarity in determining extraction efficiency and antimicrobial specificity, and suggests the potential of *Boenninghausenia albiflora* as a promising source for the development of plant-based antimicrobial agents.

Key words: *Boenninghausenia albiflora*, Phytochemicals, MIC, *Lactobacillus*, *Pseudomonas,* Rutacea

1. Introduction

India is globally recognized for its extraordinarily botanical wealth, harbouring nearly 17000 species of flowering plants, of which around a thousand are traditionally known for their medicinal value and play a vital role in the country's cultural and healthcare heritage, and *Boenninghausenia albiflora* is such plant, which is valued in traditional medicine but remains relatively under investigated scientifically [1] [2] [3].

Boenninghausenia albiflora which belongs to family Rutaceae, commonly known as White Himalayan Rue in English and Pissumar Booti in Hindi, is a herbaceous perennial plant and grows in tropical and subtropical areas, usually at high altitude between 500 and 3000 meters above sea level and is native to India and other countries like Nepal, Tibet, Pakistan, Sri Lanka, Japan, China, Thailand, Indonesia, Myanmar, North Vietnam and Laos [3]

This plant has antiseptic and healing properties and is used in traditional medicine for healing cuts and wounds, headache, scabies, and as a flea repellent. Some previous studies also show that it has calcium-blocking effects and promising anti-malarial potential. In-vitro tests have shown that crude ethanolic extract from aerial parts of *Boenninghausenia albiflora* exhibits antiplasmodial activity against both chloroquine-sensitive and chloroquine-resistant strains of *Plasmodium falciparum*, indicating it as potential source for novel anti-malarial compounds [4] [5] [6]. Additionally, Boenninghausenia albiflora essential oil has been proposed as a possible agent for integrated pest management against *Spilarctia obliqua,* commonly known as Jute hairy caterpillar [7].

As antimicrobial resistance (AMR) continues to be a serious global issue, there is an urgent need to explore natural sources for safe and effective antimicrobial agents [4]. Studying the antimicrobial properties of *Boenninghausenia albiflora* could help in developing such natural therapeutic formulations. However, there is limited comparative information on how different extraction solvents affect their phytochemical content and microbial activity, as the choice of solvent is critical, as it directly influences the yield and diversity of extracted compounds, thereby impacting the resulting biological activity. Hence, this study aims to perform a comparative phytochemical screening and antimicrobial evaluation of aqueous and methanol extracts of *Boenninghausenia albiflora* to validate its traditional use and identify the most theoretically potent extract.

2. Material & method

2.1 Plant material:

The aerial parts (flowers, leaves, and stems) of Boenninghausenia albiflora were collected from their natural habitat in the Hathipaon region of Mussoorie, Uttarakhand, India.

Fig.1. Flowers and leaves of plant *Boenninghausenia albiflora*

2.2 Plant extraction:

The collected parts of the plant were thoroughly cleaned and shade-dried at room temperature, and then the completely dried plant material was crushed into powder form by using a mortar and pestle. Two different solvents were used for the extraction: distilled water (aqueous) and methanol by using a Soxhlet apparatus.

i. **Aqueous extract:** About 30 g of powdered plant material was loaded onto filter paper and then placed into the Soxhlet extraction apparatus with 200 ml of distilled water.

ii. **Methanol extract:** 30 g of powdered plant material was loaded onto filter paper and then was placed into a Soxhlet apparatus with a solvent mixture of 100 ml methanol and 100ml of distilled water.

Fig.2: Aqueous and methanol extract of plant *Boenninghausenia albiflora*

Fig.3: Soxhlet apparatus

2.3 Phytochemical screening: The Following qualitative tests were performed to detect the presence of phytoconstituents such as alkaloids, flavonoids, phenolics, saponins, steroids, glycosides, and terpenoids by standard methods.

2.3.1 Tests for alkaloids

i. **Mayer's test:** 1ml of each sample (aqueous and methanol plant extract) was taken in two different test tubes, and a few drops of Mayer's reagent were added to both test tubes. Formation of a yellowish precipitate confirms the presence of an alkaloid in both test tubes.

ii. **Wagner's test:** 1ml of each sample (aqueous and methanol plant extract) was taken in two different test tubes, and a few drops of Wagner's reagent were added to both test tubes. Formation of reddish brown precipitate confirms the presence of alkaloid in both test tubes.

iii. **Dragendorff's test:** 2ml of each sample (aqueous and methanol plant extract) was taken in two different test tubes, and 1ml of Dragendorff's reagent was added to both test tubes. Formation of an orange precipitate confirms the presence of an alkaloid in both test tubes.

2.3.2 Test for flavonoids

i. **Alkaline reagent test:** 2ml of each sample (aqueous and methanol plant extract) was taken in two different test tubes, and 2-3 drops of sodium hydroxide (NaOH) were added to each test tube. After that, 5ml of dilute hydrochloric acid (HCl) was added to each of them. The disappearance of the yellow colour upon the addition of HCl (acidification) indicated the presence of flavonoids.

2.3.3 Tests for phenolic compounds and tannin

i. **Ferric chloride test:** 1ml of each sample (aqueous and methanol plant extract) was taken in two different test tubes, and 3 drops of 1% (w/w) ferric chloride solution ($FeCl_3$) were added to each test tube. Formation of blue-green colouration confirms the presence of phenols and tannins in both test tubes.

ii. **Lead acetate test:** 1ml of each sample (aqueous and methanol plant extract) was taken in two different test tubes, and add few drops of 10% lead acetate solution were added to each sample. The formation of white precipitate in both samples indicates the presence of phenolic compounds.

2.3.4 Test for saponins

i. Froth test: 2ml of each sample (aqueous and methanol plant extract) was taken in two different test tubes, and 8ml of distilled water was added to each test tube, and they were shaken vigorously by hand for 15 sec. The formation of froth in the test tubes indicates the presence of saponins in the sample.

2.3.5 Test for glycosides

i. Keller killani test: 1ml of each sample (aqueous and methanol plant extract) was taken in two different test tube and 2ml of glacial acetic acid containing a drop of ferric chloride solution was added to each test tubes and then 1ml of concentrated sulphuric acid (H_2SO_4) was added slowly down the side of the both test tubes until the ring is formed at the interface between two liquid layers, this appearance of the ring indicates presence of the glycosides in both of the sample.

2.3.6 Test for terpenoid

i. Salkowski's test: 2ml of each sample (aqueous and methanol plant extract) was taken in two different test tubes, and 2ml of chloroform was added to each of them. Then 1-2 drops of sulphuric acid (H_2SO_4) were added to each test tube and mixed, and allowed to stand for 30 seconds. The appearance of a reddish-brown interface indicates the presence of terpenoids in both samples.

2.3.7 Test for steroid

i. Libermann Burchard test: 1ml of each sample (aqueous and methanol plant extract) with acetic anhydride was taken in separate test tubes and boiled for a few minutes, then allowed to cool. After cooling, 1ml of concentrated sulphuric acid (H_2SO_4) was added slowly down the side of each test tube until a ring formed at the interface. The appearance of a brown ring at the junction and a green layer on top indicates the presence of a steroid in both samples.

2.4 Antimicrobial screening

Antimicrobial screening is performed to measure a substance's ability to inhibit or kill the microorganism being tested, and agar disk diffusion is one of the primary methods to do it. In this method, test microorganisms spread on agar plates are exposed to small discs containing antimicrobial agents. After incubation, clear inhibition zones around the discs indicate the effectiveness of that compound [8].

The antimicrobial screening of *Boenninghausenia albiflora* was checked against *Lactobacillus* and *Pseudomonas* species by the agar disc diffusion method. For Lactobacillus, a Chilli bacterial sample containing Lactobacillus was used for preparing a spread plate, and three discs were placed on the plate, one impregnated with 20 microliters of antibiotic as a control, one with 20 microliters of methanol plant extract, and one with 20 microliters of aqueous plant extract. After that, the plates were incubated at 37°C for 24 hours and were observed for zone inhibition.

For *Pseudomonas,* the bacterial sample was first used to prepare a spread plate, and then two discs, each with 20 microliters of methanol plant extract and aqueous plant extract, were placed on the plate. Then, after 24 hours of incubation at 37°C, the zones were observed.

3. Result

3.1 Phytochemical screening: Both plant solvent extracts (aqueous and methanol) show positive results for phytochemical tests, showing the presence of bioactive molecules, such as alkaloids, phenolic compounds, steroids, saponins, terpenoids, flavonoids, and glycosides in the plant *Boenninghausenia albiflora.*

The methanol extract of the plant showed a stronger positive reaction, as it produces more colour and precipitation intensity compared to the aqueous extract, suggesting an easier solubility of these compounds in methanol.

PHYTOCHEMICAL		TESTS	AQUEOUS EXTRACT	METHANOL EXTRACT
1.	Alkaloid	Mayers test	+	++
		Wagners test	+	++
		Dragendorffs test	+	++
2.	FLAVANOID	Alkaline reagent test	++	+++
3.	PHENOLIC COMPOUNDS	Ferric chloride test	+	++
		Lead acetate test	+	++
4.	SAPONINS	Froth test	+	++
5.	GLYCOSIDES	Keller Killani test	+	++
6.	TERPENOIDS	Salkowski test	+	++
7.	STEROIDS	Libermann Burchard test	+	++

Table no.1. Phytochemical test of aqueous extract and methanol extract of the plant *Boenninghausenia albiflora*

Key: + = positive, ++ = moderately positive, +++ = strongly positive

3.2 Antimicrobial assay: Both extract shows inhibitory effect against the *lactobacillus* and *pseudomonas* organisms.

For Lactobacillus, the aqueous extract produced larger zones of inhibition compared to the methanol extract, while for *Pseudomonas*, the methanol extract produced slightly larger zones of inhibition compared to the aqueous extract.

Notably, both extracts were more effective against *Pseudomonas* species than against *Lactobacillus* species, as indicated by the formation of larger zones of inhibition for both extracts when tested on *Pseudomonas.*

Fig.4: MIC against *Lactobacillus* from chilli bacterial sample

Fig.5: MIC against *Pseudomonas*

4. DISCUSSION & CONCLUSION

Boenninghausenia albiflora has a long history in traditional medicine, which this study supports through positive phytochemical test results indicating the presence of key bioactive compounds such as alkaloids, phenols, glycosides, flavonoids, and terpenoids. These molecules are associated with various therapeutic effects, including antibacterial, anti-diabetic, anticancer, and anti-inflammatory activities.

The stronger reactions observed in the methanol extract suggest that methanol is a more effective solvent for extracting bioactive constituents due to its intermediate polarity, and the antimicrobial result demonstrated selective activity of the extracts. The methanol extract exhibited stronger inhibition against *Pseudomonas* (Gram-negative), whereas the aqueous extract was more effective against *Lactobacillus* (Gram-positive). The overall stronger inhibition against *Pseudomonas* species suggests that *Boenninghausenia albiflora* possesses potent compounds capable of penetrating Gram-negative bacterial cell walls.

In conclusion, both aqueous and methanol extracts of *Boenninghausenia albiflora* exhibit significant phytochemical diversity and antimicrobial potential. The methanol extract proved more efficient for phytochemical extraction, while antimicrobial activity varied depending on the bacterial strain. These findings support the traditional medicinal use of the plant and highlight that the choice of

extraction solvent is critical for targeting specific pathogens. This study also points out the plant potential for developing plant-based antibacterial agents.

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