GC-MS Analysis of phytochemical constituents in stem bark extract of *Vitex negundo* [L.]

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**A R T I C L E  I N F O**

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- GC-MS analysis, Bioactive compounds, *Vitex negundo*, Stem bark, Methanol extract.

**A B S T R A C T**

The present effort was intended to investigate *Vitex negundo* [L.] for phytochemical compounds and characterize the chemical constituent of plant using GC-MS. The shade dried stem bark powder *Vitex negundo* [L.] was extracted with methanol, ethanol overnight and filtered and concentrated. The SHIMADZU QP-2010 GC used in the investigation employed a column packed with Elite-5MS (5% Diphenyl / 95 % Dimethyl poly siloxane, 30mm×0.25mm × 0.25µm df ) and the components were separated using Helium [1ML/min]as the carrier gas. The GC-MS analysis provided peaks determining the presence of eleven different phytochemical compounds. The presence of various bioactive compounds proves the purpose of *Vitex negundo* [L.] for various disorders. However, seclusion of individual phytochemical constituents may proceed to find an innovative drug.

**Introduction**

India is one of the largest producers of herbs and herbal products. The large resources of vegetable mineral and animal kingdom have been used continuously for the treatment of various diseases and other related problems. Herbal medicines are prepared from various plant parts like leaves, stems, roots, barks, rhizomes and seeds, which usually contain many bioactive compounds and used primarily for mild or chronic ailments. Increasing demand of herbal medicines, there is urgent need for application of this knowledge in authentication, detailed study and practical utilization of crude drugs (Kishor Kumar et al., 2012).

*Vitex negundo* [L.] (Verbenaceae) is a traditionally valuable plant. It is a large shrub or small tree; stem and branches obtusely 4 angled, white, gray or purple pubescent, stem bark thin, yellowish grey. Leaves 3-5 foliolat; leaflets lanceolate, apex acute, glabrous above, covered with a fine white tomentum beneath, base acute. Flowers in pedunculate branched tomentose. Cymes, opposite along the quadrangular tomentose rachis of a large terminal offen compound pyramidal panicle; bracts lanceolate, caducous. Flowers fragrant; calyx campanulate, 5 toothed ; teeths acute; corolla 5 lobed, 2- lipped, blue with pink tinge or purple; stamens 4, didy namous; filaments slender, exserted, white or purple, drupe globose, (Bhagat et al., 2008).

The genus consists of 250 species of which about 14 Species are found in India and some have commercial medicinal importance. *Vitex negundo* [L.] commonly known as five-leaf chaste tree or Monk’s pepper [Hindi Sambhalu, Telugu-Vavili, Tamil-Nironchi, Sanskrit – Nirgundi] is used as medicine fairly throughout the greater part of India (Chopra et al., 1956). Stem bark is useful in odontalgia, verminosis and Ophthalmopathy (Vishal Tandan et al., 2005). Previous studies on stem barks of *Vitex negundo* [L.] have resulted in the isolation of many terpenes, sterols, phenolic, compounds, flavonoids, alkaloids, organic acids, glycosides and anthocyanines (Zaware et al., 2010). Reported actions are astringent, resolvent, demulcent, deobstruent and expectorant (Anonymous, 1987). There is no extensive report of chemical constituents isolated from this plant. The objective of this study was evaluate the phytochemical compounds [quantitative method] using GS-MS analysis. Reported chemical compounds are P-Hydroxy benzoic acid and â-sitosterol (Ramchandra Dhakal et al., 2008).

**Material and Methods**

**Collection and Preparation of Powder:**

The *Vitex negundo* [L.] stem bark was collected from the natural habitats of Nalgonda District, Telangana state, India, in the month of December 2014. Collected plants specimens were identified taxonomically with the help of the local floras. The plant was authenticated by Prof. Ramachandra Reddy, Taxonomy and Anatomy Laboratory, Department of Botany, Osmania University, Hyderabad. Voucher specimens were deposited at the

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Figure - 1A: GC-MS chromatogram of the methanol extract of stem bark of *Vitex negundo* [L.]

Figure - 1B.1: Mass spectrum of showing Hepta methyl–phenyl-cyclo tetra siloxane

Figure - 1B.2: Mass spectrum of showing Cyclo heptasiloxane, tetra decamethyl

Figure - 1B.3: Mass spectrum of showing Nona methyl, phenyl-cylopenta siloxane

Figure - 1B.4: Mass spectrum of showing Cyclo octa siloxane, hexadeca methyl
Figure - 1B.5: Mass spectrum of showing Borazine, 2, 4, 6 triphenyl1, 3, 5-tryophl

Figure - 1B.6: Mass spectrum of showing Nonamethyl, phenyl-cyclopenta siloxane

Figure - 1B.7: Mass spectrum of showing Trtracosamethylyclododeca siloxane

Figure - 1B.8: Mass spectrum of showing Penta methyl phenyl-Disilane

Figure - 1B.9: Mass spectrum of showing Heptasiloxane 1,1,3,3,5,5,7,7,9,9,11,11,13,13,-tetra deca methyl
Table - 1: Components detected in the stem bark of methanol extract of *Vintex negundo* [L.]

<table>
<thead>
<tr>
<th>S.NO.</th>
<th>RT</th>
<th>Name of the component</th>
<th>Molecular formula</th>
<th>MW</th>
<th>Peak %</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.960</td>
<td>Hepta methyl –phenyl-cyclotetra siloxane</td>
<td>C_{13}H_{26}O_{4}Si_{4}</td>
<td>358</td>
<td>0.60</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>9.513</td>
<td>Cyclo heptasiloxane,tetra decamethyl</td>
<td>C_{14}H_{42}O_{7}Si_{17}</td>
<td>518</td>
<td>4.56</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>11.325</td>
<td>Nona methyl, phenyl-cylopenta siloxane</td>
<td>C_{15}H_{32}O_{5}Si_{5}</td>
<td>432</td>
<td>0.21</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>22.696</td>
<td>Cyclo octa siloxane,hexadeca methyl</td>
<td>C_{16}H_{48}O_{8}Si_{8}</td>
<td>592</td>
<td>2.63</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>17.709</td>
<td>Borazine, 2, 4, 6 triphenyl1, 3, 5-tryophl</td>
<td>C_{27}H_{36}B_{3}N_{3}</td>
<td>435</td>
<td>34.18</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>20.420</td>
<td>Nonamethyl, phenyl-cyclopenta siloxane</td>
<td>C_{15}H_{32}O_{5}Si_{5}</td>
<td>432</td>
<td>17.57</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>22.696</td>
<td>Trtrcosamethylcyclododeca siloxane</td>
<td>C_{25}H_{52}O_{12}Si_{12}</td>
<td>888</td>
<td>1.74</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>23.450</td>
<td>Penta methyl phenyl-Disilane</td>
<td>C_{11}H_{30}Si_{2}</td>
<td>208</td>
<td>4.91</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>31.543</td>
<td>Heptasiloxane 1,1,3,3,5,5,7,7,9,9,11,11,13,13,-tetra deca methyl</td>
<td>C_{14}H_{44}O_{6}Si_{7}</td>
<td>504</td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>33.076</td>
<td>3a, 3a-Dichloro-2 alpha, 3 alpha-ethano-3beta-methyl-cholestan, 2a-one</td>
<td>C_{30}H_{48}C_{2}O</td>
<td>494</td>
<td>0.39</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>41.412</td>
<td>Octadecamethyl,cyclonona siloxanes Cyclo octa siloxane,hexadeca methyl</td>
<td>C_{18}H_{54}Si_{9}</td>
<td>666</td>
<td>3.82</td>
<td></td>
</tr>
</tbody>
</table>
The stem bark samples were washed thoroughly in running tap water to remove soil particles and finally washed with sterile distilled water. Stem bark pieces were shade dried and ground in to fine powder. The powdered materials were stored in air tight polythene bags until use. 25gm of the powdered stem bark was soaked in 95% methanol for 12 hrs. The extract was then filtered through Whatman filter paper no. 41 and the filtrate was concentrated through the rota vapour. One gram of extract [powder] diluted with 10 ml methanol and filtered. 2µl sample of the solution was employed in GS-MS for analysis of different compound (Merlin et al., 2009).

**GC-MS Analysis**

GC-MS analyses of methanol extract were performed using a SHIMADZU QP-2010 Gas-Chromatography – Mass spectroscopy. It employed a fused silica column packed with Elite -5 ms [5% Diphenyl 95% Dimethyl poly siloxane, 30 mm × 0.25 mm × 0.25 µm df] and the components were separated using helium as carrier gas at a constant flow of 1ml / min. The 2 µl sample extract injected in to the instrument. It was detected by the turbo mass detector with aid of Turbo mass 5.2 software. During the GC Process the oven was maintained at temperature of 110°C with 2 min holding. The injector temperature was set at 250°C.

The inlet line temperature was 200°C and source temperature was 200°C. Mass spectra were taken at 70 eV, a scan period of 0.5 S and fragment from 45 - 450 Da. The MS detection was completed in 36 min.

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute standard and technology[NIST] having more than 62,000 patterns. The spectrum of unknown components stored in the NIST library. The Name, molecular weight and structure of the components of the test materials were ascertained (Merlin et al., 2009).

**Results and Discussion**

The consequences concerning to GC-MS investigation led to the recognition of lot of compounds from the GC fractions of the methanolic extract of *Vitex negundo* [L.] stem bark. These compounds were acknowledged through mass spectrum attached with GC. The active principles with their retention time [RT], molecular formula (MF), Molecular weight (MW) and concentration (%) are accessible in Table 1.

The GC-MS chromatogram of the 11 peaks of the compounds detected was shown in figure -1stembark showed the presence of major peaks and the components corresponding to the peaks were determined as follows. The results revealed that Borazine, 2, 4, 6-triphenyl1, 3, 5-tripropyl[(C₆H₅)₂B(N)] peak area% 34.18 was found as the one major component in the methanol extract and the ten minor components such are. [1]. Hepta methyl – phenyl-cyclotetra siloxane (C₆H₅O₅Si₄) peak area% 0.60. [2]. Cyclo, heptasiloxane,tetra decamethyl (C₁₂H₂₄O₈Si₄) peak area % 4.56 [3]. Nonamethyl, phenyl-cyclopenta siloxane (C₁₀H₁₅O₅Si₃) peak area%17.17. [4]. Cyclo octa siloxane, hexadeca methyl (C₆H₁₄O₂Si₈) Peak area% 2.63. [5]. Borazine, 2, 4, 6-triphenyl1, 3, 5-tri(propyl[(C₆H₅)₂B(N)] peak area % 34.18. [6]. Nona methyl, phenyl-cyclopenta siloxane (C₆H₅O₅Si₃) peak area % 0.21. [7]. Tetracosa methyl cyclodecac siloxane (C₂₄H₄₀O₁₂Si₁₂) peak area % 1.74. [8]. Penta methyl phenyl-Disilane (C₅H₅O₂Si₂) peak area% 4.91. [9]. Heptasiloxane 1, 1, 3, 3, 5, 7, 7, 9, 9, 11, 11, 13, 13-tetra deca methyl (C₁₃H₂₄O₂Si₈) peak area % 0.98. [10]. 3a,3a-Dichloro-2 alpha, 3,4,5 alpha-ethano-3 beta-methyl-cholestan, 2a-one (C₁₅H₂₀O₂O) peak area % 0.39. [11]. Octadecamethyl, cyclononan siloxanes (C₁₈H₃₆Si₈) peak area % 3.82. Prevailing major chemical constituents are the spectrum profile of GS-MS confirmed the presence of 11 components with the retention time 7.960, 9.513, 11.325, 22.696, 17.709, 20.420, 22.696, 23.450, 31.543, 33.076 and 41.412 which shows in Table I.

**Conclusion**

Eleven chemical constituents have been identified from methanol extract of the stem bark of the *Vitex negundo* L. by GC-MS analysis. Occurrence of various bioactive compounds confirms the application of *Vitex negundo* L. stem bark for a variety of ailments, by traditional practitioners, a quantity of compounds has previously been reported from a number of other plant species. Thus the detection of a good number of compounds from *Vitex negundo* L. stem bark might have some biological connotation. Further research is in progress for isolation of individual phytochemical constituents which may act as templates for novel drug molecules.

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