PHYTOCHEMICAL SCREENING AND GC-MS ANALYSIS OF METHANOL EXTRACT OF THE LEAVES OF *IPOMOEA CARNEA*

G. B. Alaka Kar¹*, Dr. Susanta Kumar Rout² and Debashisa Mishra¹

¹IMT Pharmacy College, Puri, New Nabakalabara Road, Sai Vihar, Gopalpur, Puri, Odisha.
²Patent Information Centre, Science & Technology Department, Secretariat, Odisha.

**ABSTRACT**

*Ipomoea carnea* is one of the medicinally important plants belonging to the family Convolvulaceae has been studied for different biological activities with special reference to Central Nervous System (CNS) disorders. The experimental results showed that *Ipomoea carnea* has effective in various CNS disorders along with woundhealing and anti inflammatory activity. Methanol extract of *Ipomoea carnea* at 400mg/kg showed highly significant result towards different animal models mimicking the different CNS disorders. Similarly the sub maximal dose i.e. 200 mg/kg showed significant effect in different animal models. Different literature study revealed that the plant possess antimicrobial, anticancer, anti-inflammatory activity. *I. carnea* which was introduced into some regions as an ornamental plant, is a globally distributed invasive shrub found in tropical and subtropical regions. Preliminary qualitative phytochemical screening of *I. carnea* revealed the presence of phenolic compounds, terpenoids, flavonoids and steroids. Some of them have antioxidant and antimicrobial activities. GC-MS analysis in the leaf powder’s methanol extract was done by using National Institute Standard and Technology (NIST) database 2005 to identify the compounds present. The spectra of unknown compounds were compared with that of known compounds stored in the NIST library by matching the molecular weight and retention time. 22 bio active phytochemical compounds were identified in leaf powder. The compounds predominantly Phenolic compounds, Flavonoids derivatives, Carbohydrate, Glycoside, Saponin, Phytosterols on basis of the molecular formula, molecular weight, peak area percentage etc. These different active phytochemicals have been found to possess a wide range of activities, which may help in the protection against different diseases.
KEYWORDS: Phytochemicals, GCMS analysis, spectra, antibacterial activity.

1. INTRODUCTION

Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well being. Medicinal plants represent a rich source of antimicrobial agents and natural antioxidants. (Rout et al., 2013) Many of the plant materials used in traditional medicines are readily available in rural areas at relatively cheaper than modern medicines. Medical plants and plant products are the oldest and tried health-care products. Their importance is growing not only in developing countries but in many developed countries. The allopathic drugs are good in onset and have good therapeutic activity but side effects associated are poignant. Thus, the herbal medicines from natural sources with least or no side effect having similar or better therapeutic activity are best. The herbal medicines have wide therapeutic actions and safety profile. (Rout et al., 2012) Approximately 80% of the world inhabitants rely on traditional medicine for their primary health care and play an important role in the health care system of the remaining 20% of the population. (Rout et al., 2014) The World Health Organization (WHO) is encouraging, promoting and facilitating the effective use of herbal medicine in developing countries for health programs. It has been proved that various plants extracts possess bacteriostatic and bactericidal effects, and most of these plants contain many active compounds. Consequently, they are multipurpose drugs at the same time and have formed the basis of sophisticated traditional medicine system and natural products make excellent leads for new drug development. (Rout et al., 2011) *Ipomoea carnea* (Fam. Convolvulaceae) is a plant of tropical American origin but is now widely distributed in the tropical regions of the world. This plant is erect, densely leafed, and almost unbranched, growing as shrubs to 2-3 m high. It is widely cultivated as an ornamental shrub or as an informal hedge in warm-temperate and dry subtropical regions. (Rout et al., 2013) The preliminary phytochemical screening of different extracts were carried out presence of various phytoconstituents such as Alkaloids, Carbohydrates, Glycosides, Flavonoids, Tannins and Phenolic compound. It is used to have stimulatory allelopathic effect. (Rout et al., 2013) Roots are boiled to use as laxative and to provoke menstruation. The milky juice of plant has been used for the treatment of Lecucoderma and other related skin diseases. (Gaur et al., 2009) It has depressant effect on central nervous system. Also shows muscle relaxant property. The juice is collected and applied externally on affected parts, anti-inflammatory. (Ríos et al., 2008) With this
background the present study is to identify the phytoconstituents through GC-MS of the methanol extract from the leaves of *I. carnea*.

2. MATERIALS AND METHODS

**Plant material**

*I. carnea* leaves were collected from Anandapur, Keonjhar district of Odisha, India. The leaves were authenticated in the Department of Biosciences, Sardar Patel University, Gujarat. The plants were collected in bulk and washed with running tap water to remove adhering soil and dirt particles and then shade dried. A voucher specimen was deposited at the school of pharmaceutical science, SOA University, Bhubaneswar, Odisha. The dried plant materials were coarsely powered and stored in airtight, non-toxic polyethylene bags until used. (Rout et al., 2013).

**Preparation of extract and fractions**

The powdered leaves of *I. carnea* was extracted with petroleum ether (60 – 80 °C) for 72h to de-fat it and then the residue plant materials were macerated using methanol as solvent with constant stirring. The solvent incorporating the extractives were filtered and the marc pressed to squeeze out residual extractives. This process was repeated thrice to achieve complete extraction. The extracts obtained during the three cycles were combined and reduced to 1/8th of its original volume in a rotary evaporator at 45 °C and then lyophilized in a freeze dryer to obtain the yield. The extract was again dissolved in distilled water and then successively extracted by the following solvents with increasing polarities; chloroform, ethyl acetate and methanol. The so obtained different fractions were concentrated dried and preserved for further study. Phytochemical screening give positive tests for alkaloids, glycosides, saponins, Flavonoids, carbohydrates, tannins, phenolic compounds, protein, and fats. All the extracts of plant leaves were prepared 10% w/v in normal saline consisting of 0.1% propylene glycol. (Rout et al., 2013).

**Gas Chromatography- Mass Spectrum Analysis (GC-MS)**

The GC-MS analysis for identification of compounds present in different fractions prepared from crude methanol extract of *I. carnea* had been carried to identify the compounds. GC-MS analysis was done by using GC Clarus 500 Perkin Elmer system and gas chromatograph interfaced with mass detector Turbo mass gold-Perkin Elmer (GC-MS). (Hema et al, 2010) Column: Elite-5MS (5% Diphenyl / 95% Dimethyl poly siloxane), 30 x 0.25mm x 0.25m df, Carrier gas: Helium (99.999%) with constant flow rate of 1ml per min, (Split ratio: 10:1),
Sample Injection volume 2μl, Software: Turbomass 5.2, Oven operating in electron impact mode at 70 eV, oven temperature was fixed from 110°C (isothermal for 2 min.), with an increase rate of 10°C/min up to 200°C with no hold and then at rate of 5°C/min up to 280°C ending with a 9 min hold. Injector temperature was 2500°C, Ion-source temperature 280°C and total GC running time was 36 minutes.


**Identification of compounds**

Interpretation on mass spectrum generated during GC-MS analysis was done by using National Institute Standard and Technology (NIST) database 2005 to identify the compounds present. The spectra of unknown compounds were compared with that of known compounds stored in the NIST library by matching the molecular weight and retention time. The Name, Molecular weight and Structure of the components of the test materials were confirmed. (Hema et al., 2010) The major compounds identified were searched in Dr. Duke's Phytochemical and Ethnobotanical Database for any reported anti-hyperglycemic or hypoglycemic property of the compounds. (Vanitha et al., 2011).

3. RESULT

The results of the GC-MS analysis of *I. carnea* is presented in Table 2. The GC-MS analysis revealed presence of twenty two compounds in crude methanol extract of *I. carnea*. The compounds with their Retention Time (RT), Molecular Formula, Molecular Weight (MW) and Peak Area (%) have been presented in different tables. The major compounds had been identified on basis of the percentage Peak Area in the Chromatograph. The compounds identified in the crude methanol extract (Table 10.4.2) are; Camphene (RT – 6.27, Peak Percentage – 77.18%); Myrcene (RT – 6.9, Peak Percentage – 9.41%); Eucalyptol (RT-7.6, Peak Percentage – 45.26%); 1,6-Octadien-3-ol, 3,7-dimethyl- (RT – 8.6, Peak Percentage – 10.9%); 2,6-Octadienal, 3,7-dimethyl-, (Z)- (RT – 11.4, Peak Percentage – 28.6 %); Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)- (RT – 13.06, Peak Percentage – 5.72%); Elemene (RT – 13.5, Peak Percentage – 6.6%); Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl- (RT – 14.2, Peak Percentage – 81.87%); Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene (RT – 14.74, Peak Percentage – 44.9 %); Cubenol (RT – 15.9, Peak Percentage – 6.3%); 2-Naphthalenemethanol, decahydro- -trimethyl-8-methylene- (RT –
16.2, Peak Percentage – 11.7%); geranyl-p-cymene (RT – 19.2, Peak Percentage – 0.17%).

The molecular formulae of the compounds had been mentioned in Table 5.2 whereas the chromatograph has been represented in Figure 10.4.2. Search for any reported hypoglycemic or anti-hyperglycemic potential of major compounds in Dr. Dukes Phytochemical and Ethnobotanical Database revealed no result.

Table 1: GC-MS analysis of crude methanol extract of *I. carnea*

<table>
<thead>
<tr>
<th>S.No.</th>
<th>RT</th>
<th>Compound</th>
<th>Molecular Formula</th>
<th>MW</th>
<th>Peak Area %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>5.46</td>
<td>2-Heptanol</td>
<td>C&lt;sub&gt;7&lt;/sub&gt;H&lt;sub&gt;16&lt;/sub&gt;O&lt;sub&gt;5&lt;/sub&gt;</td>
<td>116.0</td>
<td>0.93</td>
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<tr>
<td>2.</td>
<td>6.277</td>
<td>Camphene</td>
<td>C&lt;sub&gt;10&lt;/sub&gt;H&lt;sub&gt;16&lt;/sub&gt;</td>
<td>136.0</td>
<td>77.18</td>
</tr>
<tr>
<td>3.</td>
<td>6.931</td>
<td>Myrcene</td>
<td>C&lt;sub&gt;10&lt;/sub&gt;H&lt;sub&gt;16&lt;/sub&gt;</td>
<td>136.0</td>
<td>9.41</td>
</tr>
<tr>
<td>4.</td>
<td>7.619</td>
<td>Eucalyptol</td>
<td>C&lt;sub&gt;10&lt;/sub&gt;H&lt;sub&gt;18&lt;/sub&gt;O</td>
<td>154</td>
<td>45.26</td>
</tr>
<tr>
<td>5.</td>
<td>7.993</td>
<td>2-Octenal, (E)-</td>
<td>C&lt;sub&gt;8&lt;/sub&gt;H&lt;sub&gt;14&lt;/sub&gt;O</td>
<td>126</td>
<td>2.09</td>
</tr>
<tr>
<td>6.</td>
<td>8.669</td>
<td>1,6-Octadien-3-ol, 3,7-dimethyl-</td>
<td>C&lt;sub&gt;10&lt;/sub&gt;H&lt;sub&gt;18&lt;/sub&gt;O</td>
<td>154</td>
<td>10.99</td>
</tr>
<tr>
<td>7.</td>
<td>9.696</td>
<td>Endo-Borneol</td>
<td>C&lt;sub&gt;10&lt;/sub&gt;H&lt;sub&gt;18&lt;/sub&gt;O</td>
<td>154</td>
<td>8.17</td>
</tr>
<tr>
<td>8.</td>
<td>9.941</td>
<td>1,7,7-Trimethylbicyclo[2.2.1]heptan-2-ol</td>
<td>C&lt;sub&gt;10&lt;/sub&gt;H&lt;sub&gt;18&lt;/sub&gt;O</td>
<td>154</td>
<td>7.72</td>
</tr>
<tr>
<td>9.</td>
<td>10.75</td>
<td>2,6-Octadienal, 3,7-dimethyl-, (Z)-</td>
<td>C&lt;sub&gt;10&lt;/sub&gt;H&lt;sub&gt;16&lt;/sub&gt;O</td>
<td>152</td>
<td>16.09</td>
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<tr>
<td>10.</td>
<td>11.44</td>
<td>2,6-Octadienal, 3,7-dimethyl-, (E)-</td>
<td>C&lt;sub&gt;10&lt;/sub&gt;H&lt;sub&gt;16&lt;/sub&gt;O</td>
<td>152</td>
<td>28.61</td>
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<tr>
<td>11.</td>
<td>11.69</td>
<td>2-Undecanone</td>
<td>C&lt;sub&gt;11&lt;/sub&gt;H&lt;sub&gt;22&lt;/sub&gt;O</td>
<td>170</td>
<td>2.24</td>
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<td>12.</td>
<td>12.76</td>
<td>1,2,4-Metheno-1H-indene, octahydro-1,7a-dimethyl-5-(1-methylethyl)-</td>
<td>C&lt;sub&gt;15&lt;/sub&gt;H&lt;sub&gt;24&lt;/sub&gt;</td>
<td>204</td>
<td>0.81</td>
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<tr>
<td>13.</td>
<td>13.06</td>
<td>Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-</td>
<td>C&lt;sub&gt;15&lt;/sub&gt;H&lt;sub&gt;24&lt;/sub&gt;</td>
<td>204</td>
<td>5.72</td>
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<td>14.</td>
<td>13.59</td>
<td>Elemene</td>
<td>C&lt;sub&gt;15&lt;/sub&gt;H&lt;sub&gt;24&lt;/sub&gt;</td>
<td>204</td>
<td>6.61</td>
</tr>
<tr>
<td>15.</td>
<td>14.24</td>
<td>Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-</td>
<td>C&lt;sub&gt;15&lt;/sub&gt;H&lt;sub&gt;22&lt;/sub&gt;</td>
<td>202</td>
<td>81.87</td>
</tr>
<tr>
<td>16.</td>
<td>14.74</td>
<td>Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-</td>
<td>C&lt;sub&gt;15&lt;/sub&gt;H&lt;sub&gt;24&lt;/sub&gt;</td>
<td>204</td>
<td>44.99</td>
</tr>
<tr>
<td>17.</td>
<td>15.11</td>
<td>1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)-</td>
<td>C&lt;sub&gt;13&lt;/sub&gt;H&lt;sub&gt;26&lt;/sub&gt;O</td>
<td>222</td>
<td>4.43</td>
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<tr>
<td>18.</td>
<td>15.90</td>
<td>Cubenol</td>
<td>C&lt;sub&gt;13&lt;/sub&gt;H&lt;sub&gt;26&lt;/sub&gt;O</td>
<td>222</td>
<td>6.34</td>
</tr>
<tr>
<td>19.</td>
<td>16.2</td>
<td>2-Naphthalenemethanol, decahydro- -trimethyl-8-methylene-</td>
<td>C&lt;sub&gt;15&lt;/sub&gt;H&lt;sub&gt;26&lt;/sub&gt;O</td>
<td>222</td>
<td>11.79</td>
</tr>
<tr>
<td>20.</td>
<td>16.7</td>
<td>2,6,10-Dodecatrienal, 3,7,11-trimethyl-</td>
<td>C&lt;sub&gt;15&lt;/sub&gt;H&lt;sub&gt;24&lt;/sub&gt;O</td>
<td>220</td>
<td>2.72</td>
</tr>
<tr>
<td>21.</td>
<td>19.21</td>
<td>geranyl-p-cymene</td>
<td>C&lt;sub&gt;20&lt;/sub&gt;H&lt;sub&gt;30&lt;/sub&gt;</td>
<td>270</td>
<td>0.176</td>
</tr>
<tr>
<td>22.</td>
<td>19.46</td>
<td>geranyl-a-terpinene</td>
<td>C&lt;sub&gt;20&lt;/sub&gt;H&lt;sub&gt;32&lt;/sub&gt;</td>
<td>272</td>
<td>0.123</td>
</tr>
</tbody>
</table>
4. DISCUSSION

Drugs from plants have a long history in both traditional and modern societies as herbal remedies or crude drugs and as purified compounds. The present study revealed that the selected Plant extract and some fractions of the crude extracts produced anti epileptic, anxiolytic, antimicrobial, antioxidant and wound healing efficacy with dose dependent manner. The observed activities of leave extract might be attributed to the presence of secondary metabolites such as flavonoids and phenolic compounds. The leaves can be used to prevent oxidative damage caused by free radicals and to treat infections caused by pathogenic bacteria not to fungus. Further studies with purified constituents are needed to understand the complete mechanism of wound healing activity of the test plants. However, it needs further evaluation in clinical settings before consideration for the treatment of different disorders.

The GC-MS analysis of crude methanol extract of *I. carnea* showed presence 22 different compounds in it. On basis of the peak area percentage compounds like; Camphene; Myrcene; Eucalyptol; 1,6-Octadien-3-ol, 3,7-dimethyl; 2,6-Octadienal, 3,7-dimethyl-, (Z)-; Cyclohexane, 1-ethyl-1-methyl-2,4-bis(1-methylethenyl); Elemene, Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-; Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene; 2-Naphthalenemethanol, decahydro- trimethyl-8-methylene-; geranyl-p-cymene. Camphene may produce antimicrobial, antibacterial, anti-oxidant, antifungal and anti-inflammatory activity. (Brown et al, 1997; Petrovic et al, 2003) From different literature it is observed that myrcene produces antimicrobial and antioxidant activity. (Sarac et al, 2013) Eucalyptol (1,8-cineole), a terpenoid oxide has been found to show the biological activity like anti-inflammatory and antioxidant effects in various diseases, including respiratory disease, pancreatitis, colon damage, and cardiovascular and neurodegenerative diseases. etc. (Seol et al, 2016; Kumar et al, 2010; Rahuman et al, 200) 1,6-Octadien-3-ol, 3,7-dimethyl has been used as antiseptic, anti-neuralgic, analgesic effect. (Mehani et al, 2015) Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene is the phytochemical substance responsible for the medicinal value of the plants including antioxidant, anticancer, antimicrobial activities, Anticonvulsant, Anticancer, Anti-inflammatory activity. (Baskaran et al, 2016; Ramamurthy et al, 2017) Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl produces antioxidant, antiinflammatory, antimicrobial, anti-cancer, anticonvulsant, and sedative activity. (Subashini et al, 2015) Biological activity of 1,6,10-Dodecatrien-3-ol has producing Antitumor, analgesic, sedative, anti-inflammatory activity etc. (Balamurugan et al, 2012; Devi et al, 2015) Ethanone, 2-(2-benzothiazolylthio)-1-(3,5-dimethylpyrazolyl) has producing antimicrobial
activity. (Velayutham et al, 2015) Cubenol has been producing antimicrobial and anti-inflammatory activity. (Pati et al, 2014) Geranyl acetate and p-cymene also presented some antioxidant effects, but with a varying profile according the free radical-generating system studied. (Rabelo et al, 2012) So, presence of these compounds may be responsible for the observed anti-convulsant, sedative, muscle relaxant, anti-inflammatory, antimicrobial and wound healing activity. The search for reported different activities of the major compounds in Dr. Duke's Phytochemical and ethno botanical database yielded no result, and hence are subjected to further investigation.

5. CONCLUSION
In the present study sixteen chemical constituents have been identified from methanolic extract by Gas Chromatogram Mass spectrometry (GC-MS) analysis. The presence of various bioactive compounds justifies the use of plant in various ailments by traditional practitioners. The GC-MS analysis of methanol extract revealed out of the twenty two identified compounds; 1,6-Octadien-3-ol, 3,7-dimethyl-; 2,6-Octadienal, 3,7-dimethyl-, (Z)-; Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-; Elemene , Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-; Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene; 2-Naphthalenemethanol, decahydro- -trimethyl-8-methylene-; geranyl-p-cymene. Camphene may produce antimicrobial, antibacterial, anti-oxidant, antifungal and anti-inflammatory activity were considered as major compounds on basis of the percentage peak area shown on the chromatograph.

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7. REFERENCES


