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PHYTOCHEMICAL ANALYSIS OF *VITEX NEGUNDO* BY TLC, UV-VIS, FTIR TECHNIQUES

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ABSTRACT

In the present study phytochemical components of *vitex negundo* (accession no: 3017/ Botany/St.Joseph college/Trichy) plant extract was analysed using TLC, UV, and FT-IR techniques in this study. flavonoids, saponins, cardiac glycoside, terpenoids, quinones, alkaloids, and Phlobatannins were found in preliminary phytochemical investigation. TLC was performed with chloroform, ethanol, ethyl acetate, hexane, and acetic acid in a ratio of (10:2:5:1:1) and the Rf value was determined. TLC analysis revealed the presence of a spot with an Rf value of 0.80. The presence of an absorption peak at 330 nm was confirmed by UV-Vis spectroscopy investigation. The presence of carboxylic acid, aromatic compound, and halo alkane functional groups was confirmed by FTIR analysis. The TLC, UV-VIS, and FTIR spectrum profiles of the medically significant *vitex negundo* plant were developed as a result of our research.

Keywords: *vitex negundo*, TLC, UV-VIS, FTIR, Phytochemical analysis

INTRODUCTION

Taxonomical classification:

Kingdom: Plantae- Plants

Subkingdom: Tracheobionta – Vascular plants

Super division: Spermatophyte – Seed plants

Division: Magnoliophyta – Flowering plants

Class: Magnoliopsida – Dicotyledons

Subclass: Asteridae

Order: Lamiales

Family: Verbenaceae

Genus: Vitex Linn.

Species: *Vitex negundo* Linn.

Vitex negundo (*V. negundo* belongs to the Verbenaceae family) is a valuable medicinal plant that can be found in Bangladesh, India and other tropical and temperate areas of the world. Even though almost all of its parts are used in Ayurvedic and Unani systems of medicine, the extracts from its leaves and roots are the most important in the field of medicine and drug **[1]**. Its leaves **[2]** and seeds **[3]** are commonly used externally for rheumatism and joint inflammations and have insecticidal properties. Several researches have previously reported on the antimicrobial activity and chemical components of essential oil extracted from *V. negundo* leaf **[4-6]**. *V. negundo* leaves are commonly used as traditional medicine in Bangladesh for many types of gastroenteritis, and it is extremely efficient in various types of diarrhoea and dysentery. Pesticidal, antifungal, and antibacterial activities have been observed in the leaves of *V. negundo* **[7]**. TLC stands for thin layer chromatography and is a common chromatographic technique for separating non-volatile substances. TLC is commonly performed on glass sheets, aluminium foil and polymers that were coated with a thin layer of absorbent materials such as silica gel, cellulosic materials and aluminium oxides **[8]**. The mobile phase is the solvent that is involved in the separation, whereas the stationary phase is the absorbent materials. The polarity of both phases is different. Separation by the TLC is highly convenient as the components are separated on the plane. Separation occurs due to polarity and the fact that some migrate less than others **[9]**. UV-Visible one of the most commonly used techniques in pharmaceutical analysis is spectropotometry which involves measuring the quantity of UV absorbed by a chemical in solution. Ultraviolet-Visible spectrophotometers are instruments that measure the ratio or function of the intensity of two beams of light in the UV-Visible area. In qualitative analysis, organic compounds can be identified by use of spectrophotometer, if any recorded data is available and quantitative spectrophotometric analysis is used to ascertain the quantity of molecular species absorbing the radiation. Spectrophotometric technique is simple, rapid, moderately specific and applicable to small quantities of compounds **[10]**. FTIR is one of the most extensively used technologies for identifying chemical constituents and elucidating compound structures, and it is required for identifying medicines in many **[11]** Pharmacopoeias. FTIR can be used to find out the structure of unknown compositions as well as the intensity of absorption spectra related to molecular composition or chemical group

content **[12, 13]**. The FTIR method provides a spectrum that can be considered as a biochemical or metabolic "fingerprint" of the sample by measuring the vibrations of bonds within chemical functional groups. This may be able to detect minor changes in primary and secondary metabolites by obtaining IR spectra from plant samples **[14]**. FTIR is presently to be used to detect the concrete structure of certain plant secondary metabolites, particularly in phytochemistry **[15-17]**. Terpenoids, Flavonoids, Steroids, Anthroquione, Glycosides, Sugars, Alkaloid, Quinones, Phenols, Tannins, Saponins, Coumarin are all confirmed by phytochemical investigation of *Vitex negundo* **[18]**. The focus of this research is to establish a UV-VIS and FTIR spectrum profile of the *vitex negundo* plant.

MATERIALS AND METHOD

Collection of plant:

Leaves of *Vitex negundo* was collected and washed thoroughly in water to remove mud and dust particles. The leaves were shade dried and then powdered coarsely in mixer and stored in separate air tight containers at room temperature for further use.

Preparation of plant extract

10 g of fine powdered sample was taken and mixed with 200 ml ethylaetate. Samples were initially soaked in respective solvent for 24 h under refrigeration and subjected to ultra sonication at 450Hz for 5 cycle. Ultrasound assistant extraction was carried out using Bandelin Sonorex brand ultrasonic bath.

Phytochemical Analysis

Test for Carbohydrates:

To 2ml of plant extract, 1ml of Molisch's reagent and few drops of concentrated sulphuric acid were added. Presence of purple or reddish colour indicates the presence of carbohydrates.

Test for phenols and tannins

Crude extract was mixed with 2 ml of 5% solution of $FeCl_3$. A blue-green or black coloration indicated the presence of phenols and tannins.

Test for flavonoids (Shinoda test)

One to five drops of concentrated hydrochloric acid (HCl) were added to little amount of ethanolic extract of the plant material. Immediate development of a red colour indicates the presence of flavonoids.

Test for saponins

The extract was mixed with 5ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

Test for glycosides

Salkowski's test

Crude extract was mixed with 2ml of chloroform. Then 2ml of concentrated

H_2SO_4 was added carefully and shaken gently. A reddish brown colour indicated the presence of steroidal ring, i.e., glycone portion of the glycoside.

Keller-kilani test

Crude extract was mixed with 2ml of glacial acetic acid containing 1-2 drops of 2% solution of $FeCl_3$. The mixture was then poured into another test tube containing 2ml of concentrated H_2SO_4. A brown ring at the interphase indicated the presence of cardiac glycosides.

Test for terpenoids

Crude extract was dissolved in 2ml of chloroform and evaporated to dryness. To this, 2ml of concentrated H_2SO_4 was added and heated for about 2 minutes. A greyish colour indicated the presence of terpenoids.

Test for quinones

A small amount of extract was treated with concentrated HCl and observed for the formation of yellow colour precipitate.

Test for alkaloids

Two mL of extract was taken in a test tube and then 0.2 mL dilute HCl was included, followed by 1 mL of Meyer's reagent. A yellowish coloration indicates alkaloid's presence.

Test for phlobatannins:

To 1ml of plant extract few drops of 2% HCL was added appearance of red colour precipitate indicates the presence of phlobatannins.

TLC-Thin Layer Chromatography

Precoated thin layer chromatography (TLC) plates (silica gel 60F-254) with the adsorbent layer thickness of 0.25 mm (E-Merck), were used. Chloroform-ethanol-ethyl acetate-hexane and acetic acid (10:2:5:1:1) is used as mobile phase. Samples were placed on TLC using capillary tube at the bottom of plate and allowed to dry. The plate is kept under mobile phase and separation of compounds permitted until the solvent reached ¾ the distance and exposed under UV at 365 and 254 nm .

UV

The ethyl acetate plant extract was examined under UV-Visible spectral analysis. The sample was diluted to 1:10 with the same solvent. The extract was scanned in the wavelength ranging from 200-800nm using Perkin Elmer Spectrophotometer and the characteristic peaks were detected.

FTIR

FTIR analysis of the aqueous extract was carried out through the potassium bromide (KBr) pellet (FTIR grade) method in 400 – 4000 cm^{-1} and spectrum was recorded using Jasco FT/IR-6300 Fourier transform infrared spectrometer equipped with JASCO IRT-7000 Intron Infrared

Microscope using transmittance mode operating at a −1 resolution of 4 cm (JASCO, Tokyo, Japan).

RESULT & DISCUSSION

Phytochemical test

The phytochemical analysis of *vitex negundo* extract was summarized in **Table 1**. Flavonoids, Saponins, Cardiac glycoside, Terpenoids, Quinones, Alkaloids, Phlobatannins were present in ethyl acetate extract of *vitex negundo*. Carbohydrate, Phenols, Tannins are absent of ethyl acetate extract for *vitex negundo*.

TLC

Thin layer chromatogram of ethyl acetate extract of *vitex negundo* was given in **Figure 2** and TLC of ethyl acetate extract of *vitex negundo* revealed the presence of a spot having R_f value of 0.80 when a solvent phase of Chloroform, ethanol, ethyl acetate, hexane and acetic acid in the ratio of (10:2:5:1:1) a solvent system was used.

UV

UV-Vis spectrophotometer is related to the spectroscopy of photons in the UV-visible region. UV-Vis spectroscopy uses light in the visible ranges or its adjacent ranges. The colour of the chemicals involved directly affects the absorption in the visible ranges. Molecules undergo electronic transitions in these ranges of the electromagnetic spectrum **[19]**. In the present study UV-Vis spectral profile showed the peaks 330nm **Figure 3**.

FTIR

The FT-IR has proved to be an effective instrument for identifying and characterising compounds or functional groups (chemical bonds) **[20, 21]**. It allows for the qualitative identification of organic compounds by observing the appearance of bands in the infrared spectrum at a given frequency, which is modified further by the functional groups in the region **[22]**. The results of FTIR peak values and functional groups were represented in **Table 2** and **Figure 4**. When the plant extract was passed into the FTIR spectrum, the functional groups of the components were separated based on its peaks ratio. The results of FTIR analysis confirmed the presence of functional groups such as carboxylic acid (30024.90, 2935.38), Aromatic compound (1569.23), Halo alkane (1397.83,1043.76,1012.06,672.86).

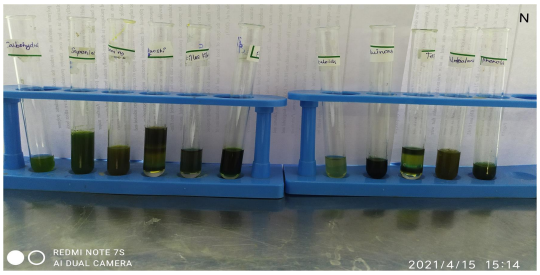

Figure 1: Phytochemical Test For *vitex negundo* Plant

Table 1: Phytochemical test for *vitex negundo*

S. No.	PHYTOCHEMICAL TEST	Interferen
1.	Carbohydrate test	-
2.	Phenols test	-
3.	Tannins test	-
4.	Flavonoids test	+
5.	Saponins test	+
6.	Cardiac glycoside i. Salkowski's test- ii. keller- kilani test-	+ +
7.	Terpenoids test	+
8.	Quinones test	+
9.	Alkaloids test	+
10.	Phlobatannins test	+

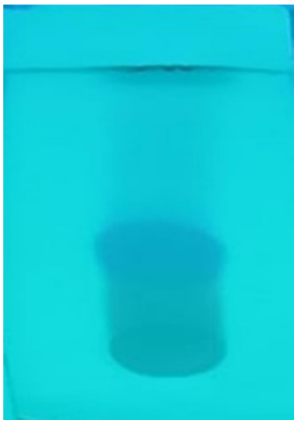

Figure 2: TLC For *vitex negundo* Plant

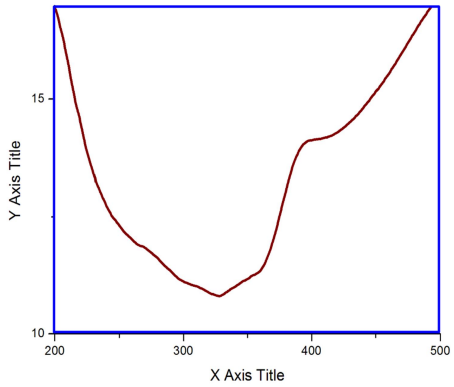

Figure 3: UV Analysis of *vitex negundo*

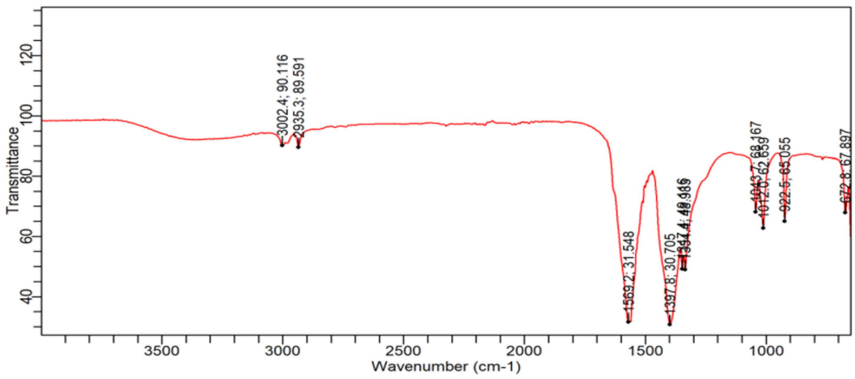

Figure 4: FTIR Analysis of *vitex negundo*

Table 2: FT-IR spectral peak values and functional groups obtained for *vitex negundo*

S. No.	Peak value (cm^{-1})	Stretch	Functional group
1	30024.90	O-H	Carboxylic acid
2	2935.38	O-H	Carboxylic acid
3	1569.23	C=C	Aromatic compound
4	1397.83	C-F	Halo alkane
5	1043.76	C-F	Halo alkane
6	1012.06	C-F	Halo alkane
7	922.56	-	-
8	672.86	C-Cl	Halo alkane

CONCULSION

The qualitative phytochemical test confirmed the presence of flavonoids, saponins, cardiac glycoside, terpenoids, quinones, alkaloids, and phlobatannins in this study, which was carried out to identify the spectroscopic characterisation of ethyl acetate leave extract of *vitex negundo*. The results of the FTIR analysis revealed the presence of carboxylic acid, aromatic compound, and halo alkane functional groups. As a result of this study, it was concluded that the leave extract of *vitex negundo* is used in the pharmaceutical industry to treat a various diseases.

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