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Phytochemical screening, antioxidant activities and quantification of compounds by HPLC of the leaf extracts from two varieties of *Vitex negundo* to explore their potential for textile uses

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Abstract
The present study was designed to explore phytochemical bioactive compounds present in *Vitex negundo* leaves (Black and White). Medicinal plants are an important source of phytochemicals that offer traditional medicinal treatment of various ailments. Leaf samples of the both selected varieties growing in the ecological conditions were collected, washed, air-dried and milled. The samples were extracted with two solvent namely ethyl alcohol and distilled water. Results revealed that ethanolic extraction depicted the higher concentration of saponins, alkaloids tannins, steroids, terpenoids and flavonoids in both the varieties of *Vitex negundo* as compared to aqueous medium. Total phenolic content of V_1 leaves extract estimated as gallic acid equivalent was found to be higher in ethnolic medium 3.17 mgGAE/g as compared to V_2 i.e 2.76 mgGAE/g. Also, Total flavonoid content of leaves extract and estimated as catechin equivalent was found to be higher in ethanoic medium of V_1 2.79 mgCE/g as compared to V_2 1.85 mgCE/g respectively. Antioxidant activity is assessed by ABTS radical scavanging activity was also found to be higher in ethanolic extract of V_1 91.64% as compared to V_2 69.32%. FTIR analysis reveals the presence of various functional groups, *viz*. alkenes, amines, carboxylic acids, alcohols, phenols, carboxylic acids and aromatic compounds. Its major constituents myrcetin, quercetin, kaempferol and chlorogenic acid were determined by high performance liquid chromatography (HPLC) and found to be higher in ethanolic medium of V_1 as compared to V_2. This study suggests that the ethanolic extract from *Vitex negundo* leaves has strong antioxidant potential and could be a significant source of natural antioxidants and antimicrobials for functional finishing of textiles.

Keywords: Phytochemical screening, antioxidant activities, HPLC, *Vitex negundo*, potential

1. Introduction
In the current pandemic scenario, sustainable green products particularly antiviral, antioxidant and antibacterial in nature are gaining worldwide fame in almost every walk of life. *Vitex negundo* (*Nirgundi*) has been known as a source of having antibacterial and mosquito repellent property. *Vitex negundo* belongs to Verbenaceae family, an important medicinal plant is found throughout India. It is a large, silvery-tomentose shrub or small tree with bluish purple flowers in terminal. Leaflets are 3-5 in number. Though almost all plant parts are used, the extract from leaves is the most important in the field of medicine and is sold as drugs. The leaf extract is used in Ayurvedic systems of medicine for treatment of various ailments. (Saklani *et al*. 2017) [3]. Flavonoids have been proven to display a wide range of biochemical and pharmacological actions such as anti-carcinogenic, anti-viral, anti-microbial, anti-thrombotic, anti-inflammatory, and antimutagenic activities. In addition, flavonoids can act as free radical scavengers and terminate the radical chains reaction that occurs during the oxidation of triglycerides in food system. (Turkoglu *et al*., 2007) [14].
Through phytochemical analysis, It is understood that dominant compounds in *Vitex Negundo* includes Quercetin, Chlorogenic acid, Myricetin, Kaempferol, Catechin and 4-hydroxybenzoic acid. Moreover its antioxidative and anti-inflammatory properties are very prominent in the field of medicine. There is very less research studies are available on comparative study of different varieties of *Vitex negundo* on the basis of phytochemical screening, Total phenolic content, Total flavonoid content and HPLC profile. Hence the basic aim of this study was executed to explore the comparative evaluation of two varieties of *Vitex negundo* leaves extract on the basis of Preliminary phytochemical screening, TPC, TFC, Antioxidant and HPLC chromatogram.

2. Experimental section

2.1 Collection/ Processing of plant material

Two varieties of Fresh *Vitex negundo* leaves (Black and white) without any plant disease were collected manually from the Hi-Tech Horticulture Saidapur farm, UAS, Dharwad, Karnataka and surroundings of local area during the month of March, 2021 where the plant grows widely under natural conditions. The details of plant source presented in Plate 1.

Scientific name: *Vitex Negundo* (V_1)	Scientific name: *Vitex Negundo* (V_2)
Family name: Lamiaceae	Family name: Lamiaceae
English name: Chinese chaste tree	English name: Chinese chaste tree
Local name: Kari lakki	Local name: Bili Lakki
Part used :Leaves	Part used: Leaves

Plate 1: Photograph and Details of selected plant source

2.2 Preparation of *Vitex negundo* leaves crude extract

The collected quantities of *Vitex negundo* plant (leaves) were washed with luke warm water to remove the dirt and other contaminates, dried under shade in a clean and dust free environment for 5 days. Crispy dried leaves were cut into small pieces for further size reduction from coarse fragments to fine powder with the help of electrical grinder and passed through 24 mesh sieve size to obtain even particle size to have better mixing during extraction of bioactive compounds. The grounded herbal powder was packed in air tight zipper bag at 4 °C until further analysis.

Extraction refers to separating the desired material by physical or chemical means with the aid of a solvent. (Roshima and Jayalakshmi, 2020) [12]. Extraction of phenolic compounds was done according to the method described (Manne *et al*. 2021) [9] with little modification.

2.2.1 Optimization of extraction conditions

2.2.1.1 Extraction with water: The quantity of *Vitex negundo* leaves powder 20g in 200ml of distilled water at room temperature After 24 hours, the supernatant was decanted and residue was resoaked in fresh solvent. Supernatant was filtered through Whatsmann filter paper, and centrifuged at 10000 rpm for 10min at 4 ^{0}C. Supernatant is a mixture of bioactive compounds and water. To evaporate water, solution was heated at 60 ^{0}C and extract was used for further analysis and finishing of textiles.

2.2.1.2 Extraction with ethyl alcohol: Ethyl alcohol LR grade was used to extract the bioactive compounds for multifunctional properties *i.e*. Ultraviolet protection factor, anti-bacterial and insect repellent finishes from *Vitex negundo* leaves extract. Extraction of medicinal plants with solvent yields both volatile and nonvolatile bio-active compounds. They need to be subsequently separated by employing different techniques based on the chemicals of interest one wants to obtain from the plant materials. (Endris and Govindan, 2020) [5]

Powdered dry leaves were soaked in ethyl alcohol and distilled water in ratio (80:20, 4:1) at room temperature to enable extraction of ethanol and water-soluble components from plant material. After 24hours, the supernatant was collected and rapidly filtered through Whatsmann No. 1 filter paper to remove all the residues and residue was resoaked in fresh solvent. Supernatant was centrifuged at 10000 rpm for 10 min at 4 ˚C. Ethanol content of the resulting filterate was evaporated by boiling the solution at 60^{0}C and the extract was used for further analysis. (Functional group assessments). Extract was used as a stock solution for the present research.

2.3 Characterization of extracted solution

2.3.1 Quantitative Phytochemical Analysis of extracted solution

Leaves powder were extracted with ethyl alcohol and distilled water successively. Qualitative phytochemical analysis of plant extracts was conducted for the evaluation of various classes of active chemical constituents like alkaloids, flavonoids, tannins, saponins and terpenoids using different methods. (Prabhavathi, *et al*. 2016; Si Said *et al.* 2016). These secondary metabolites are non-nutritive components which are produced by plants and known for medicinal as well as physiological activities. (Endris and Govindan, 2020) [5]

Phytochemical screening was analyzed as per the previously reported method (Vastrad *et al*., 2014) [15].

Test for alkaloids

1. Dragendroff's test: One gram powder samples of *Vitex negundo* were taken in a conical flask and few drops of reagent was added and allowed to stand for few minutes. A yellow colour precipitation was obtained immediately that showed the presence of alkaloids.

2. Wagners test: In 1 ml of extract few drops of Wagner's reagent were added. A reddish brown precipitate confirms the presence of alkaloids.

3. Mayers test: In 1ml extract was stirred with 5 ml of 1% HCl on a steam bath. The solution obtained was filtered, and 1 mL of the filtrate was treated with a few drops of Mayer's reagent. The turbidity of the extract filtrate on addition of Mayer's reagent was taken as evidence of the presence of alkaloids in the extract.

Test for Flavanoids

1. Ammonia test: In 1 ml of extract few drops of ammonia solution were added. A yellow precipitate confirms the presence of alkaloids.

2. Sodium hydroxide test: Few drops of 20% NaOH solution was added to 1 ml of the extract. The changed yellow colour of the extract turns to a colourless solution that shows the presence of flavonoids.

Test for tannins

1. Ferric chloride test: 1 ml of the extract was separately stirred with 10 ml of distilled water and then filtered. A few drops of 5% $FeCl_3$ were added to the filtrate. Blue-black or blue-green colouration or precipitation was taken as an indication of the presence of tannins.

2. Gelatin test: 2 ml of 1% solution of gelatin containing 10% NaCl was added to 1 ml of the extract. White precipitate indicates the presence of phenolic compounds.

3. Lead acetate test: 3 ml of 10% lead acetate solution was added to 1 ml of the extract. Appearance of bulky white precipitate confirms the presence of phenolic compounds.

Test for saponins

1. Foam test: About 1 ml of the sample extract was boiled in 20 ml of distilled water in a water bath and filtered 10 ml of the filtrate was mixed with the 5 ml of distilled water and mixed vigorously for 15 min to form a stable persistent froth. The presence of froth after 5 min was taken as an indication of presence of saponins.

Test for terpenoids

1. Salkowski test: 1 ml of each extract was mixed with 0.5 ml of chloroform and 1 ml of concentrated H_2SO_4 was carefully added carefully to form a layer. A reddish brown colouration of the interface formed to show positive results for the presence of terpenoids.

2.3.2. Examination of Total phenolic content (TPC)

The total phenolic content in the *Vitex negundo* extract was measured by Folin-Ciocalteu colorimetric method described by Singleton and Rossi using gallic acid as the standard. and results are expressed as gallic acid equivalent (GAE) in milligrams per gram.

TPC was analyzed as per the previously described method (Manne *et al.* 2021) [9]. Briefly, plant extracts were diluted to appropriate volumes and were mixed with 2 ml of 10% saturated Na_2CO_3 solution and incubated at room temperature for 30 min with intermittent shaking, 100µl of Folin-Ciocalteu reagent was added to the mixture. The solution was covered with silver foils and reaction mixture was incubated for 90 min at room temperature under dark, absorbance was measured at 765nm using ethanol as blank by Visible Spectrophotometer.(Specord S600 Analytik Jena, Germany). Three replications were done for all the samples and the mean values were calculated.

The phenolic compounds in mg from one gram of extracted powder were assessed by using the formula. (Ketema and Worku, 2020)

TPC (mgGAE/m) = C*V/m (1)

Where,
TPC= Total phenolic content in mg
m=dry mass of a sample in gram
C= concentration of Gallic acid in mg/l
V= volume of sample taken in a millimeter

2.3.3. Examination of Total flavonoid content (TFC*)*

TFC of both extarcts was determined using Catechin reagent as per the previously described method (Manne *et al.* 2021) [9]. TFC of extracted solution was determined by aluminium chloride colorimetric assay. Appropriately diluted sample extracts (250µL) were made to react with sodium nitrite (5%) and aluminium chloride (10%) further incubated after the addition of sodium hydroxide (1M) for 30 min. The absorbance of the reaction mixture was subsequently recorded at 415 nm by UV–Visible Spectrophotometer**.** (Specord S600 Analytik Jena, Germany). Catechin was used as standard reference and the values were expressed as milligram of catechin equivalent (CE) per gm.

TPC (mgCE/m) = C*V/m (2)

Where,
TFC= Total flavanoid content in mg
m=dry mass of a sample in gram
C= concentration of catechin in mg/l
V= volume of sample taken in a millimeter

2.3.4. Determination of Antioxidant capacity

2.3.4.1 ABTS radical scavenging activity

Antioxidant activity of the *vitex negundo* can be determined from its ability to scavenge $ABTS^+$ radicals. It is the stable free radical, which shows the highest absorption in the range of 520 to 515 nm due to its odd electron, however upon receiving the proton from hydrogen donor species such as phenolics and flavonoids it changes its colour and becomes yellow. $ABTS^+$ radical scavenging method is economic, reliable and widely used method to measure the antioxidant activity.

Reducing power of both the extracts was determined according to the previously reported method (Lakshmi *et al.* 2021) [8]. $ABTS^+$ cation radical was generated by the reaction between 7mM ABTS in water and 2.45mM potassium persulfate (1:1), stored in the dark at room temperature for 12-16 h before use. $ABTS^+$ solution was then diluted with ethanol and adjusting the Abs at 734 nm to 0.700 with ethanol. After the addition of 5µl of extract to 3.995 ml of diluted $ABTS^+$ solution, reaction mixture was allowed to stand at room temperature for 15 min and the absorbance were analyzed spectrophotometrically using a UV-Visible Spectrophotometer (Specord S600 Analytik Jena, Germany) at 734 nm. All the measurements were carried out in triplicates. Percent inhibition of absorbance at 734 nm was calculated using the formula:

$ABTS^+$ scavenging effect (%) = ((AB–AA)/ AB)×100 (3)

Where,
AB is absorbance of ABTS radical + methanol;
AA is absorbance of ABTS radical + sample extract/standard.

2.3.5 FTIR Characterization: FTIR spectroscopy is a powerful tool for identification of functional groups present in extracted active compounds from plants based on the peak

value in the region of infrared radiation. Each extract was loaded to Fourier transform infrared spectrophotometer (System 2000, Perkin Elmer) for functional group analysis. The IR peak absorbance (wave number, cm^{-1}) was recorded in the range of 4000 cm^{-1} to 400 cm^{-1} of two leaves extract.
It can also be used to study the chemical reactions between natural dyes and textile fibres. (Ebrahimi and Parvinzadeh, 2016). Chemical bonds in a molecule were determined by interpreting the infrared absorption spectrum. Averages of 8 scans were recorded for FTIR analysis. (Bag, G., 2015) [2]

2.3.6 HPLC analysis (High performance liquid chromatography)
HPLC is a chromatographic technique that can separate a mixture of compounds for phytochemical and to identify, quantify and fully characterize its individual components of the mixture. (Cannell, 1998 and Piana *et al* 2016) [3, 11]. The antioxidative compounds in the *Vitex negundo* extract were analyzed by High performance liquid chromatography was performed with a Dionex Ultimate 3000 RSLC equipped with a Photo Diode Array (280nm) detector connected to a DGU 20A5 degasser with a CBM 20A integrator, software under gradient conditions using column Dionex PA2 RSLC120A C_{18} (100mm x 2.1mm, 2.1µm). The mobile phase was water containing 50 mM phosphate buffer (A) and methanol (30:70, B)

2.3.7 Quantification of compounds: Extraction of phenolic compounds was done according to the previously described method (Goudar and Sathisha, 2016) [6] with little modification. In brief, 1.0g of sample was extracted with ethanol/distilled water (2x25 ml) with agitation for 3h each time, the supernatants were obtained by centrifugation at 12,000 rpm for 10 min at 4˚C and the volume of extract was reduced using a rotary evaporator. The concentrated supernatants were hydrolyzed with 4M NaOH (20ml) for 2 h under nitrogen and pH was set to 2.00 using HCl. The separation was carried out using ethyl acetate (50ml x 2) and then the fractions were pooled by treating with sodium sulphate and dried. The residue obtained is reconstituted with methanol (5mL) and filtered through 0.45µm, used for HPLC analysis.

3. Results and Discussion

3.1 Quantitative phytochemical screening of *Vitex negundo* leaves extract

Phytochemical preliminary screening of leaf extracts of both plants revealed the presence of six different compounds (Alkaloids, Iridoids, Flavonoids, Tannins, Saponins, Terpenoids) as summarized in Table 1. For both plants two solvents i.e. Water and ethyl alcohol were used for extraction. Out of the two solvents ethanol was proven to be the best solvent for extraction of the phytochemicals as evident from the table 1.
Result reveals that Saponins and terpenoids were not detected in the aqueous extrcation of V_1. Alkaloids, Iridoids, Flavonoids, Tannins and terpenoids were present in higher concentration in ethanolic leaves extract of V_1 variety of *Vitex negundo*. However in case of V_2, except iridoids and terpenoids, all phytochemicals were present in higher concentration.
Comparatively all the compounds were found to be higher in V_1 as compared to V_2. V_2 leaves extract had proven to be a good source of flavonoids. The qualitative chemical screening test confirmed that the ethanol extract showed maximum phytochemicals including flavonoids which are responsible for having good antioxidants, activity against pathogens and therefore aid the antimicrobial activity.

Table 1: Qualitative phytochemical investigation of *Vitex negundo* leaves extract in aqueous and ethanol medium

Phytochemical group	Phytochemical test	V_1 (Black)		V_2 (White)	
		Ethanol	Aqueous	Ethanol	Aqueous
Alkaloids	Dragendorff's	+++	+++	+++	++
	Wagner's	+++	++	++	++
	Mayer's	++	++	++	++
Iridoids	Trim-Hill	+++	++	++	+
Flavonoids	Ammonia	+++	++	+++	+
	Sodium hydroxide	+++	++	+++	+
Tannins	Ferric chloride	+++	++	++	++
	Gelatin	++	++	++	++
	Lead acetate	+++	++	+++	++
Saponins	Foam	+	-	+	-
Terpenoids	Salkowski	+++	-	++	-

*High concentration (+++), medium concentration (++), less concentration (+), absent (-)

3.2 Examination of Total Phenolic content: Total phenolic content is the method used to determine the phenolic level in plant extracts. (Kabra *et al*. 2019) [7]. As a basis, total phenolic content in extract was determined by the Folin-Ciocalteu reagent described above in materials and methods section, using gallic acid as the reference. The absorbance values obtained at different concentrations of gallic acid were used to get the calibration curve. Result indicates that the ethanolic extract of Black variety of *Vitex negundo* (V_1) exhibited higher TPC comparatively to the aqueous, which were about 3.17±0.06 mg GAE/g for ethanol extract, 2.20 ± 0.05 mg GAE/g for aqueous medium. Comparatively less extraction of phenolics were found with water solvent is due to the extraction with high percentage of impurities. (Chirinos *et al*. 2007) [4]. However, ethanolic and aqueous extract of V_2 depicted 2.76 ±0.12 and 1.87±0.07 mg GAE/g respectively. (Table 2)
The greater phenolic level in the ethanolic extract may suggest higher bioactivity, *viz*. antioxidant and antimicrobial activities (Kabra *et al*. (2019) [7]. In alcoholic media, a wide range of bio active constituents and facilitates the easy purification of extracted colouring pigments (Jin *et al*. 2011).

Table 2: Total phenolic contents (mg GAE/g) and total flavonoid contents (mg CE/g) values of V_1 (Black) and V_2 (White) extracts in aqueous and ethanol medium of *Vitex Negundo* leaves (mean ± SE)

Parameters analysed / Extracting solvents	TPC (mg GAE/g)		TFC (mg CE/g)	
	V_1 (Black)	**V_2 (White)**	**V_1 (Black)**	**V_2 (White)**
Aqueous	2.20 ± 0.05	1.87 ± 0.07	1.08 ± 0.02	0.62 ± 0.08
Ethanol	3.17 ± 0.06	2.76 ± 0.12	2.79 ± 0.08	1.85 ± 0.07

Note: Each value is average of three replications ± standard deviations (SD)

3.3 Examination of Total flavonoid content: Total flavonoid content were observed in the extract of Black variety of *Vitex negundo* in ethanoilc medium 2.79 mgCE/g as compared to aqueous medium 1.08 mgCE/g. Further TFC of White variety of *Vitex negundo* was also found to be higher in ethanolic solvent 1.85 mgCE/g as compared to aqueous medium 0.62 mgCE/g. (Table 2)

To our knowledge, there is no data available in the literature about the TPC and TFC of two varieties of *Vitex negundo*, thus only a few studies could be found related to the TPC and TFC of other species of the same families.

3.4 Antioxidant capacities using ABTS assay: Table 3 depicted the antioxidant activity of the *Vitex negundo* extracts (V_1 and V_2) based on ABTS Assay. In the ABTS assay, the $ABTS^+$ radical scavenging capacity observed to ethanolic extract was found to be very high. V_1 leaves extract exhibited stronger antioxidant activity as compared to the extract of V_2, which is in accordance with TPC and TFC. Phenolic compounds possess redox properties which allow them to act as potential antioxidants. (Baba and Malik, 2015) [1]

Among the selected plant extracts, *Vitex negundo* leaf extract showed the highest (91.64 ± 1.92) and lowest (48.96 ± 1.42) (Table 3). Scavenging effects of samples on ABTS radical were in the following order V_1 (Ethanol) 91.64%>V_2 (Ethanol) 69.32%> V_1 (Aqueous) 48.96%> V_2 (Aqueous) 36.70%. The study revealed that both the varieties of *Vitex negundo* have prominent antioxidant activity in ethanolic medium.

Table 3: ABTS [2, 2″-azino-bis (3-ethylbenzothiazoline-6-sulphonic) acid] radical scavenging activity of Vitex *negundo* leaves extracts

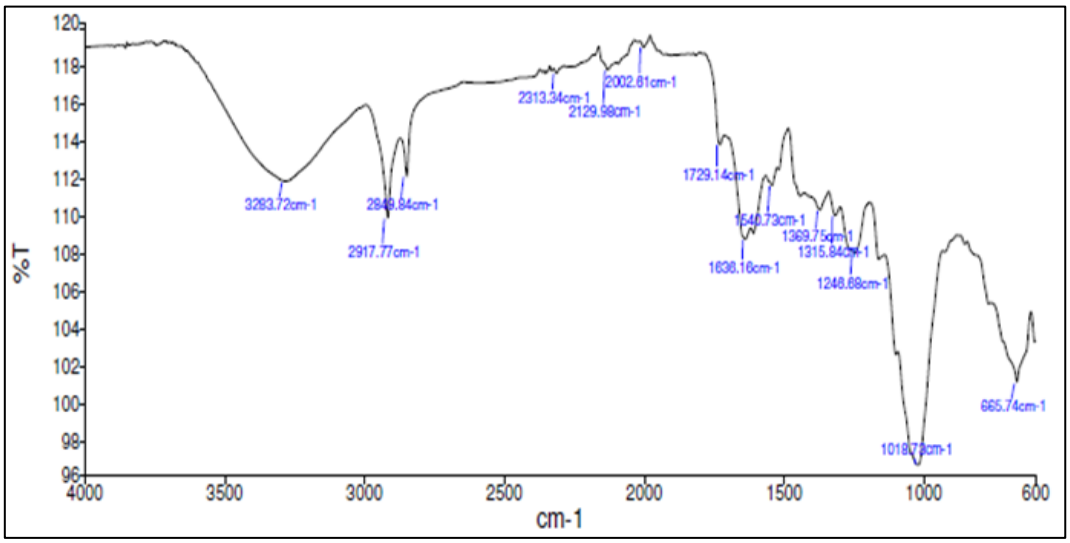
Parameter analyzed / Extracting solvents	ABTS (%)	
	V_1 (Black)	**V_2 (White)**
Aqueous	48.96 ± 1.42	36.70 ± 0.71
Ethanol	91.64 ± 1.92	69.32 ± 0.56

Note: Each value is average of three replications ± standard deviations (SD)

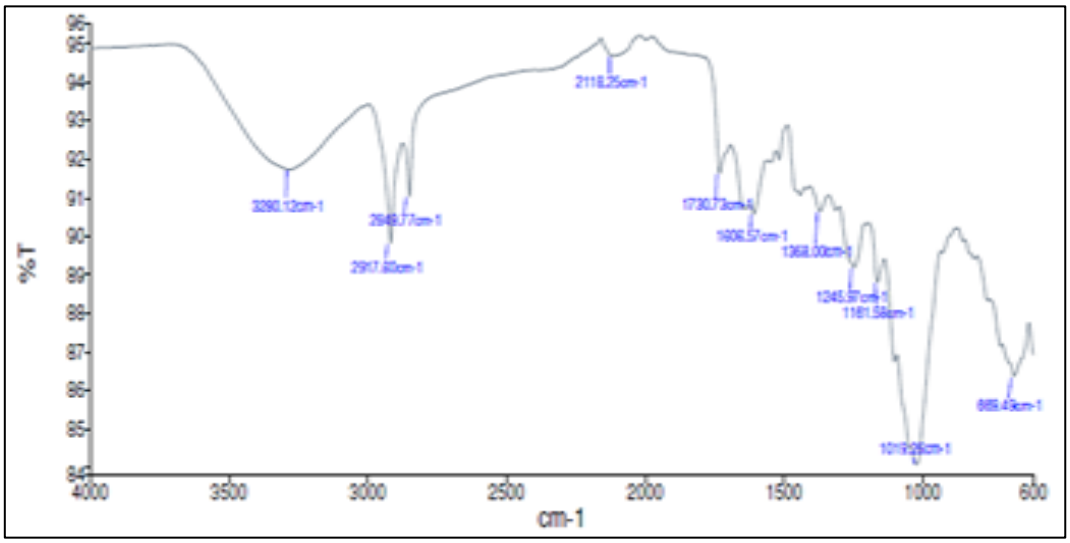
Table 4: FTIR spectra of ethanolic extract of V_1 and V_2

S. No.	Peak (cm^{-1}) V_1	Peak (cm^{-1}) V_2	Frequency Range	Functional groups
1	3283.72	3280.12	3200-3500	O-H stretching vibration presence of alcohols, phenols
2	2917.77	2917.60	2850-3000	C-H stretching vibration presence of alkenes
3	2849.84	2849.77	2500-3000	O-H stretching vibration presence of carboxylic acids
4	2313.34, 2129.98, 2002.61	2118.25	2100-2260	C=C alkyne
5	1729.14	1730.73	1705-1725,1700–1725	C=O stretching vibration presence of ketone and carboxylic acid
6	1636.16, 1540.73	1606.57	1600-1650	C=C- stretching vibration presence of alkenes
7	1540.73	-	1400-1500	C-C stretching vibration presence of aromatics
8	1369.75, 1315.84	1368.00	1375–1300	S=O stretching vibration presence of sulfones, sulfonyl chlorides, sulfates and sulfonamides
9	1246.68, 1018.73	1245.97, 1161.58, 1019.26	1250–1010	C-N stretching vibration presence of aliphatic amines
10	665.74	669.49	910–665	N-H stretching vibration presence of primary and secondary amines

3.5 Evaluation of extracted compounds using Fourier transforms infrared spectroscopy: The functional groups of compounds were examined by Fourier-transform infrared spectroscopic studies by their peak values (cm^{-1}) and presented in Table 4 and Fig. 1. Aromatics, sulfones, and aliphatic amines showed main peaks at 1540.73, 1369.75, 1315.84 cm^{-1} and 1246.68, 1018.73 cm^{-1} for V_1 and 1368.00, 1245.97, 1161.58, 1019.26 cm^{-1} for V_2. Different intensity peaks were identified for primary and secondary amines at (665.74, 669.49 cm^{-1}) carboxylic acids at (2849.84, 2849.77 cm^{-1}), alkenes at (2917.77, 2917.60 cm^{-1}) Table 4. Two small bands at 2313.34 and 2002.61 cm^{-1}were absent in V_2 sample. The strong and sharp band at 1019.2 cm^{-1} suggest the presence of carbonyl groups in the extracts as this band is associated with the IR band of carbonyl(C=O) groups.



A



B

Fig 1: FTIR spectrum of ethanolic extract of *Vitex negundo leaves* within the range of 600-4000 cm^{-1}a) V_1 and b) V_2

3.6 Identification and Quantification of phenolic using HPLC (High performance liquid chromatography)

High performance liquid chromatography (HPLC) method was developed for the qualitative analysis of V_1 and V_2 for various major constituent. Two solvent systems, ethyl alcohol and distilled water were tried in order to develop an effective mobile phase. The compounds identified by HPLC analysis were presented in Table 5. The quantitative HPLC profile of aqueous and ethanolic extracts of Black and White *Vitex Negundo* leaves was selected at a wavelength of 320nm due to the sharpness of peak and proper baseline. A total of 11 compounds were identified in the different extracts of *Vitex negundo* leaves, in particular phenolics and flavonoids. It is observed from the table 5 that all the compounds are similar in both varieties of *Vitex negund*o except two i.e. Luteolin and Catechin which are not present in white variety of *Vitex negundo* leaves extract.

The quantities of bioactive constituents are in following order in V_1:

Quercetin (18.57mg/g)>Chlorogenic acid (6.29 mg/g)>Myricetin (4.02 mg/g) > Kaempferol (3.36 mg/g) > Catechin (2.49 mg/g) > 4-hydroxybenzoic acid (2.08 mg/g) > Vitexin (1.54 mg/g) > Protocatechuic acid (0.978 mg/g) > Apigenin (0.86 mg/g) > Luteolin (0.57 mg/g) > Caffeic acid (0.147 mg/g).

The quantities of bioactive constituents are in following order in V_2: Quercetin(15.29 mg/g) > Chlorogenic acid(4.72 mg/g) > Myricetin (3.69 mg/g) > Vitexin (1.29 mg/g) > Kaempferol (l0.95 mg/g) > 4-hydroxybenzoic acid (0.92 mg/g) > Apigenin (0.63 mg/g) > Protocatechuic acid(0.249 mg/g) > Caffeic acid (0.012 mg/g).

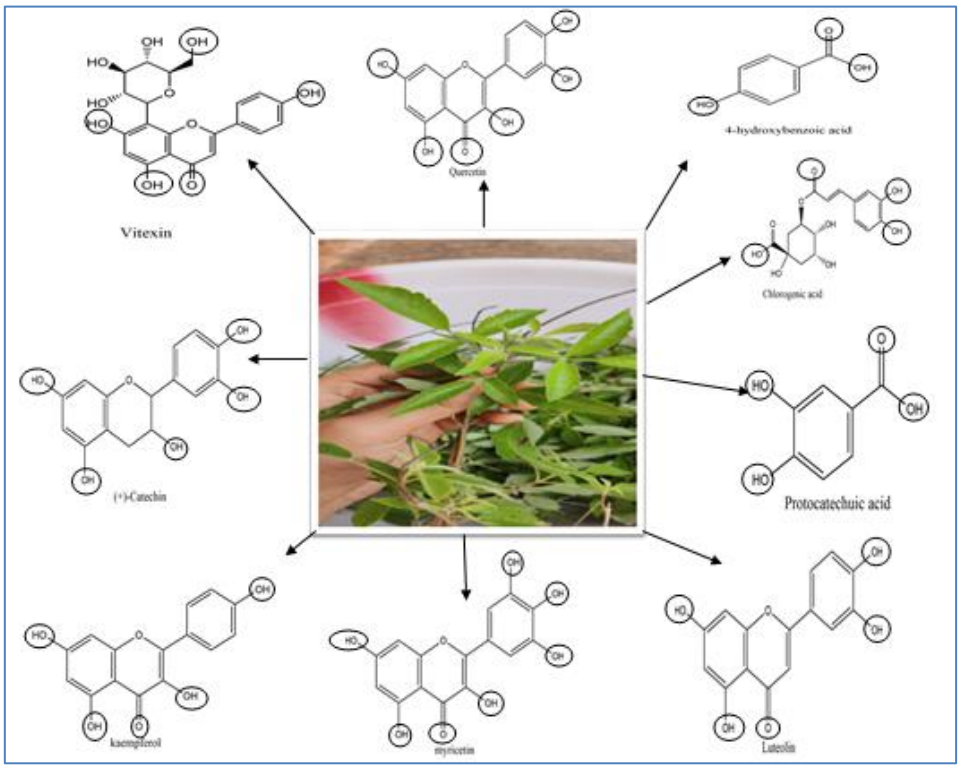


Fig 2: Chemical structure of major bio-active/ phenolic components of *Vitex negundo* leaves extract, functional groups of each molecule are identified by circles

Table 5: Phenolic acids and flavonoids composition of *Vitex negundo* (Black and White) extracts Quantitative chromatographic analysis by HPLC (mg/g)

Phenolic compounds	V_1 (Black)		V_2 (White)	
	Aqueous	Ethanol	Aqueous	Ethanol
Protocatechuic acid	0.306 ± 0.01	0.978 ± 0.04	0.071 ± 0.005	0.249 ± 0.02
Caffeic acid	0.091 ± 0.006	0.147 ± 0.03	0.004 ± 0.0008	0.012 ± 0.001
4-hydroxybenzoic acid	1.30 ± 0.04	2.08 ± 0.045	0.028 ± 0.005	0.92 ±0.02
Chlorogenic acid	2.50 ± 0.17	6.29 ± 0.15	1.83 ± 0.08	4.72 ± 0.18
Vitexin	0.65 ± 0.05	1.54 ± 0.21	0.25 ± 0.09	1.29 ± 0.07
Kaempferol	1.10 ± 0.24	3.36 ± 0.28	0.27 ± 0.05	0.95 ± 0.04
Apigenin	0.31 ± 0.05	0.86 ± 0.07	0.019 ± 0.002	0.63 ± 0.04
Quercetin	9.56 ± 0.05	18.57 ± 0.25	7.17 ± 0.17	15.29 ± 0.14
Myricetin	1.90 ± 0.08	4.02 ± 0.11	1.31 ± 0.155	3.69 ± 0.12
Luteolin	0.18 ± 0.05	0.57 ± 0.05	-	-
Catechin (+)	0.85 ± 0.08	2.49 ± 0.18	-	-

Note: Each value is average of three replications ± standard deviations (SD)

Ethanolic medium allows the separation of maximum number compounds with better resolution. Myricetin, quercetin, kaempferol and Chlorogenic acid (Table 5) that might contribute to the antimicrobial behaviour were identified in V_1 and V_2.

The results showed in Table 5 indicate the presence of higher myricetin, quercetin, kaempferol and Chlorogenic acid contents in V_1 as compared to V_2.

Conclusion

This study revealed that *Vitex negundo* leaf extracts have interesting antioxidant attributed to their richness in phytochemicals, such as phenolics and flavonoids. The findings unveil the ethanolic leaf extract of *Vitex negundo* as a natural source of potent antibacterial bioactives and mosquito repellency due the presence of various chemical constituents hence could be used to develop ecofriendly textiles. *Vitex negundo* was the richest in terms of TPC and TFC, and the HPLC analysis revealed the presence of simple phenolics, flavonoids served as a toxicants to impart antibacterial and mosquito repellent property to the textiles. Chlorogenic acid obtained from this plant may contribute to the natural dyeing to the textiles. TPC and TFC quantification of ethanolic extract of both the samples exhibited maximum phenolic and flavonoid contents compared to aqueous medium. Therefore it is considered as a suitable solvent to extract the bioactive constituents from the plant material.

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