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PHYSICOCHEMICAL PROPERTIES AND PHYTOCHEMICAL CONSTITUENTS OF INDIGENOUS MEDICINAL PLANT *VITEX NIGUNDO* LEAF.

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ABSTRACT

Vitex negundo L. (Verbenaceae) is an important medicinal plant extensively used in traditional systems of medicine for the treatment of inflammation, pain, fever, skin disorders, and other ailments. The present study was conducted to investigate the phytochemical composition of *V. negundo* leaves using methanolic, aqueous, and chloroform extracts. Preliminary qualitative phytochemical screening was performed using standard chemical tests to detect alkaloids, flavonoids, tannins, saponins, glycosides, phenolic compounds, terpenoids, steroids, carbohydrates, and proteins. The methanolic extract exhibited the richest phytochemical profile, showing positive reactions for most of the tested constituents, whereas the aqueous extract contained a moderate range of predominantly polar compounds. The chloroform extract showed comparatively fewer constituents and was mainly enriched with non-polar

compounds such as terpenoids and steroids. The presence of alkaloids, flavonoids, tannins, phenolic compounds, and terpenoids suggests that *V. negundo* possesses a diverse array of bioactive metabolites that may contribute to its reported pharmacological activities. The findings support the traditional medicinal use of the plant and indicate that methanol is the most effective extraction solvent among those investigated for obtaining a broad spectrum of phytochemicals. This study provides baseline phytochemical information that may facilitate future quantitative analysis, isolation of active constituents, and evaluation of the plant's medicinal and biopesticidal potential.

KEYWORDS: *Vitex negundo* L.; phytochemical screening, bioactive compounds; qualitative analysis; traditional medicine.

INTRODUCTION

Medicinal plants are the backbone of traditional medicine and remain an important source of therapeutic agents worldwide. *Vitex negundo* L. (Verbenaceae): commonly known as the "five-leaved chaste tree," is a large aromatic shrub widely used in traditional healthcare systems (Nyeem et al., 2017). In the Indian traditional medicine system, *Vitex negundo* is referred to as *Sarvaroganivarani*, meaning "the remedy for all diseases" (Ladda & Magdum, 2012). A popular proverb among the Bhangali communities of the Western Himalayan region of India states that "a man cannot die of disease in an area where *Vitex negundo*, *Adhatoda vasica*, and *Acorus calamus* are found, provided that he knows how to use them" (Singh et al., 2006). In Sanskrit, *Nirgundi* means "that which protects the body from diseases" (Raji, 2013).

Although all parts of *V. negundo* are used in traditional systems of medicine, the leaves possess the greatest medicinal potency (Keerti & Padma, 2012). Fresh leaves are widely used to treat rheumatism, fever, pain, inflammation, and various skin diseases (Sharath et al., 2022). Previous studies have reported anti-inflammatory, anti-arthritic, immunostimulant, anti-androgenic, insecticidal, pesticidal, anti-snake venom, antioxidant, analgesic, hepatoprotective, mosquito repellent, anti-estrogenic, and anti-HIV activities of *V. negundo* (Bhargava, 1989; Chaturvedi & Singh, 1965; Dharmasiri et al., 2003; Kadir et al., 2013; Kannan et al., 2012).

Phytochemical screening is an essential preliminary step for identifying bioactive compounds with pharmaceutical and biopesticidal potential and provides valuable insights into the

structure and function of plant metabolites (Khameneh et al., 2021). By screening medicinal plants, researchers can discover new lead compounds for the development of environmentally friendly biopesticides (Divekar et al., 2022). However, a comprehensive phytochemical analysis of *V. negundo* leaves and a stem using different solvent extracts remains limited. Therefore, the present study was undertaken to investigate the phytochemical composition of *V. negundo* leaves and stems using methanolic, aqueous, and chloroform extracts.

To date, approximately 7,000 phytomolecules and more than 1,200 biologically active compounds have been isolated from medicinal plants (Kumar et al., 2023). In Bangladesh, traditional medicinal systems, particularly Ayurveda and Unani, continue to play an important role in the management of both communicable and non-communicable diseases (Pandey et al., 2013). Plant-derived phytochemicals are generally considered relatively safe and are often associated with fewer adverse effects than many synthetic therapeutic agents (Baba & Onanuga, 2011). Consequently, the present phytochemical investigation was undertaken to provide a scientific basis for the medicinal and potential biopesticidal applications of *V. negundo*.

MATERIALS AND METHODS

Plant Materials Collection and Authentication

The fresh leaf sections of the *Vitex negundo* plant were obtained in December 2021 in Hamdard University Bangladesh campus, Gazaria, Munshiganj, Bangladesh. A qualified taxonomist examined the material of the plant to authenticate it and a voucher specimen (HUB/HB/LB No. 91) was lodged in the university herbarium as a future reference material.

Plant extraction Preparation Methods

Approximately 600 grams of *Vitex negundo* was dried for a period of 15 days, ensuring it did not come into direct sunlight. Once the moisture content of the *Vitex negundo* sample was determined to be below 5% through loss on drying (LOD) at 105˚C, the material was ground into a powder and weighed (350 grams). Subsequently, solvents were used in a Soxhlet apparatus with 500 mL of methanol, distilled water, and chloroform, following a 6 to 8-hour extraction time as outlined in standard methods (Harborne, 1998; Kokate, 2010). The extraction process was continued until the solvent in the siphon tube appeared clear. Chloroform was utilized to isolate non-polar compounds, methanol was employed for extracting compounds of intermediate polarity, and water was used for highly polar constituents. The extracts were then filtered.

Physicochemical analysis

The physicochemical characteristics of the powdered leaves of *Vitex negundo* were assessed following the guidelines set by the World Health Organization (2011) and established protocols (Haruna et al., 2023; AOAC, 2006; De & Sharma, 2023; Khandelwal, 2008; Kokate et al., 2010). The findings are shown in Table 1. All values are represented as % w/w, unless indicated otherwise.

Table 1: Physicochemical analysis.

Parameter	Procedure	Calculation	Value
Moisture content (Loss on Drying)	4.53 g of powdered sample was dried at 105°C; weight loss was recorded	Direct reading	8.14 % w/w
Total Ash Value	Burning of 5 g sample 450-600°C till the sample is carbon free	% Total Ash = (Ash weight / Sample weight) × 100	6.92% w/w
Acid-Insoluble Ash	Treatment of total ash with 10 percent HCl and then filtration and incineration	Percent concentration acid-insoluble ash = (weight of residue/ weight of sample) x 100	1.28 % w/w
Water-Soluble Ash	Dissolution of total ash in water, filtration and combustion	Water-Soluble Ash = (Weight of residue/Weight of sample) x100	2.74% w/w
Alcohol-Soluble Extractive Value	Maceration of 24 hours in 90 percent ethanol	Direct reading	13.02% w/w
pH Determination	A 1% aqueous solution was taken in a calibrated digital pH meter	Direct reading	6.17
Swelling Index	A 1 g sample in 25 mL water was measured after 24 hours	Swelling Index = (Final Volume – Initial Volume)/Sample Weight	3.48 mL/g
Bulk Density	0.50 g sample in 100 mL grad cyber	Bulk Density = Mass / Volume	0.60 g/mL

Preliminary phytochemical screening

Preliminary phytochemical screening of the plant extract was performed using standard qualitative procedures for the detection of major secondary metabolites following established pharmacognostic methods. The results are summarized in Table 2.

Table 2: Preliminary phytochemical screening.

Test for Alkaloids		
Test	**Procedure**	**Observation**
Mayer's test	One milliliter of the acidic plant extract filtrate was transferred into a clean test tube. Two to three drops of Mayer's reagent were added and the mixture was gently shaken and allowed to stand for 1–2 minutes (Maheshwaran et al., 2024).	The formation of a cream, white, or pale yellow precipitate indicates the presence of alkaloids, whereas the absence of precipitate indicates a negative result (Jain et al., 2012).
Wagner's test	One milliliter of the acidic filtrate was treated with 2–3 drops of Wagner's reagent and mixed gently (Maheshwaran et al., 2024).	The appearance of a reddish-brown or brown flocculent precipitate confirms the presence of alkaloids (De Silva et al., 2017).
Dragendorff's test	Two milliliters of the acidic filtrate were taken in a test tube and 1 mL of Dragendorff's reagent was added. The mixture was gently shaken and allowed to stand briefly (Raal et al., 2020).	The formation of an orange, orange-red, or reddish-brown precipitate indicates the presence of alkaloids (Raal et al., 2020).
Hager's test	Two milliliters of the acidic filtrate were treated with a few drops of Hager's reagent and mixed gently (Maheshwaran et al., 2024).	The formation of a yellow crystalline or yellow precipitate confirms the presence of alkaloids (Jain et al., 2012).
Test for Flavonoids		
Shinoda test	To 2 mL of the methanolic or ethanolic plant extract, add a small piece of magnesium ribbon followed by 3–5 drops of concentrated HCl.	Development of a pink, crimson, red, or orange coloration indicates the presence of flavonoids (Harborne, 1998)
Alkaline reagent test	To 2 mL of the plant extract, add 2 mL of 10% NaOH solution, then add dilute HCl dropwise.	Formation of an intense yellow color that becomes colorless after acid addition confirms the presence of flavonoids (Evans, 2009).
Ferric chloride test	To 2 mL of the plant extract, add 2–3 drops of 5% $FeCl_3$ solution.	Appearance of a green, blue-green, dark green, or blackish coloration indicates the presence of phenolic flavonoids (Harborne, 1998).
Lead acetate test	To 2 mL of the plant extract, add 1–2 mL of 10% lead acetate solution and shake gently.	Formation of a yellow precipitate indicates the presence of flavonoids (Evans, 2009).
Test for Tannins		
Ferric chloride test	To 2 mL of the aqueous or methanolic plant extract, add 2–3 drops of 5% ferric chloride ($FeCl_3$) solution.	Formation of a blue-black color (hydrolysable tannins) or greenish-black color (condensed tannins) indicates the presence of tannins (Evans, 2009).
Gelatin test	To 2 mL of the plant extract, add 1 mL of 1% gelatin solution containing sodium chloride.	Formation of a white precipitate indicates the presence of tannins (Evans, 2009).
Lead acetate test	To 2 mL of the plant extract, add 1–2 mL of 10% lead acetate solution and mix thoroughly.	Formation of a yellowish-white or bulky precipitate indicates the presence of tannins (Harborne, 1998).

Potassium dichromate test	To 2 mL of the plant extract, add a few drops of 1% potassium dichromate solution.	Formation of a brown precipitate indicates the presence of tannins (Evans, 2009).
Test for Saponins		
Froth test	To 2 mL of the aqueous plant extract, add 5 mL of distilled water in a test tube and shake vigorously for 30 seconds. Allow the mixture to stand for 10–15 min.	Formation of a stable persistent froth (approximately ≥1 cm in height) lasting for at least 10 min indicates the presence of saponins (Evans, 2009).
Foam test	To 2 mL of the plant extract, add 10 mL of distilled water and shake vigorously for 30–60 seconds. Allow the mixture to stand undisturbed for 10 min.	Formation of a persistent honeycomb-like foam or froth layer that remains for 10 min or longer confirms the presence of saponins (Evans, 2009).
Test for Glycosides		
Keller–Killiani test	To 2 mL of the plant extract, add 1 mL of glacial acetic acid containing one drop of 5% $FeCl_3$ solution, then carefully underlay with 1 mL of concentrated H_2SO_4.	A brown or reddish-brown ring at the interface, often with a bluish-green upper layer, indicates the presence of cardiac glycosides (Harborne, 1998).
Legal's test	To 2 mL of the extract, add 1 mL of pyridine and a few drops of sodium nitroprusside solution, followed by sodium hydroxide solution.	Formation of a pink to blood-red color indicates the presence of cardiac glycosides containing an unsaturated lactone ring (Evans, 2009).
Borntrager's test	Boil the extract with dilute H_2SO_4, cool and filter; extract with chloroform and add dilute ammonia.	A pink, red, or violet color in the ammoniacal layer indicates the presence of anthraquinone glycosides (Harborne, 1998).
Test for Phenolic compounds		
Ferric chloride test	To 2 mL of the aqueous or methanolic plant extract, add 2–3 drops of 5% ferric chloride ($FeCl_3$) solution.	Formation of a blue, blue-green, green, purple, or black coloration indicates the presence of phenolic compounds (Evans, 2009; Harborne, 1998).
Folin–Ciocalteu test (qualitative)	To 1 mL of the plant extract, add 1 mL of Folin–Ciocalteu reagent followed by 2 mL of 7.5% sodium carbonate solution; allow the mixture to stand for 10–15 min.	Development of a deep blue coloration indicates the presence of phenolic compounds (Singleton et al., 1999).
Ellagic acid test	To 2 mL of the plant extract, add a few drops of 5% glacial acetic acid and 5% sodium nitrite solution.	Formation of a muddy brown or brownish precipitate suggests the presence of phenolic compounds, particularly ellagitannins (Harborne, 1998).
Test for terpenoids		
Salkowski test	To 2 mL of the chloroform extract, add 2 mL of concentrated sulfuric acid (H_2SO_4) carefully along the side of the test tube to form a separate layer.	Formation of a reddish-brown or brown ring at the interface indicates the presence of terpenoids or triterpenoids (Evans, 2009).
Liebermann–Burchard test	To 2 mL of the chloroform extract, add 1 mL of acetic anhydride followed by 1–2 drops of concentrated H_2SO_4.	Development of a deep green, blue-green, or bluish coloration indicates the presence of triterpenoids or sterols (Harborne, 1998).

Copper acetate test	To 2 mL of the extract dissolved in chloroform, add a few drops of saturated copper acetate solution.	Formation of an emerald green coloration indicates the presence of diterpenes and other terpenoid compounds (Harborne, 1998).
Vanillin–sulfuric acid test	To 2 mL of the extract, add 1 mL of vanillin reagent followed by concentrated H_2SO_4 and warm gently.	Development of a pink, red, purple, or violet coloration indicates the presence of terpenoids (Harborne, 1998).
Test for Steroids		
Liebermann–Burchard test	To 2 mL of the chloroform extract, add 1 mL of acetic anhydride followed by 1–2 drops of concentrated H_2SO_4.	Development of a deep green or blue-green coloration indicates the presence of steroids (Evans, 2009; Harborne, 1998).
Salkowski test	To 2 mL of the chloroform extract, add 2 mL of concentrated H_2SO_4 carefully along the side of the test tube.	Formation of a red or reddish-brown coloration at the interface indicates the presence of steroids (Harborne, 1998).
Test for Carbohydrates		
Molisch's test	To 2 mL of the extract, add 2–3 drops of Molisch's reagent (α-naphthol): then carefully add 1 mL of concentrated H_2SO_4 along the side of the test tube.	Formation of a violet or purple ring at the interface indicates the presence of carbohydrates (Evans, 2009).
Benedict's test	To 2 mL of the extract, add 2 mL of Benedict's reagent and heat in a boiling water bath for 2–5 min.	Formation of a green, yellow, orange, or brick-red precipitate indicates the presence of reducing sugars (Evans, 2009).
Test for Proteins		
Biuret test	To 2 mL of the extract, add 1 mL of 10% NaOH followed by 2–3 drops of 1% $CuSO_4$ solution.	Development of a violet or purple coloration indicates the presence of proteins (Evans, 2009).
Ninhydrin test	To 2 mL of the extract, add 2–3 drops of 0.2% ninhydrin solution and heat in a boiling water bath for 1–2 min.	Development of a blue or purple coloration indicates the presence of amino acids and proteins (Harborne, 1998).
Xanthoproteic test	To 2 mL of the extract, add concentrated HNO_3 and heat gently; after cooling, add excess NaOH.	Formation of a yellow to orange coloration indicates the presence of aromatic amino acids in proteins (Evans, 2009).

RESULTS

Preliminary phytochemical screening was carried out on the methanolic, aqueous, and chloroform extracts of the plant sample using standard qualitative phytochemical tests. The presence or absence of major secondary metabolites was determined based on characteristic color changes or precipitate formation observed during each assay. The results of the qualitative phytochemical screening are presented in Table 3.

Table 3: Qualitative phytochemical screening of methanolic, aqueous, and chloroform extracts.

Phytochemical Test		Methanol	Aqueous	Chloroform
Alkaloids	Mayer's test	+	+	−
	Wagner's test	+	+	−
	Dragendorff's test	+	+	−
	Hager's test	+	+	−
Flavonoids	Shinoda test	+	+	−
	Alkaline reagent test	+	+	−
	Ferric chloride test	+	+	+
	Lead acetate test	+	+	−
Tannins	Ferric chloride test	+	+	−
	Gelatin test	+	+	−
	Lead acetate test	+	+	−
	Potassium dichromate test	+	+	−
Saponins	Froth test	+	+	−
	Foam test	+	+	−
Glycosides	Keller–Killiani test	+	+	−
	Legal's test	+	+	−
	Bornträger's test	−	−	+
Phenolic Compounds	Ferric chloride test	+	+	+
	Folin–Ciocalteu test	+	+	+
	Ellagic acid test	+	+	−
Terpenoids	Salkowski test	+	−	+
	Liebermann–Burchard test	+	−	+
	Copper acetate test	+	−	+
	Vanillin–sulfuric acid test	+	−	+
Steroids	Liebermann–Burchard test	+	−	+
	Salkowski test	+	−	+
Carbohydrates	Molisch's test	+	+	−
	Benedict's test	+	+	−
Proteins	Biuret test	+	+	−
	Ninhydrin test	+	+	−
	Xanthoproteic test	+	+	−

Note: + = present; − = absent.

DISCUSSION

The preliminary Phytochemical screening demonstrated that the methanolic extract contained the widest range of phytochemical constituents, whereas the aqueous extract showed a moderate profile and the chloroform extract was relatively enriched in non-polar compounds such as terpenoids and steroids. This pattern is consistent with the influence of solvent polarity on extraction efficiency, as methanol is known to extract both polar and moderately non-polar phytochemicals more effectively than aqueous or chloroform solvents (Kumar et al., 2023).

The presence of alkaloids, flavonoids, tannins, saponins, glycosides, and phenolic compounds in the methanolic and aqueous extracts suggests that these extracts are rich in biologically active secondary metabolites. Alkaloids are associated with analgesic, antimicrobial, and anti-inflammatory activities, while flavonoids and phenolic compounds possess strong antioxidant properties that help protect cells against oxidative stress (Maheshwaran et al., 2024). Tannins and saponins contribute to antimicrobial, wound-healing, and immunomodulatory effects, supporting the traditional medicinal use of the plant (Baba & Onanuga, 2011).

The detection of terpenoids and steroids in the chloroform extract indicates that non-polar constituents were preferentially extracted by chloroform. These compounds are known for their anti-inflammatory, antimicrobial, and membrane-stabilizing activities and may contribute significantly to the pharmacological potential of the plant (Kumar et al., 2023). The observed phytochemical profile is comparable with previous reports on *Vitex negundo*, which have documented the presence of flavonoids, phenolics, terpenoids, and other bioactive constituents in different solvent extracts (Keerti & Padma, 2012; Sharath et al., 2022).

Overall, the qualitative screening confirms that the plant is a valuable source of pharmacologically important phytochemicals. Among the tested solvents, methanol proved to be the most efficient extraction solvent, suggesting that the methanolic extract may be the most suitable for further quantitative phytochemical analysis and biological activity evaluation.

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