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Research Article

# ANALYSIS OF BIOLOGICAL ACTIVITY AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY STUDY OF CONVENTIONAL EXTRACTION OF *VITEX NEGUNDO* LINN. LEAVES

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**ABSTRACT**

**Objective:** The main objective of the present work is to carry out the biological activity, gas chromatography-mass spectrometry (GC-MS) studies for the possible compounds present in *Vitex negundo*.

**Methods:** The aqueous extract of *V. negundo* Linn. was screened for biological activities such as antimicrobial, antituberculosis (TB), antimalarial, and antioxidant activities. The GC-MS analysis was carried out.

**Results:** The result shows that leaf extract is effective against *Escherichia Coli* and *Bacillus subtitus* while negative results for anti-TB and anti-malarial activity. The antioxidant activity of the leave extract is excellent.

**Conclusion:** The compounds present in the leaf extract of *V. negundo* are responsible for possessing the biological activity.

**Keywords:** *Vitex negundo*, Antioxidant activity, Gas chromatography-mass spectrometry study, Aqueous extraction.

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## INTRODUCTION

*Vitex negundo* Linn (Synonym: Vitex incise Linn, Vitex incise Lam varhetropylla family: Verbenaceae) is commonly known as nirgudi in India. [1]. Roots, fruits, flowers, leaves, and bark of *V. negundo* have great medicinal value and used for insect repellent purpose [2,3]. The leaves of *V. negundo* burned to repel mosquitoes from houses [4]. The leaf extract of *V. negundo* has anticancerous activity [5], it is also used in the treatment of dengue, rheumatism, dyspepsia, diarrhea [6], anti-inflammatory, antimicrobial, antifungal [7], wound healing potential [8,9], analgesic, antifertility [10], antioxidant, and anti-hyperglycemic activities [11]. Adnaik *et al*. [12] reported the laxative activity of the aqueous extract of *V. negundo* leaves. Antitumor activity was proved by Dewade *et al*. [13]. Phytoconstituents play an important role for many activities in a plant. Thombre *et al* [14] reported the phytoconstituents present in the *V. negundo* and also reported the presence of alkaloids, tannins, steroids, triterpenoids, saponin, protein, and reducing sugars from the ethanol, methanol, acetone, and aqueous extract of *V. negundo* from Pune region.

From the literature survey, it shows the medicinal importance of *V. negundo* Linn. from the different regions of the world. That is why we select the *V. negundo* Linn. plant leaves to give the evidence of some of activities from the Aurangabad region.

## METHODS

The leaf sample of the plant was collected from Vasantrao Naik College Campus, Aurangabad. The plant is identified as *V. negundo* Linn. and index in herbarium Dr. Bamu. The leaves were washed properly and dried under shade for 6 days. The dried leaves were grind using kitchen grinder to fine powder.

### Water extraction

Accurately weight 30 g of sample was introduced into the 500 ml round bottom flask (which was first clean by very dilute hydrochloric acid and then distilled water) with 300 ml double-distilled water. The sample was refluxed on flame for 6 h. The sample was cooled and filtered. The excessive water was evaporated for the preservation of the sample, and it was kept at 4°C for 12 h.

### Antioxidant property

Antioxidant properties of the samples were measured by 2, 2-diphenyl picrylhydrazyl (DPPH) method [15]. It is very stable free radical commercially available. DPPH reacts with antioxidant molecules present in the herbal extract and converts into diphenyl hydrazine. The intensity was measured at 517 nm. The different concentrations of the extract were prepared. To that 1 ml of extract, 1 ml of DPPH and 3 ml of ethanol were added. Moreover, the intensity of the color was measured at 517 nm. The DPPH radical scavenging capacity was calculated as the percentage inhibition.

$$\% \text{ Inhibition of DPPH radical} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

### Antimicrobial study

With the help of sterile wire loop, the test was inoculated into a test tube containing Mueller Hinton broth. The optical density of the inoculums was adjusted in between 0.08 and 0.1. As per the composition, Mueller Hinton Agar was prepared using sterile distilled water and was sterilized at 121°C at 15 lb pressure for 15 min in an autoclave. The medium was cooled at room temperature and poured in sterile Petri plates and was allowed to solidify. Bacterial inoculums were swabbed over the medium using sterile cotton swab. Sterile disc was placed on medium, on which 20 µl of complex suspension was added. Zone of inhibition was observed and measured after incubation at 30°C for 18–20 h.

### Gas chromatography-mass spectrometry (GC-MS)

The GC-MS analysis of the plant extract was made in a QP 2010 Plus SHIMADZU instrument under computer control at 70 eV. About 1 µL of the water extract was injected into the GC-MS using a microsyringe and

scanning was done for 45 min, in which helium is used as the carrier gas. As the compounds were separated, they eluted from the column and entered a detector which was capable of creating an electronic signal whenever the compound was detected. The greater the concentration in the sample, bigger was the signal obtained which was then processed by the computer. The time from when the injection was made (initial time) to when elution occurred is referred to as the retention time (RT). When the instrument was run, the computer-generated graph from the signal called a chromatogram [16].

## RESULTS AND DISCUSSION

In the present study, *V. negundo* Linn. leaves were tested for the analysis of antioxidant property and antimicrobial study with the standard protocol.

### Evaluation of antioxidant activity

In a healthy person, natural oxidative defence system continuously balanced the production of free radicals [17]. Butylated hydroxy toluene and butylated hydroxyl anisole are the examples of synthetic antioxidants, which need to be replaced by natural antioxidants. Because they found to be toxic and carcinogenic in animal models [18]. The plants are a source of certain bioactive molecule which act as antioxidants. There are so many procedures to determine the antioxidant activity such as DPPH, ABTS, and FRAP [19]. Gupta *et al.* [20] reported that phytoconstituents are responsible for the antioxidant property of *Terminalia bellirica* Roxb. ethanolic extract.

In the present study, DPPH method is used to determine the antioxidant activity of *V. negundo* Linn. extract which results as the % inhibition of extract increases with increase in concentration. The $IC_{50}$ is 5.77. The result is shown in Table 1. The $IC_{50}$ value of standard ascorbic acid was found to be 14.97, whereas the plant extract shows the $IC_{50}$ as 5.77. The low value of $IC_{50}$ indicates that plant extract has more potent antioxidant activity. The percentage inhibition in both ascorbic acid and plant extracts increases linearly with concentration.

### Antimicrobial study

The extract of *V. negundo* Linn. leaves was active against *Escherichia coli* and *Bacillus subtilis* bacteria and inactive against *Salmonella typhi* and *Staphylococcus aureus*. It does not give any positive result about tuberculosis (TB) and Malaria. It does not show activity against TB and malarial parasites. It may be due the decomposition of the molecule due the continues heat (Table 2).

### GC-MS

Amala and Jeyraj [21] showed the possible compounds present in triphala by GC-MS analysis. 22 peaks are observed in GC-MS study of *V. negundo* Linn. leaf extract. Four peaks are having large % area (Table 3). Library search at respective RT is shown in Table 4. It is clear that these peaks are for the phytoconstituents present in the plant which are responsible to possess many activities [22]. In the present study, there are four major peaks observed at 13.365, 15.179, 15.264, and 15.458 RT (Fig. 1)

The present study proved that hydro extract of *V. negundo* Linn. possesses good antioxidant property. This may be due to the presence of that n-hexadecanoic acid and hexadecanoic acid, ethyl ester. 9,12-octadecodienoic acid (Z,Z) shows anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, and anti-coronary activity [23]. Pentadecanoic acid-14-methyl, methyl ester shows antifungal and antimicrobial activity [24]. 9,12,15-octadecatrienoic acid, methyl ester shows the activity against several bacteria [25] such as *E. coli* and *B. subtilis*.

**Table 1: Antioxidant activity of *Vitex negundo* Linn. leaf extract**

| S. No. | Concentration (µg/ml) | % Inhibition ascorbic acid | % Inhibition extract |
|---|---|---|---|
| 1. | 5 | 40.48 | 49.33 |
| 2. | 10 | 45.09 | 53.81 |
| 3. | 20 | 55.15 | 54.06 |
| 4. | 30 | 59.39 | 54.66 |
| 5. | 40 | 67.15 | 56.72 |
| 6. | 50 | 68.00 | 60.24 |
| IC50 value (µg/ml) | | 14.97 | 05.77 |

*V. negundo: Vitex negundo*

## CONCLUSION

The plant extract of *V. negundo* Linn. possesses excellent antioxidant activity and antimicrobial activity against *E. coli* and *B. subtilis*. It shows negative results as anti-TB and antimalarial agents. The GC-MS chromatogram gives 22 peaks at different RTs. The library search reveals the presence of hexadecanoic acid methyl ester, pentadecanoic acid 14-methyl-methyl ester, heptadecanoic acid methyl ester, 9,12-octadecadienoic acid, methyl ester, octadecadienoic acid, methyl ester, 11,14-eicosadienoic acid, 9,12,15-octadecatrienoic acid, methyl ester, 11,14,17-eicosatrienoic acid, methyl ester, 9,12,15-octadecatrien-1-ol, (Z,Z,Z), nonadecanoic acid, methyl ester, hexadecanoic acid, 15-methyl, methyl ester, heptadecanoic acid, 16-methyl, and methyl ester.

## ACKNOWLEDGMENT

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**Table 2: Biological activity of *V. negundo* Linn. leaf extract**

| S. No. | Activity | Result |
|---|---|---|
| 1. | *E. coli* | Highly active |
| 2. | *B. subtilis* | Active |
| 3. | *S. typhi* | Inactive |
| 4. | *S. aureus* | Inactive |
| 5. | TB | -- |
| 6. | Malaria | -- |

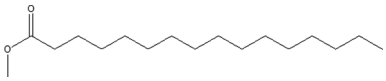
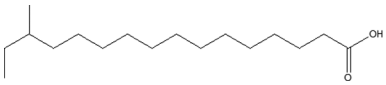
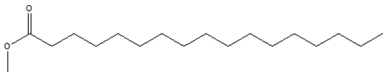
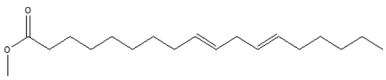
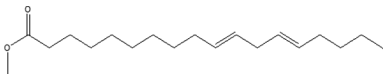
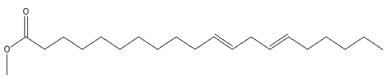
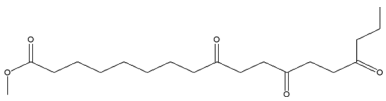
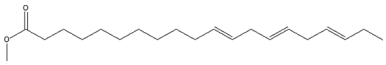
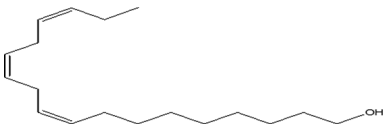
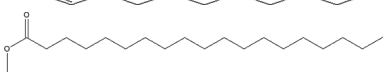
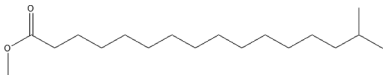
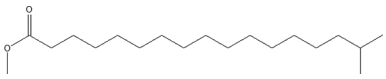
*E. coli: Escherichia coli, B. subtilis: Bacillus subtilis, Salmonella typhi: Salmonella typhi, S. aureus: Staphylococcus aureus, V. negundo Linn.: Vitex negundo* Linn., TB: Tuberculosis

**Table 3: Retention time and the peak area of the leaves of *V. negundo* plant by GC-MS**

| S. No. | Retention time | Peak area (%) |
|---|---|---|
| 1. | 8.046 | 1.13 |
| 2. | 8.967 | 1.89 |
| 3. | 9.680 | 1.64 |
| 4. | 10.091 | 1.21 |
| 5. | 10.746 | 1.26 |
| 6. | 11.362 | 0.22 |
| 7. | 12.444 | 2.12 |
| 8. | 12891 | 1.36 |
| 9. | 13.303 | 3.10 |
| 10. | 13.365 | 20.10 |
| 11. | 13.766 | 0.36 |
| 12. | 14.196 | 0.47 |
| 13. | 14.438 | 0.53 |
| 14. | 15.179 | 7.16 |
| 15. | 15.264 | 30.57 |
| 16. | 15.367 | 2.70 |
| 17. | 15.458 | 9.18 |
| 18. | 15.649 | 1.53 |
| 19. | 16.264 | 2.44 |
| 20. | 17.249 | 2.33 |
| 21. | 17.689 | 0.77 |
| 22. | 18.104 | 1.32 |

*V. negundo* Linn.: *Vitex negundo* Linn., GC-MS: Gas chromatography-mass spectrometry

**Table 4: Possible compounds present in extract as per of GC-MS analysis**

| S. No. | Retention time (min) | Structure | Molecular weight/name |
|---|---|---|---|
| 1. | 13.365 |  | Mol. wt. 270<br>MF: $C_{17}H_{34}O_2$<br>Hexadecanoic acid methyl ester |
| | |  | Mol. wt. 270<br>MF: $C_{17}H_{34}O_2$<br>Pentadecanoic acid 14-methyl-methyl ester |
| | |  | Mol. wt. 284<br>MF: $C_{18}H_{36}O_2$<br>Heptadecanoic acid methyl ester |
| 2. | 15.179 |  | Mol. wt. 294<br>MF: $C_{19}H_{34}O_2$<br>9,12-Octadecadienoic acid, methyl ester |
| | |  | Mol. wt. 294<br>MF: $C_{19}H_{34}O_2$<br>10,13-Octadecadienoic acid, methyl ester |
| | |  | Mol. wt. 322<br>MF: $C_{21}H_{38}O_2$<br>11,14-Eicosadienoic acid |
| 3. | 15.264 |  | Mol. wt. 292<br>MF: $C_{19}H_{32}O_2$<br>9,12,15-Octadecatrienoic acid, methyl ester. |
| | |  | Mol. wt. 320<br>MF: $C_{21}H_{36}O_2$<br>11,14,17-Eicostrienoic acid, methyl ester |
| | |  | Mol. wt. 264<br>MF: $C_{18}H_{32}O$<br>9,12,15-octadecatrien-1-ol, (Z, Z, Z) |
| 4. | 15.458 |  | Mol. wt. 312<br>MF: $C_{20}H_{40}O_2$<br>Nonadecanoic acid, methyl ester |
| | |  | Mol. wt. 284<br>MF: $C_{18}H_{36}O_2$<br>Hexadecanoic acid, 15-methyl, methyl ester |
| | |  | Mol. wt. 298<br>MF: $C_{19}H_{38}O_2$<br>Heptadecanoic acid, 16-methyl, methyl ester |

GC-MS: Gas chromatography-mass spectrometry

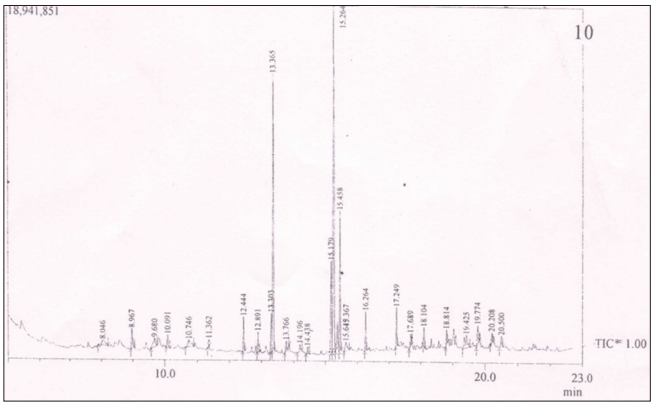



**Fig. 1: Gas chromatography-mass spectrometry chromatogram of *Vitex negundo* aqueous extract**

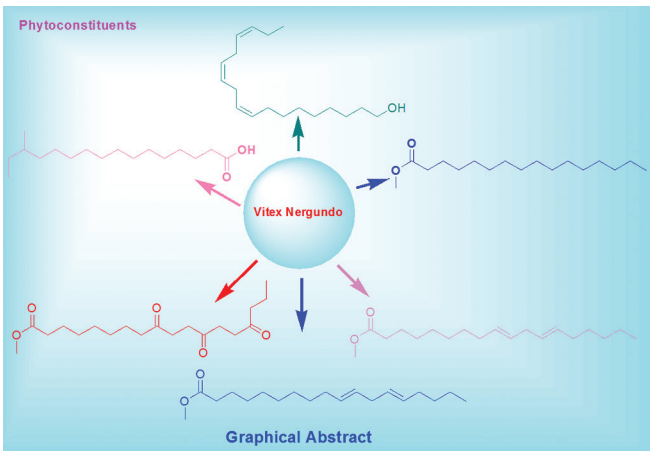
## AUTHORS' CONTRIBUTIONS

Samreen Fatema Research Scholar has majorly performed the experiments in the laboratory. Milind Ubale has provided GC-MS facility and interpretation of data. Mazahar Farooqui has provided help in preparing manuscript, refining, and proofreading of the manuscript and discussion regarding experiment process. Pathan Mohd Arif is research guide and provided the design and content protocol for conducting experiment.

## CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this article.

## Graphical abstract

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