IDENTIFICATION OF MEDICINAL ACTIVE COMPOUNDS AND QUANTIFICATION OF STRYCHNINE AND BRUCINE IN HYDRO ETHANOLIC EXTRACT OF STRYCHNOS NUX-VOMICA LEAVES

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ABSTRACT

In this study, we have analyzed the phytochemical composition of this plant. Purity and concentration of strychnine and brucine are determined. The identification of medicinal active compounds was done with GC-MS with NIST Library. Twenty one medicinal bio active compounds were identified from the Strychnos Nux-Vomica leaf ethanolic extract. Strychnine is showing 28.43% purity and brucine was not detected in GCMS analysis. Quantified the concentration of strychnine (0.6 mg in 500mg of extract) and brucine (1.6 mg in 500mg of extract) was done by HPLC against Strychnine and Brucine standard. These compounds are having natural properties of Anti-inflammatory, Hypocholesterole, Cancer preventive, Hepatoprotective, Antimicrobial, Antioxidant, Cardio protective, Antiaging, Antialzheimeran, Antidermatitic, Immunostimulant, Anthepatotoxic, biosynthesis of steroid hormones, Nematicide, Antiandrogenic, 5-alpha reductase inhibitor, antipsychotic, analgesic, apoptotic effect, antidepressant, antidote for snake poisoning and diabetic activity. From our observations, it was very clear that, this plant is a rich source of bioactive compounds.

INTRODUCTION:

The plants retain numerous pharmacological actions such as an antimicrobial, fungistatic and to reduce stress [1, 2]. The phytochemical studies and analysis statement shows that the medicinal plant main compounds are bioflavonoids, alkaloids (naturally occurring chemical compounds), poly hydroxy phenols, amphipathic glycosides and steroids (family of polycyclic chemical compounds) [3]. The Plants yield many secondary metabolites (organic compounds that are not directly involved in the normal growth, development, or reproduction of an organism), these alkaloids purpose as a actual chief class of protective compounds in human life. Plant compounds have biological activity that used in medications. Alkaloids are identical beneficial therapeutic compounds since of their natural actions [4, 5]. Strychnos Nux-vomica shown the occurrence of carbohydrate, alkaloid, tannin, steroid, triterpenoid and glycoside in the extract [6]. Strychnos Nux-vomica of the natural components of the plant is useful in the innovation of the therapeutic agent as well as
commercial ingredients like oil and gums. The most significant bioactive components of the plants are alkaloids, tannins, flavonoids and phenolic compounds. In India a huge number of plant species had selected for their pharmacological properties. Medicinal plants are of interest to the field of biotechnology, medicine industries depend for the production of therapeutic combinations [7]. Strychnos nux-vomica is a therapeutic plant distributed in India, Sri Lanka, Southeast Asia and Northern America. Usually seeds of S. nux-vomica are used for therapeutic ailments in antitumor, antimicrobial, ant convulsion, anti-amnesic and immunomodulatory effects [8]. Therapeutic and medicinal plants are widely used in the world for the treatment human illnesses from the earliest historical. In India approximately 47,000 herbal plants are identified in various regions, almost 8,000 kinds of plants have curative significant. Roughly 2,500 plants are identified in Indian for several medical applications [9]. Now a day’s medicinal plants are becoming extinct. In olden days plant materials are occurred from natural sources. India is having good number of therapeutic plants through which people are getting rid of their illness [10]. In Ayurveda Strychnos Nux vomica Linn is used in the treatment of several sicknesses and disorders [11, 12]. It is used for curing chicken pox fever and also used for snake bite [13].

Experimental
Plant Collection & authentication of Plant

Leaves of Strychnos Nux-vomica were used for investigation obtained from Nellore district, Andhra Pradesh, India. The plant was authenticated by Botanical survey of India, Coimbatore. Authentication number of plant is BSI/SRC/5/23/2013-14/Tech/682.

Extraction Procedure and preparation for GCMS and HPLC

Leaves of Strychnos Nux-vomica is washed with distilled water, shade dried, powered for the solvent extraction process. The crude extract was obtained by extracting 50 grams of dried plant powder in 200ml of 50% Ethanol in a water shaker for 72 hrs. Repeatedly solvent extraction was done with the same solvent till colour less solvent obtained. The hydro ethanolic plant extract was further concentrated by using Rota evaporator at 45-50°C. After concentration, the residue occurred was dissolved in methanol and analysis is carried out by using GC-MS, 500mg of plant extract taken into a 10ml of volumetric flask ,dissolved with methanol and used for HPLC analysis.

Mobile phase and Standard Preparation for HPLC

Composition of Mobile phase is used as Methanol, water and diethyl amine (55:45:0.2v/v) for HPLC. 10mg of strychnine and 10 mg of brucine taken into a 10ml of volumetric flask and dissolved with methanol and filtered with 0.22 micron filter.

Instrument, Column and Method of Gas Chromatography- Mass Spectrometry

Identification of medicinal active compounds was done by using Gas Chromatography- Mass Spectrometry. Shimadzu GC-MS was used for analysis, model-QP2010 and operating in Electron Impact Ionization mode at 70electron volts. A Restek-5MS column (30meters x 0.25milli meter x 0.25μicro meter) was used, Helium flow rate was kept 1mL/min for analysis.

Instrument, Column and Method of HPLC

Quantification of Strychnine and Brucine was done by using HPLC. Agilent HPLC was used for analysis, model-1100 with Chemstation software. A Phenomenex-ODS column (250mm x 46mm x 5μicro meter) was used, the Column flow rate was kept at 1mL/min, Detector at 260nm, Run time 15 minutes for analysis.

Formula for HPLC Calculation

The same formula used for quantification used for strychnine and brucine [14].
Identification of medicinal active compounds was done by using Gas Chromatography-Mass Spectrometry. Shimadzu GC-MS was used for analysis, model-QP2010 and operating in Electron Impact Ionization mode at 70 electron volts. A Restek-5MS column (30 meters x 0.25 millimeter x 0.25 micro meter) was used; Helium flow rate was kept 1 mL/min for analysis. The oven thermal program was initially kept at 50°C and hold for 5 min, rise 10°C per minute up to 150°C and hold for 8 min, rise 8°C per minute up to 250°C and hold for 2 min, rise 10°C per minute up to 280°C and hold for 29.5 min. Injector temperature was kept 280°C and injection volume was 2 μL. The EI Source temperature kept was 250°C, Quadrupole temperature kept was 150°C, Interface Temperature kept was 250°C, full scan mode, Scan range 40-600 m/z and solvent delay kept was 3 minutes.

Identification of components:

Identification of mass spectra of GC-MS was done by using the National Institute of Standards and Technology (NIST) library. Unknown components of the mass spectrum were compared with to the NIST library components and identified. The name, mass and structure of the compounds of the test sample were determined.

RESULTS AND DISCUSSION

In the present study these biological compounds were found with GCMS analysis of Strychnos nux-Vomica. These compounds are Megastigmatrienone, 1,3,4,5-

\[
\%c \text{ Estimation} = \frac{At \times Ds \times Ws}{As \times Dt \times Wt}
\]

At = Area count for sample solution.
As = Area count for standard solution.
Ds = Dilution factor for sample.
Dt = Dilution factor for standard.
Ws = Weight of standard (mg)
Wt = Weight of sample (mg)

Instrument, Column and Method of Gas Chromatography-Mass Spectrometry

Tetrahydroxy-Cyclo hexane carboxylic acid, Methyl-d-glucose, Hexadecanoic acid methyl ester, Cholest-5-EN-3-OL (3.Beta.), n-Hexadecanoic acid, 9,12,15-Octadecatrienoic acid methyl ester, 2-Hexadecen-1-OL, 1,4-Dioxaspiro[4.14]nonadecane, Hexadecanoic acid, 2,6,10,14,18,22-Tetracosahexaene, γ-Tocopherol, Vitamin E, Ergost-5-EN-3-OL, Strychnidin-10-ONE, Stigmast-5-EN-3-OL and Methyl Commate D (These are mentioned in Figure-1 for GCMS chromatogram, Table-1 for List of the Compounds and Table-2 for Structure of the compounds). Strychnine and brucine content was quantified in HPLC, these are 0.6 and 1.6 mg respectively in the 500mg of the extract. (These are mentioned in Figure-2 for HPLC Standard chromatogram and Figure-3 for HPLC Sample chromatogram).

The therapeutic properties of the significant component specify the veracity of the use of this medication. Megastigmatrienone is used as an Aroma compound and 3-O-Methyl-d-glucose is having Preservative properties. Hexadecanoic acid methyl ester is used in Antioxidant, Flavor, Hypo cholesterolmic Pesticide and 5-Alpha reductase inhibitor treatments. 9, 12, 15-Octadecatrienoic acid methyl ester are reports in Anti-inflammatory, Hypocholesterolemic, Cancer preventive and Hepatoprotective. 2-Hexadecen-1-OL showed the Antimicrobial, Anticancer and Anti-inflammatory activity. 2, 6, 10, 14, 18, 22-Tetracosahexaene is also having the Antibacterial and Antioxidant properties. γ-Tocopherol is a vitamin and used in the treatment for Anticancer, Antioxidant, Antitumor, Anti-inflammatory, Hypocholesterolemia and Cardioprotective sickness. Vitamin E is known compound used in Antiaging, Antialzheimeran, Antidermatitic, Antidiabetic, Antioxidant, Antitumor, Cancer-preventive, hypocholesterolemia and Immunostimulant. Ergost-5-EN-3-OL is an Antioxidant and also having hypocholesterolemia activity.
Figure 1: GC-MS chromatograms shows the Retention Time of the compounds of *Strychnos Nux-vomica*.

Figure 2: Retention Time of Strychnine and brucine standards chromatograms shows in HPLC.

Figure 3: Retention Time of Strychnine and brucine compounds in *Strychnos Nux-vomica* of HPLC.
### Table 1. List of the Compounds present in *Strychnos Nux-vomica* extract of the GC MS analysis

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Retention Time in minutes</th>
<th>Compound</th>
<th>Molecular Formula</th>
<th>Molecular Weight</th>
<th>Area</th>
<th>Area %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>21.302</td>
<td>Megastigmatrienone</td>
<td>C_{13}H_{18}O</td>
<td>190</td>
<td>415108</td>
<td>0.58</td>
</tr>
<tr>
<td>2</td>
<td>22.995</td>
<td>Megastigmatrienone</td>
<td>C_{13}H_{18}O</td>
<td>190</td>
<td>1403906</td>
<td>1.98</td>
</tr>
<tr>
<td>3</td>
<td>23.541</td>
<td>1,3,4,5-Tetrahydroxy-Cyclohexanecarboxylic acid</td>
<td>C_{7}H_{12}O_{6}</td>
<td>192</td>
<td>1680694</td>
<td>2.37</td>
</tr>
<tr>
<td>4</td>
<td>28.203</td>
<td>3-O-Methyl-d-glucose</td>
<td>C_{7}H_{14}O_{6}</td>
<td>194</td>
<td>2220057</td>
<td>3.13</td>
</tr>
<tr>
<td>5</td>
<td>29.662</td>
<td>Hexadecanoic acid, methyl ester</td>
<td>C_{17}H_{34}O_{2}</td>
<td>270</td>
<td>281869</td>
<td>0.40</td>
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<tr>
<td>6</td>
<td>29.867</td>
<td>Cholest-5-EN-3-OL (3.Beta.)</td>
<td>C_{27}H_{46}O</td>
<td>386</td>
<td>88151</td>
<td>1.24</td>
</tr>
<tr>
<td>7</td>
<td>30.087</td>
<td>Cholest-5-EN-3-OL (3.Beta.)</td>
<td>C_{27}H_{46}O</td>
<td>386</td>
<td>630306</td>
<td>0.89</td>
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<tr>
<td>8</td>
<td>30.294</td>
<td>n-Hexadecanoic acid</td>
<td>C_{16}H_{32}O</td>
<td>256</td>
<td>6887181</td>
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<td>9</td>
<td>30.711</td>
<td>Hexadecanoic Acid, Ethyl ester</td>
<td>C_{18}H_{36}O_{2}</td>
<td>284</td>
<td>1496754</td>
<td>2.11</td>
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<tr>
<td>10</td>
<td>32.299</td>
<td>9,12,15-Octadecatrienoic acid, methyl ester</td>
<td>C_{19}H_{32}O_{2}</td>
<td>292</td>
<td>262921</td>
<td>0.37</td>
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<tr>
<td>11</td>
<td>32.476</td>
<td>2-Hexadecen-1-OL</td>
<td>C_{20}H_{40}O</td>
<td>296</td>
<td>10492917</td>
<td>14.78</td>
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<tr>
<td>12</td>
<td>32.900</td>
<td>Cyclopropaneoctanoic acid</td>
<td>C_{22}H_{34}O_{2}</td>
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<td>3246047</td>
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<td>13</td>
<td>33.197</td>
<td>9,12,15-Octadecatrienoic acid, ethyl ester</td>
<td>C_{20}H_{36}O_{2}</td>
<td>306</td>
<td>3298198</td>
<td>4.65</td>
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<td>14</td>
<td>35.738</td>
<td>1,4-Dioxaspiro[4.14] nonadecane</td>
<td>C_{17}H_{34}O_{2}</td>
<td>268</td>
<td>418787</td>
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<td>15</td>
<td>37.922</td>
<td>Hexadecanoic acid</td>
<td>C_{19}H_{34}O_{4}</td>
<td>330</td>
<td>477387</td>
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<tr>
<td>16</td>
<td>42.134</td>
<td>2,6,10,14,18,22-Tetracosahexaene</td>
<td>C_{38}H_{80}</td>
<td>410</td>
<td>4974663</td>
<td>7.01</td>
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<tr>
<td>17</td>
<td>46.307</td>
<td>γ-Tocopherol</td>
<td>C_{28}H_{48}O_{2}</td>
<td>416</td>
<td>557217</td>
<td>0.78</td>
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<tr>
<td>18</td>
<td>47.940</td>
<td>Vitamin E</td>
<td>C_{28}H_{50}O_{2}</td>
<td>430</td>
<td>3038192</td>
<td>4.28</td>
</tr>
<tr>
<td>19</td>
<td>50.393</td>
<td>Ergost-5-EN-3-OL</td>
<td>C_{28}H_{52}O</td>
<td>400</td>
<td>2683723</td>
<td>3.78</td>
</tr>
<tr>
<td>20</td>
<td>51.044</td>
<td>Strychnidin-10-ONE (Strychnine)</td>
<td>C_{21}H_{32}N_{2}O_{2}</td>
<td>334</td>
<td>20184057</td>
<td>28.43</td>
</tr>
<tr>
<td>21</td>
<td>52.833</td>
<td>Stigmat-5-EN-3-OL, (3.Beta.)-</td>
<td>C_{20}H_{30}O</td>
<td>414</td>
<td>2688057</td>
<td>3.79</td>
</tr>
<tr>
<td>22</td>
<td>53.995</td>
<td>Methyl Commate D</td>
<td>C_{31}H_{50}O_{4}</td>
<td>486</td>
<td>2769166</td>
<td>3.90</td>
</tr>
</tbody>
</table>

List of the NIST library Compounds present in *Strychnos Nux-vomica* leaves extract of the GC MS analysis
Figure 4. Megastigmatrienone
Formula: C_{13}H_{18}O, MW: 190, Exact Mass: 190.135765, NIST#: 47233 ID#: 3584

Figure 5. 1, 3, 4, 5-Tetrahydroxy-Cyclohexanecarboxylic acid
Formula: C_{7}H_{12}O_{6}, MW: 192, Exact Mass: 192.063388, NIST#: 229684 ID#: 28354

Figure 6. 3-O-Methyl-d-glucose
Formula: C_{7}H_{14}O_{6}, MW: 194, Exact Mass: 194.079039, NIST#: 27259 ID#: 37586

Figure 7. Hexadecanoic acid, methyl ester
Formula: C_{17}H_{34}O_{2}, MW: 270, Exact Mass: 270.25588, NIST#: 333716 ID#: 40690
Figure 8. Cholest-5-EN-3-OL (3.Beta.)(Cholesterol)
Formula: C_{27}H_{46}O, MW: 386, Exact Mass: 386.354866, NIST#: 332884, ID#: 6840

Figure 9. n-Hexadecanoic acid
Formula: C_{16}H_{32}O_{2}, MW: 256, Exact Mass: 256.24023, NIST#: 151973, ID#: 8689

Figure 10. Hexadecanoic Acid, Ethyl ester
Formula: C_{18}H_{36}O_{2}, MW: 284, Exact Mass: 284.27153, NIST#: 233204, ID#: 52733

Figure 11. 9, 12, 15-Octadecatrienoic acid, methyl ester
Formula: C_{19}H_{30}O_{2}, MW: 292, Exact Mass: 292.24023, NIST#: 333199, ID#: 44319
Figure 12. 2-Hexadecen-1-OL
Formula: \(C_{20}H_{40}O\), MW: 296, Exact Mass: 296.307917, NIST#: 114703, ID#: 45842

Figure 13. Cyclopropanoic acid
Formula: \(C_{22}H_{38}O_2\), MW: 334, Exact Mass: 334.28718, NIST#: 35867, ID#: 2585

Figure 14. 9, 12, 15-Octadecatrienoic acid, methyl ester
Formula: \(C_{20}H_{34}O_2\), MW: 306, Exact Mass: 306.25588, NIST#: 235982, ID#: 44703

Figure 15. 1, 4-Dioxaspiro [4.14] nonadecane
Formula: \(C_{17}H_{32}O_2\), MW: 268, Exact Mass: 268.24023, NIST#: 141676, ID#: 65930
Figure 16. Hexadecanoic acid 2-hydroxy-1-(hydroxymethyl) ethyl ester
Formula: C_{19}H_{38}O_4, MW: 330, Exact Mass: 330.27701, NIST#: 15400, ID#: 7272

Figure 17. 2, 6, 10, 14, 18, 22-Tetracosahexaene (Supraene)
Formula: C_{30}H_{50}, MW: 410, Exact Mass: 410.391253, NIST#:227620, ID#: 32435

Figure 18. γ-Tocopherol
Formula: C_{28}H_{48}O_2, MW: 416, Exact Mass: 416.36543, NIST#: 374719, ID#: 125688

Figure 19. Vitamin E
Formula: C_{29}H_{50}O_2, MW: 430, Exact Mass: 430.38108, NIST#: 374713, ID#: 136995
Figure 20. Ergost-5-EN-3-OL (5-Cholesten-3-ol, 24-methyl-)
Formula: C_{28}H_{48}O, MW: 400, Exact Mass: 400.370516, NIST#: 214174, ID#: 6688

Figure 21. Strychnidin-10-ONE (Strychnine)
Formula: C_{21}H_{22}N_{2}O_{2}, MW: 334, Exact Mass: 334.168127, NIST#: 232994, ID#: 203578

Figure 22. Stigmast-5-EN-3-OL, (3.Beta.)-(3.Beta.)-(β-Sitosterol)
Formula: C_{29}H_{50}O, MW: 414, Exact Mass: 414.386166, NIST#: 251915, ID#: 6717

Figure 23. Methyl Commate D (Lup-20(29)-en-28-oic acid, 2, 3-dihydroxy-, methyl ester
Formula: C_{31}H_{50}O_{4}, MW: 486, Exact Mass: 486.37091, NIST#: 64982, ID#: 155663
Stigmast-5-EN-3-OL used in Antiepileptic, Antiviral, Antioxidant, Cancer preventive and hypcholesterolemic illnesses. Methyl Commate D is compound used in antimicrobial, anti-inflammatory diseases [15]. Choleste-5-EN-3-OL (3.Beta.) is essential for the biosynthesis of steroid hormones, bile acids, and vitamin D [16]. n-Hexadecanoic acid is having Antioxidant, hypcholesterolemic, Nematicide, Antandrogenic, Hemolytic, 5-alpha reductase inhibitor and antipsychotic properties [17 and 18]. Strychnidin-10-ONE is biologically active compound used in various treatments that is analgesic [19], apoptotic effect and antidepressant [20], antidote for snake poisoning [21], antitumor [22] and diabetic activity [23].

5. CONCLUSION

Bio active compounds in Silayn Nux-Vomica leaves were identified by using GCMS with NIST library and Strychnine and Brucine was quantified with HPLC. These naturally occurring compounds plays crucial role in pharmaceutical and biological industries for new drug development for various diseases.

REFERENCES:


