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# EVALUATION OF PHYTOCHEMICALS AND IN-VITRO BIOACTIVITY OF HIGH ALTITUDE ESSENTIAL OILS

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**Abstract:**

Background: High-altitude plant essential oils are prized for their distinct chemical makeup and possible medical uses. These plants, which are frequently subjected to harsh environmental circumstances, provide bioactive chemicals in their essential oils that may have important therapeutic uses. Understanding the medicinal potential of high-altitude essential oils requires assessing their phytochemical composition and in-vitro bioactivity.

This study's goal was to assess the essential oils derived from high-altitude plants' phytochemical makeup and in vitro bioactivity.

Methods: Steam distillation was used to extract essential oils from plants gathered in high-altitude areas. The essential oils' phytochemical composition was ascertained using gas chromatography-mass spectrometry (GC-MS). The medicinal potential of the essential oils was evaluated using in-vitro bioactivity assays, which included cytotoxicity, antioxidant, and antibacterial testing. The disc diffusion method was used to test the antibacterial activity against a variety of bacterial and fungal pathogens, and the DPPH radical scavenging assay was used to assess the antioxidant activity. The MTT assay was used to investigate the cytotoxicity on human cancer cell lines.

Results: A variety of substances with bioactive qualities, such as monoterpenes, sesquiterpenes, and phenolic compounds, were identified by the phytochemical study. Significant antibacterial action was demonstrated by the essential oils, especially against Staphylococcus aureus and Escherichia coli. Furthermore, the oils showed high antioxidant activity, with IC50 values that were on par with those of common antioxidants. Moderate efficacy against cancer cell lines was shown by the cytotoxicity assay, suggesting that these compounds might be developed further as anti-cancer medicines.

The study concludes that a wide range of phytochemicals with noteworthy in-vitro bioactivities, such as cytotoxic, antioxidant, and antibacterial properties, are present in essential oils derived from high-altitude plants. Additional study is necessary to fully investigate the potential of these oils for potential future pharmacological and therapeutic uses.

**Keywords:** gas chromatography-mass spectrometry, bioactivity, antimicrobial, antioxidant, cytotoxicity, high-altitude essential oils, phytochemical analysis, and medicinal potential.

## 1. Introduction

The genus Vitex (family: Verbenaceae) has 250 recognised species and exhibits a broad global range, including shrubs and trees in tropical, subtropical, and temperate climates. Members of this genus have been extensively used in traditional medicine and are highly esteemed as medicinal plants in several Asian nations, including India, Pakistan, Nepal, China, Sri Lanka, and Bangladesh. The leaves, seeds, flowers, and whole aerial portions of several species under the Vitex genus possess numerous exterior and interior applications. The predominant use of these plants include the treatment of asthma, ophthalmodynia, headaches, coughs, and premenopausal syndrome, among others, while other uses have also been documented [2]. For example, V. agnus-castus fruits are used in the management of menstrual problems (amenorrhoea, dysmenorrhea) and several female illnesses such as premenstrual dysphoric disorder, infertility, disturbed breastfeeding, acne, breast discomfort, menopause, and inflammatory disorders [3,4]. The V. negundo species serves as a tonic, vermifuge, and lactagogue, and is used in the treatment of catarrhal fever, ocular disorders, inflammation, skin ulcers, rheumatoid arthritis, and bronchitis [5]. V. trifolia is used as a sedative for headaches, an anti-inflammatory drug, and for the treatment of the common cold. The herb is used in Chinese traditional medicine for cancer therapy [6,7]. Phytochemical investigations indicated that species within the Vitex genus are abundant in bioactive components, such as essential oils (terpenoids), flavonoids, glycosides, phenolic acids, and ecdysteroids, among others. The existing literature indicates that the essential oils (EOs) and other bioactive compounds derived from Vitex species exhibit various biological activities, including antioxidant, antibacterial, oestrogenic, cytotoxic, antifeedant, antifungal, antidiabetic, enzyme inhibitory, antiproliferative, antipyretic, antimalarial, antinociceptive, and phytotoxic properties [7–13].

Contemporary pharmaceutical treatments for oxidative stress induced by free radicals are efficacious; nevertheless, they are expensive and linked to many adverse effects, including carcinogenic and teratogenic consequences [14,15]. Regarding chemical antioxidants, there is a growing pursuit of alternative supplies derived from natural, plant-based origins, since they are seen to be safer. Numerous plant-derived essential oils have been evaluated and shown to possess remarkable antioxidant activities [16–19]. In recent years, research on the pesticidal characteristics of plant-based products (botanicals) has been steadily expanding due to their environmental safety, biodegradability, and lower toxicity compared to chemical pesticides [20]. Furthermore, prior research indicates that the essential oil derived from V. negundo and V. agnus-castus has significant phytotoxic activity [21,22]; however, there are no documented findings on the phytotoxic potential of V. trifolia. Essential oils are superior alternatives among natural sources, since they include potent phytotoxic allelochemicals that influence the growth and development of both desirable and undesirable plants (weeds) [23].

Research has identified Vitex species as a source of antioxidants and phytotoxic agents according to their essential oil content [7,21,22]. The efficacy and potency of the essential oil are significantly influenced by its chemical contents, which are determined by the

plant's genotypes as well as environmental, climatic, and agronomic circumstances [24]. The essential oils of V. agnus castus and V. trifolia, sourced from the Tarai area of Uttarakhand, have yet to undergo phytochemical investigation. The current study aims to (i) assess the chemical diversity of the essential oil compositions of Vitex agnus-castus, Vitex negundo, and Vitex trifolia from the Tarai region of Uttarakhand, India; (ii) evaluate the in vitro antioxidant and phytotoxic (herbicidal) activities; (iii) conduct in silico studies on the inhibitory effects of major volatiles in the essential oils on the crystal structures of specific proteins; and (iv) perform ADMET predictions for the principal compounds identified in the essential oils under examination.

## 2. Materials and Methods

### 2.1. Collection of Plant Material

In the Tarai region of Uttarakhand, India, in Pantnagar (28°58'12.00" N, 79°24'36.00" E), fresh leaves from a variety of Vitex plant species were collected. One of the writers, the taxonomist D.S. Rawat, identified the plant specimens. With voucher numbers GBPUH-1439, GBPUH-1438, and GBPUH-1440, the voucher specimens for the newly identified Vitex species—Vitex agnus-castus, Vitex negundo L., and Vitex trifolia L.—have been added to the Department of Biological Sciences' herbarium for future use.

### 2.2. Extraction of Essential Oil

Using a Clevenger-type equipment, fresh leaves of several Vitex species were hydrodistilled for four hours in order to separate the essential oils. The extracted essential oils were given the designations VAO, VNO, and VTO for Vitex agnus-castus, Vitex negundo, and Vitex trifolia, respectively. For future usage, the extracted essential oils were kept in amber-colored glass vials at a low temperature (4 ◦C in the refrigerator) after being dried over anhydrous sodium sulphate (Na2SO4) to eliminate any remaining water. The corresponding oil yield (v/w) for VAO, VNO, and VTO was 0.9% (0.45 mL/100 gm dry matter), 0.8% (0.4 mL/100 gm dry matter), and 0.6% (0.3 mL/100 gm dry matter).

### 2.3. Chemical Composition Analysis

The essential oils were examined using GC-MS (Shimadzu QP 2010 plus) with GCMS-QP 2010 Ultra DB-5 and GCMS-QP 2010 Ultra Rtx-5MS columns (30 m × 0.25 mm i.d., 0.25 µm) in order to verify the chemical variety in the tested Vitex species. Helium was employed as the carrier gas under the following experimental circumstances (split ratio = 10.0, flow rate = 1.21 mL/min). A temperature gradient of 3 ◦C/min up to 210 ◦C (isotherm for 2 min) and then 6 ◦C/min up to 280 ◦C was used to program the oven temperature, which was set between 50 and 280 ◦C. Relative retention index (RI) values were compared with mass spectra from the NIST (NIST version 2.1) and WILEY (7th edition) libraries, and the fragmentation pattern of the mass spectral data was compared with those documented in the literature in order to identify the constituents of essential oils [25,26].

### 2.4. Antioxidant Activity

The antioxidant activity of the essential oils was assessed using a variety of in vitro procedures, and the findings were reported as mean ± SD of triplicate.

#### 2.4.1. DPPH Radical Scavenging Assay

The test has been carried out using previously suggested techniques [27, 28]. Briefly, 5 mL of freshly made methanolic solution of DPPH (0.004%) was mixed with varying concentrations of VAO, VNO, and VTO (10 µL/mL–50 µL/mL). The mixture was then allowed to sit in the dark for 30 minutes. Finally, the absorbance was measured in triplicate at 517 nm using a UV spectrophotometer (Thermo Fisher Scientific, Evolution-201, Waltham, MA, USA) at 517 nm in comparison to a blank. In the same doses as the studied essential oils (10 µL/mL–50 µL/mL), BHT was the standard antioxidant that was utilised. The following formula was used to determine the oils' and the standard's percentage inhibition of the DPPH free radical:

$$\%\ \text{DPPH radical scavenging activity} = \frac{(A_o - A_t)}{A_o} \times 100$$

where the absorbance values of the test and control essential oils are denoted by the letters Ao and At, respectively. Plotting percent inhibition versus concentrations allowed for the calculation of IC50 (half-maximal inhibitory concentration) values using the equation for the line.

### 2.4.2. Hydrogen Peroxide (H2O2) Radical Scavenging Activity

The examined samples' H2O2 radical scavenging activity was carried out in accordance with the recommended procedure previously documented [29, 30]. Here, 0.4 mL of methanolic solution containing varying quantities of essential oils and the standard (10–50 µL/mL) was mixed with 0.6 mL of H2O2 solution (40 mM) made in phosphate buffer (0.1 M; pH 7.4). For ten minutes, the aforementioned solution was incubated at room temperature. Additionally, the absorbance was measured at 230 nm in relation to the blank, which is methanol. As a positive control, L-ascorbic acid (10–50 µL/mL) was used. The following formula was used to determine the % scavenging of H2O2:

$$\%\ H_2O_2\ \text{radical scavenging activity} = \frac{(A_o - A_t)}{A_o} \times 100$$

where the absorbance values of the test and control essential oils are denoted by the letters Ao and At, respectively. Plotting percent inhibition versus concentrations allowed for the calculation of IC50 values using the line equation.

### 2.4.3. Nitric Oxide Radical Scavenging Activity

The previously published approach was used to measure the tested essential oils' nitric oxide (NO) radical scavenging capability [31]. In short, various quantities of essential oils and the standard (10–50 µL/mL) were individually mixed with 2 mL of sodium nitroprusside (10 mM) produced in phosphate buffer saline (0.5 mM, pH 7.4), and then incubated at 25 ◦C for 150 minutes. Additionally, 0.5 mL of each incubated solution was mixed with 0.5 mL of Griess reagent, which included 1.0 mL of sulphuric acid reagent. The absorbance was measured at 540 nm after the mixture was once again incubated for 30 minutes at room temperature. The conventional antioxidant was L-ascorbic acid. The following formula was used to determine the % scavenging of NO:

$$\%\ \text{NO radical scavenging activity} = \frac{(A_o - A_t)}{A_o} \times 100$$

where the absorbance values of the test and control essential oils are denoted by the letters Ao and At, respectively. Plotting percent inhibition versus concentrations allowed for the calculation of IC50 values using the line equation.

### 2.4.4. Reducing Power Assay

The previously described technique was used to determine the reducing power assay of several essential oils [32]. To 2.5 mL of phosphate buffer (200 mM, pH = 6.6), various quantities of the investigated samples (essential oils and the standard 10–50 µL/mL)) were added. Additionally, 2.5 mL of K3[FeCN6], a 1% potassium ferricyanide, was added to the solution above. After 20 minutes of incubation at 50 °C, 2.5 mL of trichloroacetic acid was added, and the mixture was centrifuged for 10 minutes at 650 rpm. The top layer received 1 mL of 0.1% ferric chloride and 5 mL of distilled water. The final solution's absorbance was measured at 700 nm, and a positive control of gallic acid (10–50 µL/mL) was used. The following calculation was used to determine the percentage lowering power:

$$\% \text{ Reducing power activity} = \frac{(A_o - A_t)}{A_o} \times 100$$

where the absorbance values of the test and control essential oils are denoted by the letters Ao and At, respectively. Regression models for the percent inhibition plotted versus concentrations were used to get the RP50 values.

### 2.4.5. Fe2+ Metal Chelating Activity

Following the established and recommended technique, the Fe2+ metal-chelation activity of VAO, VNO, and VTO was assessed [33]. Separately, 0.1 mL of FeCl2·4H2O (2 mM) and 0.2 mL of (5 Mm) ferrozine were combined with varying concentrations of oils (10–50 µL/mL) and the standard. Additionally, 4.7 mL of methanol was added to the solution, increasing its total volume to 5 mL. A spectrophotometer (Thermo Fisher Scientific, Evolution-201, USA) was used to measure the absorbance at 562 nm after the solution was agitated and incubated for 30 minutes at 25 ◦C. As a common antioxidant, Na2-EDTA (10–50 µL/mL) was used. The following formula was used to determine the samples' capacity to chelate ferrous ions:

$$\% \text{ } Fe^{2+} \text{ metal-chelation activity} = \frac{(A_o - A_t)}{A_o} \times 100$$

where the absorbance values of the test and control essential oils are denoted by the letters Ao and At, respectively. Regression equations were used to get the IC50 values for the percent inhibition vs concentration plots.

### 2.5. Herbicidal (Phytotoxic) Activity

The essential oils of Vitex species were used to test the herbicidal efficacy against the receptor plant, Raphanus raphanistrum. Using the previously described approach, many factors were used, including suppression of root length growth, inhibition of shoot development, and inhibition of seed germination [34, 35]. Radish seeds were acquired for the experiment from the Vegetable Research Centre (VRC) at G.B.P.U.A. & T. Pantnagar, Uttarakhand, India.

### 2.5.1. Seed Germination Inhibition

Different essential oil concentrations (50–200 µL/mL) were made in a Tween-20 (1%) solution of distilled water in order to assess the suppression of seed germination. Radish seeds were surface sterilised for 15 minutes in a 5% hypochlorite solution to break dormancy. Each petri plate, coated with sheets of quality filter paper, contained ten sterile radish seeds. Additionally, 2 mL of the tested sample at different concentrations (50–200 µL/mL) were put onto the plates,

and the seeds were left to germinate in an incubator with a photoperiod of 12 hours and a regulated temperature of 25 ± 1 ◦C. When a seed's root length reached 2 mm, it was said to have germinated. The bioassay was carried out in triplicate using pendimethalin (50–200 µL/mL) as a standard herbicide and distilled water as the control. The number of seeds that germinated in each petri dish was counted after 120 hours, and the percentage of seeds that were inhibited from germinating was then calculated using the method below:

$$\text{Inhibition of seed germination (\% Inhibition)} = 100 \times (1 - Gt/Gc)$$

where Gt = no. of seeds germinates in treatment, Gc = No. of seeds germinate in control.

### 2.5.2. Inhibition of Shoot and Root Elongation

Evaluations of shoot and root elongation were conducted over a 24-hour photoperiod at a regulated temperature of 25 °C. Two pre-germinated seeds were added to each Petri plate, which was then filled with 2.0 mL of the test solution. The germination bioassay and the EOs were examined at identical concentrations. The shoot and root lengths were measured after the 120-hour incubation period. The bioassays were conducted in triplicate, with pendimethalin (50–200 µL/mL) employed as a standard herbicide and distilled water as the controls treatment. The following formulas were used to calculate the inhibition of root and shoot growth:

$$\text{Inhibition of hypocotyl (shoot length) growth (\% Inhibition)} = 100 \times (1 - Ct/Cc)$$

where, Ct = shoot length growth in treatment, Cc = shoot length growth in control.

$$\text{Inhibition of radicle (root length) growth (\% Inhibition)} = 100 \times (1 - Rt/Rc)$$

where, Rt = root length growth in treatment, Rc = root length growth in control.

## 3. Results and Discussion

### 3.1. Chemical Composition

A total of 37, 45, and 43 components were identified in VAO (0.1–25.0%), VNO (0.1–19.4%), and VTO (0.1–16.2%), respectively. Twenty-two components were identified as common across all three essential oils, namely: α-thujene, α-pinene, sabinene, β-pinene, myrcene, 1,8-cineole, γ-terpinene, p-cymene, linalool, trans-sabinenehydrate, cis-p-menth-2-en-1-ol, terpinen-4-ol, α-terpineol, dihydroedulan II, β-caryophyllene, α-humulene, β-iraldeine, β-caryophyllene oxide, α-muurolol, drimenol, and manool. Nevertheless, they differed in their respective percentages. Table 1 summarises that the predominant compounds in V. agnus-castus oil are 1,8-Cineole (25.0%), sabinene (13.3%), α-pinene (8.2%), and α-terpinyl acetate (5.5%); in V. negundo oil, they are sabinene (19.4%), viridiflorol (17.8%), β-caryophyllene (7.5%), and β-iraldiene (6.4%); while in V. trifolia oil, the abundant compounds include β-caryophyllene (16.2%), 5-(1-isopropenyl-4,5-dimethylbicyclo[4.3.0]nonan-5-yl)-3-methyl-2-pentenol acetate (11.7%), 13-epi-manoyl oxide (5.6%), and caryophyllene oxide (4.6%). The chemical class composition of VAO was mostly comprised of oxygenated monoterpenes (40.6%), followed by monoterpene hydrocarbons (31.2%) and miscellaneous compounds. The only diterpenoid identified in VAO was manool (0.5%). Conversely, VNO was mostly composed of monoterpene hydrocarbons (29.4%), followed by oxygenated sesquiterpenes (24.8%) and oxygenated monoterpenoids (11.3%). The predominant class identified in VTO was sesquiterpene hydrocarbon (21.9%), followed by oxygenated sesquiterpene (15.8%) and oxygenated diterpenes (13.8%). For further information on chromatograms and mass

spectra of chemical composition, please see Supplemental Material S1.

Table 1. Comparative chemical composition of essential oil of Vitex species.

| S. No. | Compound Name | Molecular Formula | R.I. | % Composition | | |
|---|---|---|---|---|---|---|
| | | | | VAO | VNO | VTO |
| 1 | α-Thujene (MH) | $C_{10}H_{16}$ | 930 | 1.4 | 0.2 | 0.1 |
| 2 | α-Pinene (MH) | $C_{10}H_{16}$ | 939 | 8.2 | 2.6 | 1.6 |
| 3 | Sabinene (MH) | $C_{10}H_{16}$ | 975 | 13.3 | 19.4 | 2.0 |
| 4 | β-Pinene (MH) | $C_{10}H_{16}$ | 979 | 1.2 | 0.4 | 0.3 |
| 5 | Oct-1-en-3-ol | $C_8H_{16}O$ | 979 | - | 0.5 | 4.0 |
| 6 | Myrcene (MH) | $C_{10}H_{16}$ | 990 | 3.1 | 0.7 | 0.1 |
| 7 | α-Phellandrene (MH) | $C_{10}H_{16}$ | 1002 | - | - | 4.2 |
| 8 | α-Terpinene (MH) | $C_{10}H_{16}$ | 1017 | - | 2.3 | - |
| 9 | β-Phellandrene (MH) | $C_{10}H_{16}$ | 1029 | - | 1.0 | - |
| 10 | 1,8-Cineole (OM) | $C_{10}H_{18}O$ | 1031 | 25.0 | 1.2 | 2.1 |
| 11 | β-Ocimene (MH) | $C_{10}H_{16}$ | 1044 | 1.5 | - | - |
| 12 | γ-Terpinene (MH) | $C_{10}H_{16}$ | 1059 | 0.3 | 0.8 | 3.3 |
| 13 | Linalool oxide (OM) | $C_{10}H_{18}O_2$ | 1086 | - | 0.4 | - |
| 14 | α-Terpinolene (MH) | $C_{10}H_{16}$ | 1088 | - | 1.2 | - |
| 15 | *p*-Cymene (MH) | $C_{10}H_{12}$ | 1091 | 2.2 | 0.6 | 0.2 |
| 16 | Linalool (OM) | $C_{10}H_{18}O$ | 1096 | 0.8 | 0.6 | 2.2 |
| 17 | *trans*-Sabinenehydrate (OM) | $C_{10}H_{18}O$ | 1098 | 0.2 | 0.3 | - |
| 18 | Isoamyl isovalerate (Fatty Acid Ester) | $C_{10}H_{20}O_2$ | 1103 | - | 0.1 | - |
| 19 | *cis-p*-menth-2-en-1-ol (OM) | $C_{10}H_{18}O$ | 1121 | 0.2 | 0.2 | 0.1 |
| 20 | *δ*-Terpineol (OM) | $C_{10}H_{18}O$ | 1166 | 0.5 | - | - |
| 21 | Terpinen-4-ol (OM) | $C_{10}H_{18}O$ | 1177 | 1.9 | 5.4 | 1.8 |
| 22 | Cryptone (OM) | $C_9H_{14}O$ | 1185 | 0.1 | - | - |
| 23 | α-Terpineol (OM) | $C_{10}H_{18}O$ | 1188 | 2.5 | 1.4 | 0.3 |
| 24 | Myrtenol (OM) | $C_{10}H_{16}O$ | 1195 | - | 0.1 | - |
| 25 | *cis*-Piperitol (OM) | $C_{10}H_{18}O$ | 1196 | - | 0.1 | - |
| 26 | γ-Terpineol (OM) | $C_{10}H_{18}O$ | 1199 | 0.1 | - | - |
| 27 | *trans*-Piperitol (OM) | $C_{10}H_{18}O$ | 1208 | - | - | 0.8 |
| 28 | β-Citronellol (OM) | $C_{10}H_{20}O$ | 1225 | 1.4 | - | 0.5 |
| 29 | *cis*-Verbenyl acetate (OM) | $C_{12}H_{18}O_2$ | 1282 | 1.1 | - | - |
| 30 | Dihydroedulan II | $C_{13}H_{22}O_2$ | 1284 | 0.1 | 0.1 | 0.5 |
| 31 | Theaspirane A (OM) | $C_{13}H_{22}O_2$ | 1290 | - | 1.5 | 0.2 |
| 32 | α-Terpinyl acetate (OM) | $C_{12}H_{20}O_2$ | 1349 | 5.5 | - | 0.5 |
| 33 | β-Citronellyl acetate (OM) | $C_{12}H_{22}O_2$ | 1352 | 1.3 | - | - |
| 34 | β-Damascenone (OM) | $C_{13}H_{18}O$ | 1384 | - | 0.1 | - |
| 35 | β-Bourbonene (SH) | $C_{15}H_{24}$ | 1388 | - | - | 0.7 |
| 36 | β-Elemene (SH) | $C_{15}H_{24}$ | 1390 | - | - | 0.8 |
| 37 | β-Caryophyllene (SH) | $C_{15}H_{24}$ | 1419 | 3.7 | 7.5 | 16.2 |
| 38 | Methyl-isoeugenol (Phenylpropanoid) | $C_{11}H_{14}O_2$ | 1453 | - | - | 3.1 |
| 39 | α-Humulene (SH) | $C_{15}H_{24}$ | 1454 | 0.2 | 0.4 | 1.4 |
| 40 | β-Farnesene (SH) | $C_{15}H_{24}$ | 1456 | 4.5 | 0.6 | - |
| 41 | β-Selinene (SH) | $C_{15}H_{24}$ | 1490 | - | 0.3 | - |
| 42 | *epi*-Cubebol (OS) | $C_{15}H_{26}O$ | 1494 | - | - | 2.3 |
| 43 | Bicyclogermacrene (SH) | $C_{15}H_{24}$ | 1500 | 0.3 | - | - |
| 44 | α-Muurolene (SH) | $C_{15}H_{24}$ | 1500 | - | - | 1.5 |
| 45 | γ-Cadinene (SH) | $C_{15}H_{24}$ | 1513 | - | - | 0.8 |
| 46 | δ-Cadinene (SH) | $C_{15}H_{24}$ | 1523 | - | - | 0.5 |
| 47 | Hedycaryol (OS) | $C_{15}H_{26}O$ | 1548 | - | 0.5 | - |
| 48 | β-Iraldeine (ionone) | $C_{14}H_{22}O$ | 1557 | 3.8 | 6.4 | 2.0 |
| 49 | Nerolidol (OS) | $C_{15}H_{26}O$ | 1563 | - | - | 2.2 |
| 50 | Spathulenol (OS) | $C_{15}H_{24}O$ | 1578 | 1.4 | - | - |
| 51 | β-caryophyllene oxide (OS) | $C_{15}H_{24}O$ | 1583 | 1.9 | 1.3 | 4.6 |
| 52 | Globulol (OS) | $C_{15}H_{26}O$ | 1590 | - | 0.2 | 1.4 |
| 53 | Viridiflorol (OS) | $C_{15}H_{26}O$ | 1592 | - | 17.8 | - |
| 54 | Ledol (OS) | $C_{15}H_{26}O$ | 1602 | 0.4 | 1.0 | - |
| 55 | Humulene epoxide II (OS) | $C_{15}H_{24}O$ | 1608 | - | - | 1.2 |
| 56 | Humulane-1,6-dien-3-ol (OS) | $C_{15}H_{26}O$ | 1619 | 2.4 | 2.3 | - |
| 57 | *epi*-α-Cadinol (OS) | $C_{15}H_{26}O$ | 1640 | 2.1 | - | - |
| 58 | α-Muurolol (OS) | $C_{15}H_{26}O$ | 1646 | 0.1 | 0.2 | 1.7 |
| 59 | β-eudesmol (OS) | $C_{15}H_{26}O$ | 1650 | 0.1 | 1.1 | - |
| 60 | Pogostol (OS) | $C_{15}H_{26}O$ | 1653 | - | - | 1.6 |
| 61 | Drimenol (OS) | $C_{15}H_{26}O$ | 1767 | 0.3 | 0.4 | 0.8 |
| 62 | Flourensadiol (OS) | $C_{15}H_{26}O_2$ | 1870 | 0.1 | - | - |
| 63 | Cubitene (DT) | $C_{20}H_{32}$ | 1878 | - | 1.3 | - |
| 64 | Phytol (OD) | $C_{20}H_{40}O$ | 1943 | - | 1.2 | 1.3 |
| 65 | 13-epi-manoyl oxide (OD) | $C_{20}H_{34}O$ | 2002 | - | - | 5.6 |
| 66 | Manool (OD) | $C_{20}H_{34}O$ | 2057 | 0.5 | 1.9 | 1.7 |
| 67 | Sclareolide (SH) | $C_{14}H_{24}O_2$ | 2066 | - | 0.2 | - |
| 68 | Sclareol (OD) | $C_{20}H_{36}O_2$ | 2223 | - | 1.7 | 1.0 |
| 69 | 5-(1-isopropenyl-4,5-dimethylbicyclo[4.3.0]nonan-5-yl)-3-methyl-2-pentenol acetate | $C_{22}H_{36}O_2$ | 2265 | - | 5.2 | 11.7 |
| 70 | Larisol (OD) | $C_{20}H_{34}O_2$ | 2266 | - | 0.7 | - |
| 71 | Verticiol (OD) | $C_{20}H_{34}O$ | 2273 | - | - | 1.4 |
| 72 | 16-oxo-cleroda-3,13(14)-(e)-dien-15-oic acid (OD) | | - | - | - | 2.8 |
| | Monoterpene hydrocarbon (MH) | | | 31.2 | 29.4 | 11.8 |
| | Oxygenated monoterpene (OM) | | | 40.6 | 11.3 | 8.5 |
| | Sesquiterpene hydrocarbon (SH) | | | 8.7 | 8.8 | 21.9 |
| | Oxygenated sesquiterpene (OS) | | | 8.8 | 24.8 | 15.8 |
| | Diterpene hydrocarbon (DT) | | | - | 1.3 | - |
| | Oxygenated diterpene (OD) | | | 0.5 | 5.7 | 13.8 |
| | Other than terpenoids | | | 3.9 | 12.3 | 21.3 |
| | Total | | | 93.7 | 93.6 | 93.1 |

current investigation. The oxygenated sesquiterpenes found in VAO, including spathulenol, ledol, and epi-α-cadinol, have been previously reported in the essential oil of V. agnus castus leaves from Ogliastra, Sardinia, Italy [46]. However, other sesquiterpenoids such as β-eudesmol, drimenol, and flourensadiol were not previously detected in the essential oil of V. agnus castus leaves. VAO is also devoid of chemicals such as limonene, viridiflorol, and globulol, which are often found in the majority of prior studies [45–48]. The research illustrates many chemo-variants of V. agnus castus in both qualitative and quantitative terms.

Prior studies have also examined the essential oils of Vitex negundo, which are the subject of this work. For example, the predominant components identified in VNO, sabinene (19.4%), viridiflorol (17.8%), and β-caryophyllene (7.5%), were likewise recognised in varying concentrations in the hydrodistilled essential oil of Vitex negundo leaves [22,49,50]. 5-(1-Isopropenyl-4,5-dimethylbicyclo[4.3.0]nonan-5-yl)-3-methyl-2-pentenol acetate (5.2%), a significant component identified in VNO, was also isolated in substantial quantities in the essential oil of V. negundo leaves [51]. The chemical composition of the essential oil of Vitex negundo, extracted in the spring from Pantnagar, exhibited over 33 compounds, with the predominant constituents being viridiflorol (23.8%), sabinene (11.2%), an unidentified diterpene M+ = 272 (11.0%), and caryophyllene (6.7%) [50]. The composition previously lacked the compounds α-thujene, β-pinene, α-terpinene, β-phellandrene, γ-terpinene, linalool oxide, p-cymene, trans-sabinenehydrate, theaspirane A, β-iraldeine, β-caryophyllene oxide, ledol, humulane-1,6-dien-3-ol, and the diterpenes cubetene, phytol, manool, and sclereol; however, in the current study, these compounds are identified in significant quantities. Consequently, the composition may fluctuate according on the harvesting season. In a separate research from India, α-copaene (25.3%), β-elemene (19.2%), and camphene (21.1%) were identified as the primary components in the leaf essential oil of V. negundo [52]. Khokra et

al. [53] identified ethyl-9-hexadecenoate (28.5%), δ-guaiene (18.0%), and caryophyllene oxide (10.2%) as the predominant constituents in the leaf essential oil of V. negundo. Conversely, the leaf essential oil of V. negundo from China exhibited δ-guaiene (50.0%) and β-caryophyllene (38.0%) as its principal components [54]. The qualitative and quantitative differences in the essential oils of V. negundo from various geographic locations may result from differing geographical and climatic circumstances.

Thomas et al. [55] examined the essential oil of V. trifolia and identified caryophyllene (38.36%) and 1,8-cineole (25.72%) as the principal components. In the current research, the concentration of 1,8-cineole in VTO is just 2.1%. β-caryophyllene is recognised as the principal component of V. trifolia oil in several papers [56–58], corroborating the findings of the current investigation. Arpiwi et al. [59] discovered five components in V. trifolia essential oil, with cis-ocimene (44.57%), α-thujene (25.63%), and cyclopentene, 3-isopropenyl-5,5-dimethyl (18.19%) recognised as the predominant ingredients. In the current investigation, such compounds were not identified, and the quantity of α-thujene discovered was minimal (0.2%). The second predominant component identified in VTO, 5-(1-isopropenyl-4,5-dimethylbicyclo[4.3.0]nonan-5-yl)-3-methyl-2-pentenolacetate (11.7%), has also been seen in other Vitex species, including V. agnus-castus and V. negundo [51,60]. The prominent diterpenes found in VTO, 13-epi-manoyl oxide (5.6%) and 16-oxo-cleroda-3,13(14)-(e)-dien-15-oic acid (2.8%), are being reported for the first time in V. trifolia oil. The variations in essential oil composition may result from internal and external variables and their interplay.

The chemicals found in the analysed essential oils has significant biological uses. 1,8-cineole is used in cosmetic formulations and as a flavouring ingredient due to its agreeable fragrance and flavour. The chemical has additional properties: insecticidal, antioxidant, and anti-inflammatory [61]. Viridiflorol is mostly used as an anti-inflammatory, antioxidant, and anti-tuberculosis agent [62]. Literature describes sabinene as possessing antibacterial, anti-inflammatory, and antioxidant effects [63]. Additionally, the diterpene, 13-epi-manoyl oxide, has cytotoxic antibacterial and antifungal properties [64].

### 3.2. Chemometric Analysis

The primary chemical constituents prevalent in all tested species' essential oils (α-thujene, α-pinene, sabinene, β-pinene, myrcene, 1,8-cineole, γ-terpinene, p-cymene, linalool, cis-p-menth-2-en-1-ol, terpinen-4-ol, α-terpineol, dihydroedulan II, β-caryophyllene, α-humulene, β-iraldeine, β-caryophyllene oxide, α-muurolol, drimenol, and manool) were analysed using hierarchical cluster analysis with Euclidean distance as the metric for similarity. Figure 1 illustrates the heat map clustering diagram. 1,8-Cineole, sabinene, and β-caryophyllene constitute distinct clusters with varying values relative to the other examined common elements. The heat map clustering, using Euclidean distance, clearly categorises all examined species into two primary groupings based on shared chemical elements. VNO and VTO are grouped into a single cluster, but VAO is in a distinct cluster.

Figure 1. The heatmap analysis of the shared essential oil constituents and evaluated species (The distribution of the characteristic (common essential oil components) is shown by colours, with yellow denoting the largest value and blue signifying the least value).

### 3.3 Principal component analysis

Principal component analysis (PCA) is a prominent multivariate statistical approach used to discern the most significant aspects of a dataset. Distinct essential oils may be used in PCA pattern recognition to evaluate the alterations in chemical profile induced by interspecies and altitudinal factors. The PCA method established that the cumulative variance contribution of the first two principal components (PC1 and PC2) accounted for 81.2% of the variation in chemical composition changes. PC1 and PC2 were used to delineate the compositional variances in the essential oils. PC1 had a positive correlation with terpinen-4-ol, β-iraldeine, β-caryophyllene, viridiflorol, and sabinene, accounting for 48.7% of the total variance. PC2 accounts for 32.5% of the variance and has a robust positive connection with α-pinene and 1,8-cineole (Figure 2).

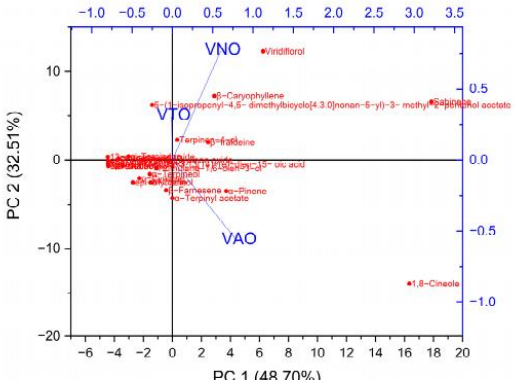



Figure 2. Principal Component Analysis of tested essential oil's chemical constituents.

### 3.4. Antioxidant Activity

The antioxidant activity was assessed utilising several chemical techniques. Figures 3A–E illustrate the antioxidant efficacy of the evaluated essential oil shown as percent inhibition. The results indicated that all antioxidant activity were concentration-dependent. The percentage inhibition of free radicals (DPPH, H2O2, NO), reducing capacity, and metal chelation enhanced with the concentration rise from 10 µL/mL to 50 µL/mL. The percentage of inhibition by the evaluated essential oils and standards for various antioxidant tests was graphed against concentrations, and the line equation was used to get the IC50 (half-maximal inhibitory concentration) values.

Figures 4A–E illustrate the antioxidant activity of the evaluated essential oils based on their IC50 values. The DPPH test uses the conversion of the stable violet radical DPPH to the yellow DPPH-H to assess the capacity of an antioxidant molecule to function as a donor of hydrogen atoms or electrons. Figure 4A indicates that VNO reduced DPPH with an IC50 value of 23.16 ± 0.5 µL/mL, which is comparable to the standard antioxidant used in the experiment, BHT (18.84 ± 0.6 µL/mL). VAO and VTO exhibited moderate and weak antioxidant activity, with IC50 values of 25.39 ± 0.0 µL/mL and 32.49 ± 0.5 µL/mL, respectively. Hydrogen peroxide (H2O2) may permeate biological membranes, thereby inflicting harm on the human body by generating reactive hydroxyl radicals (OH·) via the Fenton reaction [65]. In the H2O2 radical scavenging experiment, VAO (IC50 = 24.49 ± 0.1 µL/mL) exhibited superior scavenging activity relative to the standard, ascorbic acid (28.33 ± 0.5 µL/mL), followed by VNO (32.38 ± 0.5

µL/mL) and VTO (34.30 ± 0.5 µL/mL). The nitrite scavenging capacity of the samples was evaluated against ascorbic acid, yielding the following IC50 values: ascorbic acid (24.49 ± 0.1 µL/mL) > VNO (27.58 ± 0.1 µL/mL) > VTO (32.27 ± 0.1 µL/mL) > VAO (32.95 ± 0.5 µL/mL). The reducing power of a chemical correlates with its capacity to transport electrons, indicating its considerable antioxidant potential. Figure 4D illustrates that VNO exhibited a commendable reducing capacity (RP50 = 19.05 ± 0.6 µL/mL), which is comparable to and somewhat lower than that of the standard gallic acid (20.22 ± 0.4 µL/mL). The sequence of RP50 values for various samples is as follows: VNO (19.05 ± 0.6 µL/mL) > gallic acid (20.22 ± 0.4 µL/mL) > VAO (20.97 ± 0.5 µL/mL) > VTO (22.74 ± 0.7 µL/mL). In auto-oxidation processes, metal ions serve as potent catalysts by inhibiting the formation of oxygen radicals. The IC50 values for various samples and standards for their antioxidant capacity in terms of chelating ability were recorded as follows: Na2-EDTA (IC50 = 26.23 ± 0.26 µL/mL) > VTO (IC50 = 29.77 ± 0.2 µL/mL) > VNO (IC50 = 31.18 ± 0.2 µL/mL) > VAO (IC50 = 36.60 ± 0.1 µL/mL).

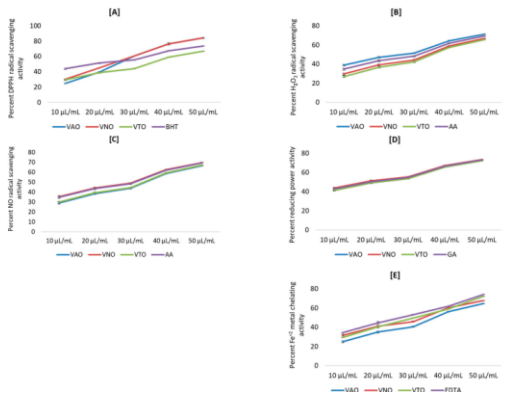



Figure 3. (A–E) Antioxidant efficacy of essential oils from Vitex species: (A) Percentage of DPPH radical scavenging activity; (B) Percentage of H2O2 scavenging activity; (C) Percentage of NO radical scavenging activity; (D) Percentage of reducing power activity; (E) Percentage of Fe2+ metal chelating activity.

The pronounced antioxidant activity of VNO against DPPH and NO radicals is likely attributable to its substantial sabinene content, along with additional compounds such as β-caryophyllene, terpinen-4-ol, and 1,8-cineole, which exhibit antioxidant potential via various mechanisms [66–68]. Kazemi [69] demonstrated that sabinene had a significant nitric oxide-scavenging action and suppressed the production of inducible nitric oxide synthase. Comparable findings were noted in prior research on the antioxidant activity of V. negundo essential oil, where sabinene was the predominant component [49]. In the H2O2 radical scavenging experiment, VAO exhibited significant scavenging activity, perhaps attributable to the presence of 1,8-cineole, sabinene, and β-caryophyllene as predominant ingredients [69,70]. Previous studies have evaluated the antioxidant activity of essential oil and extracts from the aerial portions of V. agnus castus, noting a significant presence of 1,8-cineole and β-caryophyllene in their composition, which demonstrated commendable antioxidant efficacy [64,71,72]. Due to the complexity of essential oils as combinations of several chemicals, their overall biological action is difficult to

elucidate.

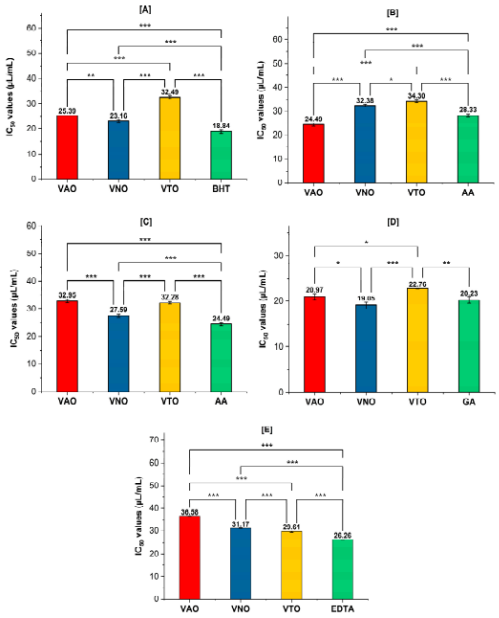



Figure 4. (A–E) Antioxidant activity measured by IC50 values (µL/mL) for VAO, VNO, and VTO: (A) DPPH radical scavenging, (B) H2O2 radical scavenging, (C) NO radical scavenging, (D) reducing power activity, (E) metal chelating activity. Statistically significant differences were analysed using one-way ANOVA and Tukey post hoc testing. Significant changes between treatment groups are shown as follows: *** $p < 0.001$, ** $p < 0.005$, * $p < 0.05$. Values are expressed as mean ± standard deviation, n = 3.

### 3.5. Herbicidal (Phytotoxic) Activity

The examined materials exhibited significant phytotoxic effects on the germination and development of wild radish (R. raphanistrum) seedlings in a concentration-dependent manner. At the maximum concentration (100 µL/mL), VAO inhibited seed germination, root growth, and shoot growth of R. raphanistrum by 66.67%, 96.66%, and 89.09%, respectively. VNO exhibited inhibition rates of 90.0%, 89.39%, and 97.57%, respectively, whereas VTO demonstrated inhibition rates of 100%, 99.39%, and 92.12%, respectively (Tables 2–4). According to the IC50 values, VAO exhibited IC50 values of 82.89, 19.468, and 37.95 µL/mL concerning seed germination, root development, and shoot growth, respectively. The IC50 values for VNO were 50.13, 47.06, and 16.75 µL/mL, respectively. The IC50 values for VTO were 29.5, 9.33, and 27.13 µL/mL, respectively. (Tables 2–4).

Table 2. Mean % inhibition and IC50 values for seed germination inhibition by tested essential oils.

| Samples | % Inhibition of Seed Germination | | | | | $IC_{50}$ Values (µL/mL) in Triplicates | | | Mean $IC_{50}$ Values (µL/mL) ± SD |
|---|---|---|---|---|---|---|---|---|---|
| | 20 µL/mL | 40 µL/mL | 60 µL/mL | 80 µL/mL | 100 µL/mL | I | II | III | |
| VAO | 3.33 ± 5.77 $^{h}$ | 3.33 ± 5.77 $^{h}$ | 40 ± 0.00 $^{f}$ | 43.33 ± 5.77 $^{ef}$ | 66.66 ± 5.77 $^{cd}$ | 78.94 | 81.17 | 88.57 | 82.89 ± 5.04 |
| VNO | 23.33 ± 5.77 $^{g}$ | 43.33 ± 5.77 $^{ef}$ | 56.66 ± 5.77 $^{de}$ | 76.66 ± 5.77 $^{bc}$ | 90.00 ± 0.00 $^{ab}$ | 51.11 | 55.294 | 44.00 | 50.13 ± 5.7 |
| VTO | 33.33 ± 5.77 $^{fg}$ | 60.00 ± 0.00 $^{cd}$ | 90.00 ± 0.00 $^{ab}$ | 93.33 ± 5.77 $^{a}$ | 100.00 ± 0.00 $^{a}$ | 31.764 | 25.00 | 31.764 | 29.50 ± 3.9 |
| Pendimethalin | 100.00 ± 0.00 | 100.00 ± 0.00 | 100.00 ± 0.00 | 100.00 ± 0.00 | 100.00 ± 0.00 | | | | |

Table 3. Mean % inhibition and IC50 values for root length inhibition by tested essential oils

| Samples | % Inhibition of Root Length | | | | | $IC_{50}$ Values (µL/mL) in Triplicates | | | Mean $IC_{50}$ Values (µL/mL) ± SD |
|---|---|---|---|---|---|---|---|---|---|
| | 20 µL/mL | 40 µL/mL | 60 µL/mL | 80 µL/mL | 100 µL/mL | I | II | III | |
| VAO | 45.15 ± 1.0 $^{h}$ | 66.36 ± 0.9 $^{d}$ | 80.90 ± 0.9 $^{c}$ | 89.69 ± 0.5 $^{b}$ | 96.66 ± 0.5 $^{a}$ | 18.963 | 19.161 | 20.28 | 19.468 ± 0.7 |
| VNO | 52.27 ± 1.6 $^{fg}$ | 45.45 ± 0.9 $^{g}$ | 58.48 ± 1.0 $^{e}$ | 68.78 ± 2.2 $^{d}$ | 89.39 ± 2.6 $^{b}$ | 46.529 | 47.00 | 47.67 | 47.06 ± 0.5 |
| VTO | 52.42 ± 2.2 $^{f}$ | 70.00 ± 1.8 $^{d}$ | 83.03 ± 1.3 $^{c}$ | 92.12 ± 1.04 $^{b}$ | 99.39 ± 1.04 $^{a}$ | 9.766 | 9.766 | 8.479 | 9.337 ± 0.7 |
| Pendimethalin | 100.00 ± 0.00 | 100.00 ± 0.00 | 100.00 ± 0.00 | 100.00 ± 0.00 | 100.00 ± 0.00 | | | | |

Table 4. Mean % inhibition and IC50 values for shoot length inhibition by tested essential oils.

| Samples | % Inhibition of Shoot Length | | | | | $IC_{50}$ Values (µL/mL) in Triplicates | | | Mean $IC_{50}$ Values (µL/mL) ± SD |
|---|---|---|---|---|---|---|---|---|---|
| | 20 µL/mL | 40 µL/mL | 60 µL/mL | 80 µL/mL | 100 µL/mL | I | II | III | |
| VAO | 37.57 ± 0.5 $^{k}$ | 52.72 ± 1.8 $^{i}$ | 63.48 ± 0.6 $^{g}$ | 77.57 ± 0.5 $^{e}$ | 89.09 ± 0.9 $^{bc}$ | 37.52 | 37.58 | 38.75 | 37.95 ± 0.6 |
| VNO | 51.21 ± 1.3 $^{i}$ | 64.24 ± 2.7 $^{g}$ | 74.54 ± 0.9 $^{e}$ | 83.78 ± 0.2 $^{d}$ | 97.57 ± 0.5 $^{a}$ | 17.43 | 17.42 | 15.41 | 16.75 ± 1.2 |
| VTO | 45.15 ± 0.5 $^{j}$ | 58.48 ± 1.0 $^{h}$ | 68.03 ± 0.6 $^{f}$ | 85.90 ± 1.2 $^{cd}$ | 92.12 ± 0.5 $^{b}$ | 28.65 | 26.417 | 26.33 | 27.13 ± 1.3 |
| Pendimethalin | 100.00 ± 0.01 | 100.00 ± 0.01 | 100.00 ± 0.0 | 100.00 ± 0.00 | 100.00 ± 0.00 | | | | |

The phytotoxic potential of essential oils from many Vitex species, including V. agnus-castus, V. negundo, and V. simplicifolia, has been documented in other plants and weeds. Nonetheless, no research has been documented about the phytotoxic potential of V. trifolia. The current investigation shown that VTO was more effective against R. raphanistrum than VNO and VAO. The inhibitory impact of VTO on R. raphanistrum may be attributed to elevated levels of β-caryophyllene (16.2%) and its synergistic interaction with other predominant and trace chemicals in the oil. Previous results indicated that β-caryophyllene inhibits

germination and seedling development in many plant species, including Brassica campestris, Raphanus sativus, Vigna radiata, and Solanum lycopersicum [22]. VNO demonstrated significant suppression of seed germination and shoot development, but VAO exhibited superior inhibition of root growth. The inhibitory impact of the samples may be attributed to the presence of phytotoxic chemicals, including β-caryophyllene, 1,8-cineole, and sabinene, which are principal constituents of essential oils exhibiting phytotoxic action [22,74]. Moreover, 1,8-cineole was shown to disrupt the normal development of Nicotiana tabacum by inhibiting DNA synthesis in the cell nuclei and organelles of root apical meristem cells [75]. Research has shown that the terpenoids in essential oils have phytotoxic effects on plants, leading to morphological and physiological changes in cells that hinder plant development [76].

### 3.6. Correlation of Essential Oil Components and Biological Activities

The Pearson correlation coefficient of major essential oil constituents, which was greater than two percent, as well as the antioxidant and herbicidal activities of Vitex species, revealed that 5-(1-isopropenyl-4,5-dimethylbicyclo[4.3.0]nonan-5-yl)-3-methyl-2-pentenol acetate and β-caryophyllene exhibited a strong positive correlation with DPPH radical scavenging activity and Fe2+ metal chelating activity. Additionally, γ-terpinene was found to have a positive correlation with Fe2+ metal chelating activity. In their study, Dahham et al. [77] have provided evidence that β-caryophyllene has a significant DPPH scavenging activity. The compounds α-terpinyl acetate, 1,8-cineole, epi-α-cadinol, α-pinene, and β-farnesene shown a slight association with the ability to scavenge H2O2 radicals. Terpinen-4-ol, α-terpinene, and viridiflorol demonstrated a link with nitric oxide radical scavenging activity and reducing power activity, although a slight one. Terpinen-4-ol was shown to induce relaxation in rabbit duodenal relaxation [78], and it is presumed that this relaxation was not mediated by the production of nitric oxide. The essential oils that were examined for their herbicidal effect indicated that 5-(1-isopropenyl-4,5-dimethylbicyclo[4.3.0]nonan-5-yl)-3-methyl-2-pentenol acetate and β-caryophyllene demonstrated a significant positive connection with the suppression of seed germination. In contrast, it was shown that linalool and β-caryophyllene exhibited a moderate association with root length inhibition. On the other hand, terpinen-4-ol, α-terpinene, and viridiflorol were connected with a substantial positive correlation with shoot length inhibition. There is evidence that plants that possess high amounts of β-caryophyllene have the ability to exert phytotoxic effects on several types of weeds [79]. The results of the correlation coefficient were supported by in vitro activities that were conducted as part of the study that was given, in addition to studies that had been published in the past. An illustration of Pearson's correlation coefficient between the components of essential oils and the biological activity of those components can be seen in Figure 5.

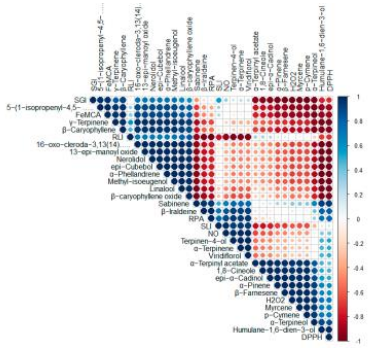


Figure 5. There is a correlation between the chemical components of essential oils and the biological activities of Vitex species. In this context, the abbreviations DPPH and H2O2 represent the percentage of inhibition of radical scavenging activity at a concentration of 50 µL/mL. The abbreviation NO represents the percentage of inhibition of NO radical scavenging activity at a concentration of 50 µL/mL. The abbreviation RPA represents the percentage of inhibition of reducing power activity at a concentration of 50 µL/mL. FeMCA represents the percentage of inhibition of Fe2+ metal chelating activity at a concentration of 50 µL/mL. The abbreviation SGI represents the percentage of seed germination at a concentration of 100 µL/mL. The abbreviation RLI represents the percentage of root length at 100 µL/mL.

### 3.7. Molecular Docking

In addition, we investigated whether the primary phytoconstituents found in VAO, VNO, and VTO physically connect with the receptors for antioxidant proteins (human peroxiredoxin 5, PDB: 1HD2) and 4-hydroxyphenylpyruvate dioxygenase (HPPD, PDB: 6J63). Due to the fact that the essential oils that were tested exhibited a high level of free radical inhibition, the enzyme human peroxiredoxin 5 was chosen for this purpose. This enzyme has a wider range of action against reactive oxygen species (ROS) and is primarily engaged in the mechanism that protects against stress [80,81]. HPPD was chosen because it is known to be the target protein for drugs that have post-emergence herbicidal action. This provided the basis for the selection of HPPD. Based on the findings of our investigation, it was discovered that the essential oils that were put through their paces had a high level of post-emergence herbicidal efficacy against the receptor species that were chosen as the target enzyme [18,38]. Thirteen-epi-manoyl oxide, out of all the phytocompounds that were chosen, had the highest binding affinity with human peroxiredoxin 5 (-6.2 kcal mol) and HPPD (-8.7 kcal/mol). It was determined that the optimal docked stance was the one that had the lowest binding energy by doing an introspective analysis of the various docked poses. As shown in Figure 6B, the best docked posture of 13-epi-manoyl oxide had several Van der Waal contacts with amino acid residues such as Phe A:424, Phe A:419, and Phe A:381. These connections included two pi-alkyl interactions, one pi-sigma interaction, and other interactions. In a similar manner, the best docked posture of 13-epi-manoyl oxide displayed alkyl contact with 1HD2 that included the amino acids Ala A:90 and Arg A:86, as well as Van der Waal interaction. The docking research of nitisinone (CID:115355) was also carried out using HPPD for the goal of providing a point of comparison. It is well known that nitisinone, also known as 2-[2-nitro-4-(trifluoromethyl)benzoyl]cyclohexane-1,3-dione (NTBC), is an inhibitor of high-potential prostate cancer. The docking research of ascorbic acid, which is a well-known antioxidant, was carried out utilising 1HD2 as the method of investigation. NTBC complexed with 6J63 exhibited a binding energy of -8.9

kcal/mol, which is in close proximity to the binding energy of 13-epi-manoyl oxide, which was -8.7 kcal/mol respectively. On the other hand, binding energy of ascorbic acid complexed with 1HD2 came out to be −5.7 kcal/mol, which was higher than most of the compounds such as 13-epi-manoyl oxide (−6.2 kcal/mol), caryophyllene oxide (−6.1 kcal/mol), 5-(1-isopropenyl-4,5-dimethylbicyclo [4.3.0]nonan5-yl)-3-methyl-2-pentenol acetate (−6.1 kcal/mol), β-caryophyllene (−6.0 kcal/mol), and viridiflorol (−5.9 kcal/mol), as shown in Figure 7. When the values of binding free energy are lower, it indicates that the interaction between the receptor and the ligand is more substantial. Previous in silico investigations that were published by Alminderej et al. [73] showed that a phenylpropanoid-rich Piper cubeba essential oil (EO) offered comparable results in terms of a postulated in vitro antioxidant activity by targeting human periredoxin 5. Our findings were compatible with these findings. The chemicals viridiflorol and caryophyllene oxide demonstrated a substantial interaction with the 1HD2 receptor in this analysis, just as they did in the previous study. In a recent research that focused on the phytotoxic potential of Calycolpus goetheanus essential oil, it was discovered that the primary constituents of the specimen, namely 1,8-cineole and β-caryophyllene, had a positive interaction with the HPPD protein [18]. There is a high degree of concordance between these findings and the findings acquired in the current investigation.

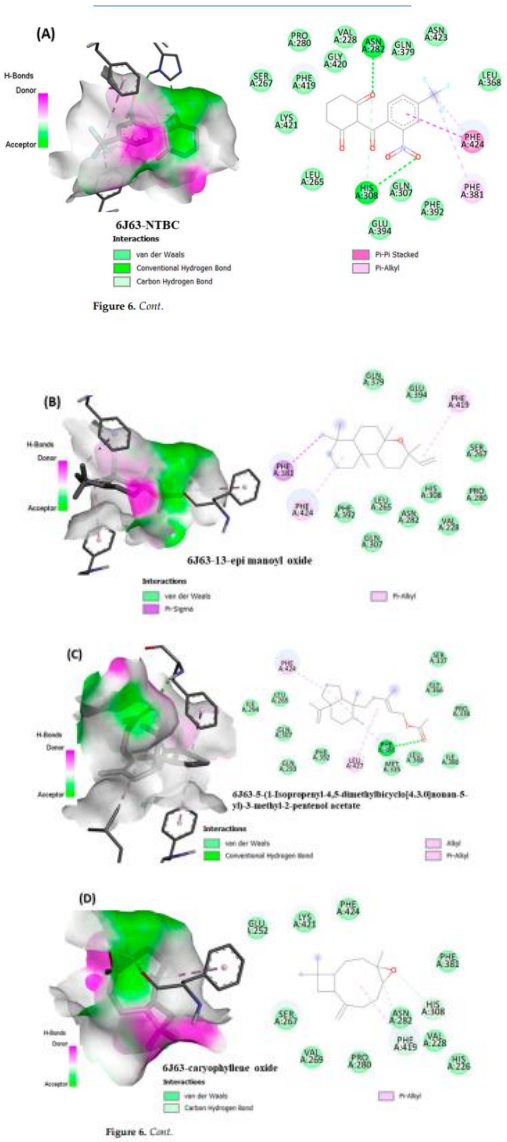



Figure 6. *Cont.*

Figure 6. *Cont.*

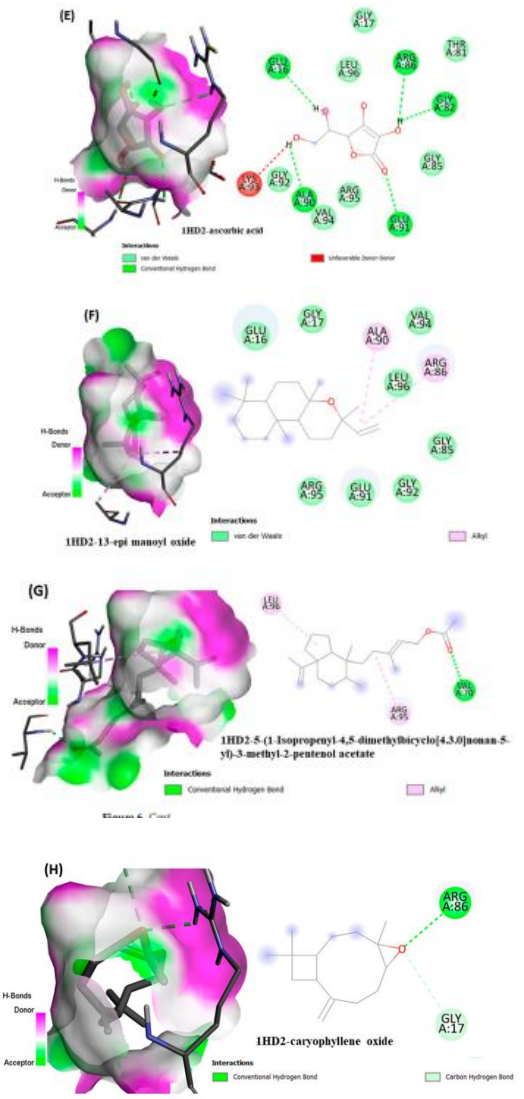
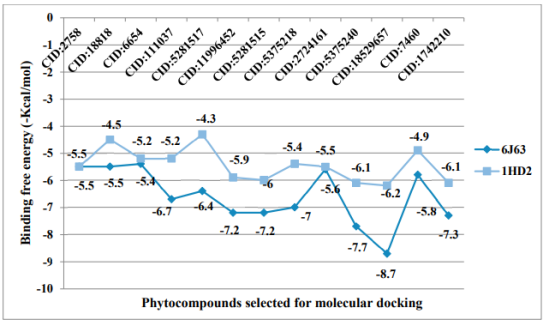



**Figure 6.** (**A**–**H**) Docked conformations of molecules in the binding cavity of HPPD (PDB: 6J63) and human periredoxin 5 (PDB: 1HD2) with least binding energies. The complex established are (**A**) 6J63-NTBC, (**B**) 6J63-13-*epi*-manoyl oxide, (**C**) 6J63-5-(1-Isopropenyl-4,5-dimethylbicyclo [4.3.0]nonan-5-yl)-3-methyl-2-pentenol acetate, (**D**) 6J63-caryophyllene oxide, (**E**) 1HD2-ascorbic acid; (**F**) 1HD2-13-*epi*-manoyl oxide, (**G**) 1HD2-5-(1-Isopropenyl-4,5-dimethylbicyclo [4.3.0]nonan-5-yl)-3-methyl-2-pentenol acetate, (**H**) 1HD2-caryophyllene oxide.




It was shown that some drugs mostly interacted with two isoenzymes belonging to the cytochrome (CYP) family, namely CYP2C19 and CYP2C9, which indicates that these compounds are effective while exhibiting a low level of toxicity. Additionally, the boiled-egg prediction (Figure 8) and the bioavailability radar graph (Figure 9) were used to illustrate the drug-like qualities and gastrointestinal absorption of certain compounds derived from VAO, VNO, and VTO products. The bioavailability radar graphs reveal that the compounds that are present in the yellow zone of the boiled-egg graph are capable of passing across the blood–brain barrier (BBB), and the pink region of the graphs demonstrates that the compounds have the characteristics of drugs.

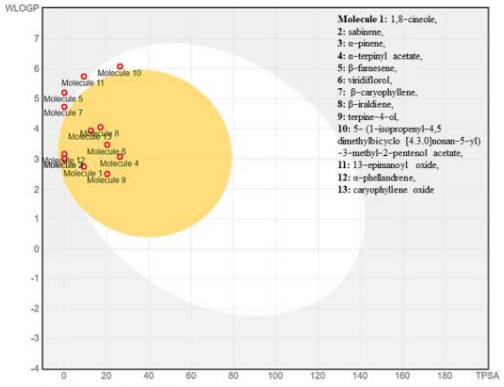



**Figure 8.** Boiled-egg graph of the selected phytoconstituents.

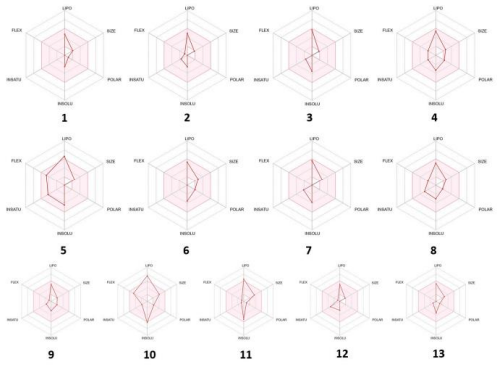



Figure 9. The drug-like characteristics of chosen compounds were shown in the pink region of the bioavailability radar for selected phytoconstituents. sabinene, 1,8-cineole, and 1,8-cineole Three: α-pinene, four: α-terpinyl acetate, five: β-farnesene, six with viridiflorol, seven with β-caryophyllene, and eight with β-iraldiene

terpine, ninefour-ol, 10-(1-isopropenyl4,5-dimethylbicyclo[4.3.0]nonan-5-yl) is the chemical formula.acetate derivative of 3-methyl-2-pentenol, 13-epimanoyl oxide, α-phellandrene, and caryophyllene oxide are the first three compounds.

Table 5. In silico ADMET analysis of major constituents of VAO, VNO, and VTO

| Entry | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TPSA ( Å²) | 9.23 | 0.00 | 0.00 | 26.30 | 0.00 | 20.23 | 0.00 | 17.07 | 20.23 | 26.30 | 9.23 | 0.00 | 12.53 |
| Consensus ᵃ Log Po/w | 2.67 | 3.25 | 3.44 | 3.06 | 4.97 | 3.42 | 4.24 | 3.56 | 2.60 | 4.25 | 5.14 | 2.97 | 3.68 |
| Mol wt (g/mol) | 154.25 | 136.23 | 136.23 | 196.29 | 204.35 | 222.37 | 204.35 | 206.32 | 154.25 | 332.52 | 293.48 | 136.23 | 220.35 |
| nRB | 0 | 1 | 0 | 3 | 2 | 0 | 0 | 3 | 1 | 7 | 1 | 1 | 0 |
| nOHA | 1 | 0 | 0 | 2 | 0 | 1 | 0 | 1 | 1 | 2 | 1 | 0 | 1 |
| nOND | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| WLOGP | 2.74 | 3.00 | 3.00 | 3.07 | 5.20 | 3.47 | 4.73 | 4.05 | 3.50 | 6.07 | 5.74 | 3.36 | 3.94 |
| Water solubility | Soluble | Soluble | Soluble | Soluble | Soluble | Soluble | Soluble | Soluble | Soluble | Moderately | Moderately | Soluble | Soluble |
| GI absorption ** | High | Low | Low | High | Low | High | Low | High | High | High | Low | Low | High |
| BBB permeant ** | Yes | Yes | Yes | Yes | No | Yes | No | Yes | Yes | No | No | Yes | Yes |
| P-gp substrate ** | No | No | No | No | No | No | No | No | No | No | No | No | No |
| CYP1A2 inhibitor ** | No | No | No | No | Yes | No | No | No | No | No | No | No | No |
| CYP2C19 inhibitor ** | No | No | No | No | No | Yes | Yes | No | No | Yes | Yes | No | Yes |
| CYP2C9 inhibitor ** | No | No | Yes | Yes | Yes | No | Yes | No | No | Yes | Yes | No | Yes |
| CYP2D6 inhibitor ** | No | No | No | No | No | No | No | No | No | No | No | No | No |
| CYP3A4 inhibitor ** | No | No | No | No | No | No | No | No | No | No | No | No | No |
| Log $K_p$ (cm/s) (Skin permeation) | −5.30 | −4.94 | −3.95 | −4.69 | −3.27 | −5.00 | −4.44 | −5.16 | −4.93 | −2.97 | −3.86 | −4.85 | −5.12 |
| Lipinski *** | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| Lipinski violation | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 |
| Bioavailability score *** | 0.55 | 0.55 | 0.55 | 0.55 | 0.55 | 0.55 | 0.55 | 0.55 | 0.55 | 0.55 | 0.55 | 0.55 | 0.55 |

Table 6. Toxicological properties of selected compounds from VAO, VNO, and VTO

| Compounds | Hepatotoxicity | | Carcinogenicity | | Cytotoxicity | | Immunotoxicity | | Mutagenicity | | Predicted $LD_{50}$ (mg/kg) | Toxicity Class |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pr | Pb | Pr | Pb | Pr | Pb | Pr | Pb | Pr | Pb | | |
| 1,8-Cineole | NH | 0.86 | NC | 0.68 | NCy | 0.75 | NI | 0.99 | NM | 0.96 | 2480 | V |
| Sabinene | NH | 0.81 | NC | 0.59 | NCy | 0.71 | NI | 0.51 | NM | 0.82 | 5000 | V |
| α-Pinene | NH | 0.86 | NC | 0.60 | NCy | 0.75 | NI | 0.99 | NM | 0.93 | 3700 | V |

| Compounds | Hepatotoxicity | | Carcinogenicity | | Cytotoxicity | | Immunotoxicity | | Mutagenicity | | Predicted $LD_{50}$ (mg/kg) | Toxicity Class |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pr | Pb | Pr | Pb | Pr | Pb | Pr | Pb | Pr | Pb | | |
| α-Terpinyl acetate | H | 0.53 | NC | 0.66 | NCy | 0.80 | NI | 0.97 | NM | 0.94 | 4800 | V |
| β-Farnesene | NH | 0.79 | NC | 0.73 | NCy | 0.81 | NI | 0.99 | NM | 0.98 | 5000 | V |
| Viridiflorol | NH | 0.77 | NC | 0.69 | NCy | 0.89 | NI | 0.87 | NM | 0.75 | 2000 | IV |
| β-Caryophyllene | NH | 0.80 | NC | 0.70 | NCy | 0.75 | I | 0.54 | NM | 0.95 | 5300 | V |
| β-Bisabolene | NH | 0.68 | NC | 0.79 | NCy | 0.78 | NI | 0.97 | NM | 0.93 | 4590 | V |
| Terpinen-4-ol | NH | 0.80 | NC | 0.72 | NCy | 0.88 | NI | 0.99 | NM | 0.85 | 1016 | IV |
| 5-(1-isopropenyl-4,5-dimethylbicyclo[4.3.0]nonan-5-yl)-3-methyl-2-pentenol acetate | NH | 0.68 | C | 0.58 | NCy | 0.76 | NI | 0.89 | NM | 0.87 | 5000 | V |
| 13-epi manoyl oxide | NH | 0.86 | NC | 0.69 | NCy | 0.75 | NI | 0.71 | NM | 0.91 | 4300 | V |
| α-Phellandrene | NH | 0.83 | NC | 0.52 | NCy | 0.80 | NI | 0.88 | NM | 0.92 | 5700 | VI |
| Caryophyllene oxide | NH | 0.80 | NC | 0.57 | NCy | 0.79 | I | 0.83 | NM | 0.88 | 5000 | V |

## 4. Conclusions

Within the scope of this research, the chemical variety of the essential oils (EOs) that were obtained from three different species of Vitex that were discovered in the Tarai region of India was investigated and disclosed. The chemical composition of essential oils was characterised by the high quantity of terpenoids that they contained. In addition, the antioxidant and phytotoxic activities of the essential oils were investigated in vitro in order to evaluate the biological potential of the plant-derived products that were obtained from these Vitex species. The essential oils that were tested shown moderate to excellent potentials for antioxidants and phytotoxicity, according to a number of different tests. The molecular docking research claimed that the compounds derived from essential oils (EOs) have the potential to be efficient phytotoxic and antioxidant agents. This conclusion was reached based on the examination of ligand interaction with proteins. According to the findings of the ADMET research, the vast majority of the primary compounds that are contained in essential oils are safe to use. All things considered, our analysis uncovered some fascinating biological activities of these essential oils, notably as natural antioxidants and phytotoxic agents, which lends credence to the use of the plant species in both the protection of crops and in traditional medicine. It is necessary to do in vivo research in order to investigate and assess the effectiveness and safety of these essential oils and the active components that they contain.

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