

Original Research Article

## Phytochemical Screening Of Different Extracts Of *Vitex Negundo* Leaves

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### ABSTRACT:

In this study, we used several solvent extracts to screen preliminary phytochemically and analyse the leaves of *Vitex negundo* using HPLC. Different solvent extracts (petroleum ether, chloroform, ethanol, and methanol) were used to extract *Vitex negundo* leaves, and the results revealed the presence of phenols, phytosterol, steroids, alkaloids, flavonoids, tannins, terpenoids, saponins, and carbohydrates. The flavonoids moiety in the ethanol extract was caffeic acid, orientin, ferulic acid, and casticin, according to the HPLC chromatogram. As a result, this study will promote further research into the phytochemical components of plants and encourage other related investigations.

**Keyword:** *Vitex negundo*, phytochemical analysis, HPLC Analysis, Flavonoids

### INTRODUCTION:

Since the beginning of human civilization, medicinal plants have been an integral element of human society as a means of illness prevention. We are well aware of plants' significance. [1] In recent years, there has been a growing understanding of the significance of medicinal plants. The plant kingdom offers a treasure trove of potential medications. Drugs made from plants are widely available, inexpensive, efficient, and safe, and they seldom ever cause side effects. The most obvious choice for looking at the current quest for therapeutically effective novel medications, such as anticancer drugs [2], antibacterial drugs [3], and antihepatotoxic chemicals, is the plants that have been selected for medical use over thousands of years.

The World Health Organization (WHO) states that the best source for a wide range of medications would be medicinal plants. In developed nations, traditional medicines with ingredients derived from medicinal plants are used by about 80% of people. To learn more about these plants' characteristics, security, and effectiveness, however, more research should be done [4]. Tannins, steroids, sugars, terpenoids, alkaloids, and flavonoids are only a few of the chemical components

found in medicinal plants that have defined physiological effects on humans [5, 6]. Since the beginning of time, plant products have been used in phytomedicines. Barks, fruits, flowers, roots, leaves, and seeds can all be used to make this. The ability to synthesise complex chemical substances will benefit from knowledge of the chemical components of plants [7, 8].

*Vitex negundo* Linn. belong to family Verbenaceae. A significant medicinal plant, it. [9] It has therapeutic powers because it contains a variety of complex chemicals with varying chemical compositions that are found in one or more of these plants as secondary plant metabolites. Anthraglycosides, arbutin, bitter substances, flavonoids, alkaloids, saponins, coumarins, phenol carboxylic acids, terpenes, and valepotriates are only a few of the several phytochemicals found in plants. [10] These phytochemicals exhibit a variety of biological actions in this plant, including anti-oxidants, anti-inflammatory, antispasmodics, emetics, and anti-cancer properties. [11] The procedure of obtaining vitex negundo leaf extract led to the considerable manifestation of several activities in petroleum ether, methanol, ethanol, chloroform, etc. Pharmaceutical research has long held that the phytochemical components of medicinal plants are a prerequisite for the development of highly effective medications and treatments for a variety of ailments. [12]

## MATERIAL METHODS

**Collection and Authentication:** The fresh plant parts (leaves) of *Vitex negundo* were collected from Botanical garden of Government Forest Department, Bareilly, Uttar Pradesh, India. The taxonomic authentication of the plant was confirmed by Professor Dr. Alok Srivastava, Department of Plant Science, Mahatma Jyotiba Phule Rohilkhand University Bareilly, Uttar Pradesh, India. The plant authentication letter no. RU/PS/19/01. The preparation of various extracts was made from the shade dried and powdered leaves of *Vitex negundo* L.

**Chemicals, Reagents, and Drugs:** Petroleum ether, chloroform, ethanol, methanol, acetone, toluene, benzene, ammonia, n-hexane (Rankem, SD Fine chemical Ltd., India),  $\alpha$ -Naphthol (E-merk, Mumbai, India), Ethyl acetate (RFCL Ltd. New Delhi), cephalixin (Medisel Ltd., Kenya), and Mercuric chloride, Lead acetate (Central Drug House New Delhi) were used in this study, and the chemical and reagents were of analytical grade.

**Collection and Extraction of *Vitex negundo* Linn.** Fresh *Vitex negundo* leaves were gathered in Bareilly, Uttar Pradesh, washed with water multiple times to remove contaminants, and then shade dried for fifteen days before being ground into powder. Using a soxhlet extraction equipment at the solvent's boiling point for 48–72 hours or until the extracted solvent became clear, 40gm of *Vitex negundo* Linn powder was extracted in each of the solvents—ethanol, petroleum ether, chloroform, and methanol. The extracts were then filtered using filter paper, and the solvent was removed from the extract using a rotary evaporator to give it a syrupy consistency. The extract was then stored for upcoming tests in a refrigerator at 4°C. [13, 14, 15]

% Yield= Weight of extract (g)/ Weight of dry powder (g)  $\times$  100

**Table 1:** Percentage yield value of different extract

Solvent(s)	Raw Material	Extract yield (%)	Color
Petroleum ether	40g	3.4	Dark brown color
Methanol	40g	2.12	Light brown color
Ethanol	40g	3.5	Grey color
Chloroform	40g	1.8	Slightly green

**Phytochemical Studies of Leaves:**

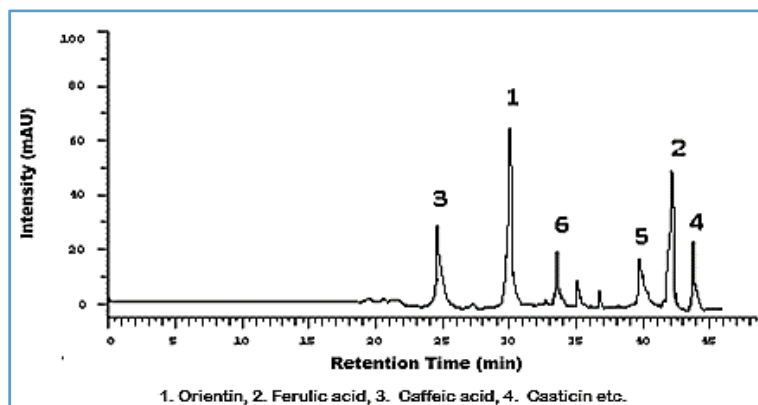
**Preliminary Phytochemical Screening:** The strength of the colour reactions was represented by the following symbols: (-) for no observable changes, which indicates a negative result; (+) for mild intensity; and (++) for strong intensity. The powdered material underwent preliminary phytochemical screening by being chemically analysed for the presence of alkaloids, phenols, tannins, flavonoids, quinones, terpenes, carbohydrates, proteins, gums, and mucilage using standardised procedures. [16–20].

**Table 2:** Preliminary of phytochemical analysis of various extracts of *V. negundo* leaves

S. No.	Phyto-constituents	Test	Successive extraction			
			Pet. Ether	Chloroform	Ethanol	Methanol
1.	Carbohydrate	Benedict's test	-	-	+	+
		Molisch's test	-	-	+	+
2.	Protein	Biuret test	-	-	+	+
		Ninhydrin reaction test	-	-	+	+
3.	Tannins	Lead sub-acetate test	+	-	+	+
		Ferric chloride test	+	-	+	+
4.	Flavonoids	Ammonium test	+	+	+	+
		Aluminum chloride test	+	+	+	+
5.	Alkaloids	Mayer's test	-	++	++	++
		Wagner's reagent	-	++	++	++
		Picric acid solution (1%)	-	++	++	++
6.	Glycosides	Borntrager's test	-	-	+	+
7.	Terpenoids	Conc. H <sub>2</sub> SO <sub>4</sub> test	+	-	+	-
		Salkowski test	+	-	+	-
8.	Saponin	Emulsion test:	-	-	-	+
		Frothing test	-	-	-	+
9.	Amino acid	Cysteine test	-	-	+	-
		Tryptophan test	-	-	+	-

Key: - (Absent), + (Mild intensity positive result), ++ (High intensity positive result)

**HPLC-analysis:** HPLC-analysis High performance liquid chromatography (HPLC) was performed on a FARE Labs Pvt. Ltd. MG Road sector-25 Gurugram (H.R)-122002. The HPLC analysis of ethanolic extract was carried out with Chromatographic system (YL 9100, Korea) consist of autosampler (YL 9150) with 100 µl fixed loop and an YL9120 UV-Visible detector. The separation was performed on a SGE Protecol PC18GP120 (250mm×4.6 mm, 5µm) column at ambient temperature. The mobile phase consists of methanol to water (70:30 v/v) and the separations were performed by using isocratic mode, elution performed at a flow rate of 1 ml/min. The samples were run for 15 minutes, and detection was done at 254 nm by UV detector. All chromatographic data were recorded. [21, 22]



**Figure 1:** HPLC chromatogram of *Vitex negundo* leaves extract

### RESULTS AND DISCUSSION:

The percentage yield of the leaf extracts in petroleum ether, methanol, ethanol, and chloroform was the highest (3.4, 2.12, 3.5, and 1.8%, respectively), while crude hexane (8.2 and 5.5%, respectively) had the lowest yields (Table 1). This suggests that ethanol and methanol leaf extracts of *V. negundo* contain more chemicals than petroleum ether and chloroform extracts. The ethanol and methanol extracts contained phytochemicals such as carbohydrates, tannins, phenols, flavonoids, alkaloids, and glycosides (Table 2).

Figure 1 shows the *Vitex negundo* HPLC fingerprints. The chromatogram obtained at different retention periods from the HPLC examination of the *Vitex negundo* ethanolic extract (Figure 1) at 254 nm demonstrates the presence of numerous compounds. These constituents included orientin, ferulic acid, caffeic acid, and casticin.

### CONCLUSION

The findings showed that the plants under study had components with significant therapeutic value. There is a wealth of evidence from past investigations that supports the bioactivity of the discovered phytochemicals. Numerous studies have shown that the presence of these phytochemicals gives the plants under study physiological and medicinal properties that can be used to treat a variety of diseases. This study demonstrates that the methanol and ethanol extract fraction of *vitex negundo* contains bioactive phytochemicals like flavonoids, alkaloids, and tannins. Casticin, ferulic acid, orientin, and caffeic acid were discovered as components in the *Vitex negundo* leaves extract after HPLC examination. It is therefore highly advised to investigate *vitex negundo* for additional phytochemical and biological research.

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### CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest. The research received no specific grant from any funding agency in the public, community, or non-for profit sectors.

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