CHARACTERIZATION OF BIOACTIVE COMPOUNDS IN THE METHANOLIC EXTRACT OF MOSS *HERPETINEURON Toccoae* (SULL. & LESQ.) CARDOT USING GC-MS ANALYSIS

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Bryophytes are the simplest and most primitive land plants due to absence of conductive tissue system. Being small, this group of plants have been neglected for a long time. The pharmaceutical investigations of their chemical compositions in last few decades have proven their medicinal importance. *Herpetineuron toccoae* (Sull. & Lesq.) Cardot is a pluerocarpic moss belonging to family Anomodontaceae. The moss is cosmopolitan in distribution. The objective of present study is to analyze the presence of bioactive secondary metabolites from the methanolic extract of *Herpetineuron toccoae*. The phytochemical screening was carried out through gas chromatography- mass spectrometry technique. A total of 28 bioactive phytochemical compounds were isolated from the methanolic extract. These compounds were Pterin-6-carboxylic acid, Thieno [2,3-c]furan-3-carbonitrile, 2-amino-4,6-dihydro-4,6,6,6-tetramethyl-, Ethylbenzene, Dodecylcyclohexane, 1,7-Dimethyl-4-(1-methylethyl)cyclodecane, 1-Hexadecanol, 2-methyl, cis-13-Eicosenoic acid, Phen-1,4-diol, 2,3-dimethyl-5-trifluoromethyl-, trans-13-Octadecenoic acid, 3-Trifluoroacetoxypentadecane, n-Tetracosanol-1, 2-Hexyl-1-octanol, 1-Hexadecanol, 8-Octadecenal, 5-Octadecenal, 9-Hexadecenoic acid, 1-Hexadecanol, 2-methyl-, 17-Pentatriacontene, 10-Octadecenal, Cholestan-3-one, cyclic 1,2-ethanediyl aetal, (5aï,- Octadecane, 3-ethyl-5-(2-ethylbutyl)-, Benzenepropanoic acid, 3,5-bis(1,1-dimethyleryl)-4-hydroxy-, methyl ester, Ethyl iso-allocholate, cis-11-Eicosenoic acid, 1-Heptatriacontanol, Cyclopropanedodecanoic acid, 2-octyl-, methyl ester, 9,10-Secholestena-5,7,10(19)-triene-3,24,25-triol, (3ai,5Z,7E)-, Oleic acid, 3-(octadecyloxy)propyl ester. The identification of these bioactive phytochemical compounds was based on peak area, retention time, molecular formula and weight. Subsequently, anti-microbial, anti-inflammatory, anti-cancerous properties of the extracted phytochemicals indicate the medicinal value of *Herpetineuron toccoae*.

**Key words :** Bryophytes; *Herpetineuron toccoae*, Bioactive compounds, GC-MS analysis, Phytochemicals.

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Bryophytes are the simplest and most primitive land plants due to absence of conductive tissue system. Being small, this group of plants have been neglected for a long time. The pharmaceutical investigations of their chemical compositions in last few decades have proven their medicinal importance. *Herpetineuron toccoae* (Sull. & Lesq.) Cardot is a pluerocarpic moss belonging to family Anomodontaceae. The moss is cosmopolitan in distribution. The objective of present study is to analyze the presence of bioactive secondary metabolites from the methanolic extract of *Herpetineuron toccoae*. The phytochemical screening was carried out through gas chromatography- mass spectrometry technique. A total of 28 bioactive phytochemical compounds were isolated from the methanolic extract. These compounds were Pterin-6-carboxylic acid, Thieno [2,3-c]furan-3-carbonitrile, 2-amino-4,6-dihydro-4,6,6,6-tetramethyl-, Ethylbenzene, Dodecylcyclohexane, 1,7-Dimethyl-4-(1-methylethyl)cyclodecane, 1-Hexadecanol, 2-methyl, cis-13-Eicosenoic acid, Phen-1,4-diol, 2,3-dimethyl-5-trifluoromethyl-, trans-13-Octadecenoic acid, 3-Trifluoroacetoxypentadecane, n-Tetracosanol-1, 2-Hexyl-1-octanol, 1-Hexadecanol, 8-Octadecenal, 5-Octadecenal, 9-Hexadecenoic acid, 1-Hexadecanol, 2-methyl-, 17-Pentatriacontene, 10-Octadecenal, Cholestan-3-one, cyclic 1,2-ethanediyl aetal, (5aï,- Octadecane, 3-ethyl-5-(2-ethylbutyl)-, Benzenepropanoic acid, 3,5-bis(1,1-dimethyleryl)-4-hydroxy-, methyl ester, Ethyl iso-allocholate, cis-11-Eicosenoic acid, 1-Heptatriacontanol, Cyclopropanedodecanoic acid, 2-octyl-, methyl ester, 9,10-Secholestena-5,7,10(19)-triene-3,24,25-triol, (3ai,5Z,7E)-, Oleic acid, 3-(octadecyloxy)propyl ester. The identification of these bioactive phytochemical compounds was based on peak area, retention time, molecular formula and weight. Subsequently, anti-microbial, anti-inflammatory, anti-cancerous properties of the extracted phytochemicals indicate the medicinal value of *Herpetineuron toccoae*.

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**Introduction**

The significance of natural and herbal medicines is increasing day by day as people are now more attracted towards them due to their potential, cost effectiveness and least side effects. Almost 80% population worldwide depends on herbal medication directly or indirectly (Ekor, 2014). Plants are rich source of active ingredients called secondary metabolites having anti-microbial, anti-inflammatory, anti-cancerous activities, which makes them pharmacologically important (Chavhan, 2017). The screening of plants is important to detect the presence of bioactive compounds, which can be used in pharmacological studies. However, the method used for identification should be easy and repeatable (Ganesh et al., 2017). Gas chromatography- mass spectrometry technique can separate volatile compounds very efficiently, thus, making it very compatible for identification and quantification prospect (Vinodh et al., 2013). *Herpetineuron toccoae* (Sull. & Lesq.) Cardot is yellowish- green plant, growing in compact turfs. Stem is ±5cm long, horizontal. Rhizoids are present. Stem is covered with leaves. The secondary branches emerging
from the main stem are erect and branched. Leaves are compactly arranged, lanceolated with acuminated tip and broad base, margins serrated in the upper region. The presence of tortous vein inspired Cardot to name this moss, *Herpetineuron* (snake costa). Costa is the main identifying feature of this genus. It is strong, ends almost at the apex. Laminal cells are thick, almost hexagonal in shape. Perichaetial leaves are present. Setae erect with slightly inclined capsule. Peristome teeth double. The moss is cosmopolitan in distribution.

Many plants especially bryophytes are phytochemically unexplored as compared to other plant groups. Due to the fact that bryophytes are small, simple plants and shortfall of the knowledge of their contribution towards the ecosystem, they have always been overlooked by conservation groups. Phytochemical studies on liverworts have shown the presence of various bioactive compounds which shows anti-bacterial, anti-fungal and anti-cancerous properties (Asakawa, 1995). However, a very little work has been done on the phytochemical analysis of mosses. The literature study revealed that no GC-MS analysis has been done so far on *Herpetineuron toccoae*. Therefore, the present work has been taken up to reveal the presence of phytochemicals, which are medicinally important.

**Materials and Methods**

**Collection and preparation of plant material**

The sample was collected from various locations of the study area (District Solan, Himachal Pradesh). The collected plant was identified and the specimen was deposited under PAN no. 6410 in the herbarium, Department of Botany, Panjab University, Chandigarh.

**Preparation of plant extract**

To prepare the methanolic extract of *Herpetineuron toccoae*, 500mg of plant material was crushed in methanol and kept on rotatory shaker for 24 hrs. The filtrate was then subjected to GC-MS.

**Gas Chromatography-Mass spectrometry (GC-MS) analysis**

The GC-MS analysis of whole plant ethanol extract was done using Thermo Trace 1300GC with thermo TSQ8000 Triple Quadrupole MS at CIL/SAIF, P.U. Chandigarh with 250°C injector temperature, 10ul injector volume with 29.08 run time. TG 5MS (30m × 0.25 mm, 0.25um) column with 5% diphenyl and 95% dimethyl polysiloxane column make up was used for the analysis. In this chromatography, helium works as a carrier gas at 1.5ml/minute constant flow with 45ml/min split flow because in GC extract separated through heating and heated gases of individual substances are carried out to column through helium (an inert gas). Mass spectrum was taken at 70eV in 50-700 range along with MS transfer line temperature of 280°C. Ion source temperature was 230°C.

**Identification of compounds**

The obtained mass spectra were interpreted by comparing with National Institute of Standards and Technology (NIST) database. NIST 2.0 is a compendium of 62000 mass spectrum patterns which denotes chemical compounds (Kumar *et al.*, 2018). The bioactivity of these compounds was documented from the literature.

**Results**

The GC-MS analysis of *Herpetineuron toccoae* has shown the presence of 28 bioactive compounds. Results showed the identified compounds along with their molecular weight, retention time, area percentage and their biological activities and full GC-MS chromatogram. (Table 1, Fig. 1).

**Fig. 1**: GC-MS chromatograph of methanolic extract of *H. toccoae*. 
Table 1 : Bioactive compounds identified in *H. toccae* by GC-MS analysis along with their biological activity.

<table>
<thead>
<tr>
<th>S. no.</th>
<th>Compound name</th>
<th>Molecular Formula</th>
<th>RT</th>
<th>Area %</th>
<th>Biological activity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Pterin-6-carboxylic acid</td>
<td>C7H5N5O3</td>
<td>3.33</td>
<td>1.63</td>
<td>Anti-cancerous</td>
<td>Kumar et al. (2018)</td>
</tr>
<tr>
<td>2.</td>
<td>Thieno[2,3-c]furan-3-carbonitrile, 2-amino-4, 6-dihydro-4,4,6,6-tetramethyl-</td>
<td>C11H14N2OS</td>
<td>3.86</td>
<td>1.11</td>
<td>Analgesic, Antianginal, Analgesic, non-opioid, Antihypertensive, Antiarthritic, Dementia treatment, Neurotransmitter uptake inhibitor</td>
<td>Brintha et al. (2017)</td>
</tr>
<tr>
<td>3.</td>
<td>Ethylbenzene</td>
<td>C8H10</td>
<td>4.43</td>
<td>1.64</td>
<td>Used as solvents in insecticide sprays, rubber and chemical manufacturing, and household degreasers, paints, adhesives and rust preventives. As an antiknock agent in aviation and motor fuels</td>
<td>Jang et al. (2001)</td>
</tr>
<tr>
<td>4.</td>
<td>Dodecylcyclohexane</td>
<td>C18H36</td>
<td>10.31</td>
<td>1.86</td>
<td>As a source of carbon and energy for cultures</td>
<td>Beam and Perry (1974)</td>
</tr>
<tr>
<td>5.</td>
<td>1,7-Dimethyl-4-(1-methylethyl)cyclodecane</td>
<td>C15H30</td>
<td>12.07</td>
<td>3.92</td>
<td>Anti-microbial, Anti-inflammatory</td>
<td>Krishnamoorthy and Subramaniam (2014)</td>
</tr>
<tr>
<td>6.</td>
<td>1-Hexadecanol, 2-methyl-</td>
<td>C17H36O</td>
<td>12.50</td>
<td>1.93</td>
<td>Anti-inflammatory</td>
<td>Kim et al. (2013)</td>
</tr>
<tr>
<td>7.</td>
<td>cis-13-Eicosenoic acid</td>
<td>C20H38O2</td>
<td>12.76</td>
<td>0.88</td>
<td>Oleochemical used in Pharmaceutical, cosmetic and food applications</td>
<td>Kassab et al. (2019)</td>
</tr>
<tr>
<td>8.</td>
<td>Phen-1,4-diol, 2,3-dimethyl-5-trifluoromethyl-</td>
<td>C9H9F3O2</td>
<td>13.89</td>
<td>1.11</td>
<td>Antioxidant, Anti-thrombotic and Anti-tuberculosis activity</td>
<td>Hameed et al. (2015)</td>
</tr>
<tr>
<td>10.</td>
<td>3-Trifluoroacetoxypentadecane</td>
<td>C17H31F3O2</td>
<td>14.47</td>
<td>1.15</td>
<td>Anti-nephrotic, Anti-oxidant activities</td>
<td>Hussein et al. (2016)</td>
</tr>
<tr>
<td>11.</td>
<td>n-Tetracosanol-1</td>
<td>C24H50O</td>
<td>15.02</td>
<td>2.83</td>
<td>Anti-microbial</td>
<td>Lakshmi and Nair (2017)</td>
</tr>
<tr>
<td>12.</td>
<td>2-Hexyl-1-octanol</td>
<td>C14H30O</td>
<td>15.02</td>
<td>2.83</td>
<td>Anti-microbial</td>
<td>Witkowska-Banaszczak and Dlugaszewska (2017)</td>
</tr>
<tr>
<td>13.</td>
<td>1-Hexadecanol</td>
<td>C16H34O</td>
<td>15.10</td>
<td>2.85</td>
<td>Used in surfactants, lubricants, detergents, pharmaceuticals and cosmetics.</td>
<td>Feng et al. (2016)</td>
</tr>
<tr>
<td>14.</td>
<td>8-Octadecenal</td>
<td>C18H38O</td>
<td>15.28</td>
<td>2.23</td>
<td>Anti-inflammatory activity, Anti-microbial activity</td>
<td>Hussein et al. (2016)</td>
</tr>
<tr>
<td>15.</td>
<td>5-Octadecenal</td>
<td>C18H32O</td>
<td>16.19</td>
<td>1.85</td>
<td>Food flavouring agent, Anti-microbial, Anti-inflammatory</td>
<td>Anjali et al. (2019)</td>
</tr>
<tr>
<td>16.</td>
<td>9-Hexadecenoic acid</td>
<td>C16H30O2</td>
<td>16.89</td>
<td>1.00</td>
<td>Non-cytotoxic</td>
<td>Ismail et al. (2013)</td>
</tr>
</tbody>
</table>

Table 1 continued...
The analysis revealed the presence of bioactive compounds, which includes Pterin-6-carboxylic acid, Thierno [2,3-c]furan-3-carbonitrile, 2-amino-4,6-dihydro-4,4,6,6-tetramethyl-, Ethylenbenzene, Dodecylcyclohexane, 1,7-Dimethyl-4-(1-methylthyl) cyclodecane, 1-Hexadecanol, 2-methy1, cis-13-Eicosenoic acid, Phen-1,4-diol, 2,3-dimethyl-5-trifluoromethyl-, trans-13-Octadecenoic acid, 3-Trifluoroacetoxy pentadecane, n-Tetracosanol-1, 2-Hexyl-1-octanol, 1-Hexadecanol, 8-Octadecenal, 5-Octadecenol, 9-Hexadecenoic acid, 1-Hexadecanol, 2-methy1-, 17-Pentatriacontene, 10-Octadecenal, Cholestane-3-one, cyclic 1,2-ethanediyl, (5aì)-, Octadecane, 3-ethyl-5-(2-ethylbutyl)-, Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester, Ethyl iso-allocholate, cis-11-Eicosenoic acid, 1-Heptatriacontanol, Cyclopropanedodecanoic acid, 2-octyl-, methyl ester, 9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol-, (3aì,5Z,7E)-, Oleic acid, 3-(octadecylxylo) propyl ester. Moreover, the GC-MS spectrum of the identified phytochemicals are depicted individually in Fig. 2.

**Discussion**

The identified bioactive compounds possess various biological activities, which have significant use in pharmaceutical studies such as Pterin-6-carboxylic acid (Brintha et al., 2017), 17-Pentatriacontene (Bertel et al., 2021) and Cholestane-3-one, cyclic 1,2-ethanediyl ester, (5ai)- (Al-Marzoqi et al., 2015) shows anti-cancerous activities. The compounds 1,7-Dimethyl-4-(1-methylthyl) cyclodecane (Kim et al., 2013), 2-Hexyl-1-octanol (Feng et al., 2015), 8-Octadecenal (Anjali et al., 2019), 5-Octadecenol (Ismail et al., 2013), 1-Hexadecanol, 2-methy1-(Odiase-Omoighe et al., 2022), Ethyl iso-allocholate (Abubacker et al., 2016), 1-Heptatriacontanol (Abubacker et al., 2014), Cyclopropanedodecanoic acid, 2-octyl-, methyl ester (Al-Marzoqi et al., 2015) and Oleic acid, 3-(octadecylxylo) propyl ester (Abubacker et al., 2014) have been reported for their anti-microbial properties. The

Fig. 2 continued...
Fig. 2 continued...
Characterization of Bioactive Compounds in the Methanolic Extract of Moss Cardot using GC-MS Analysis

Fig. 2 continued...

Fig. 2 continued...
compounds showing anti-inflammatory properties such as 1,7-Dimethyl-4-(1-methylethyl) cyclodecane (Kim et al., 2013), 1-Hexadecanol, 2-methyl- (Kassab et al., 2019), trans-13-Octadecenoic acid (Hussein et al., 2016), 8-Octadecenal (Anjali et al., 2019), 5-Octadecenal (Ismail et al., 2013), 17-Pentatriacontene (Bertel et al., 2021), Octadecane, 3-ethyl-5-(2-ethylbutyl)- (Bashir et al., 2012) and Ethyl iso-allocholate (Abubakar et al., 2016) were also found to be present in the plant extract. Moreover, the compound cis-11-Eicosenoic acid has been used as raw material for medical supplies and cosmetic industry (Kalaiarasan et al., 2011). 1-Hexadecanol is used as surfactants, lubricants, detergents, pharmaceuticals and cosmetic industry (Hussein et al., 2016).

**Conclusion**

A total of 28 biologically active compounds were isolated from methanolic extract of *Herpetineuron toccoa*. The results have clearly shown the importance of the studied moss in pharmaceutical, cosmetic and food industry.

**Acknowledgments**

Meenal Sharma sourced the plant materials, collected the data and wrote the paper. Shiwani Latwal contributed to data and helped in the analysis tools. Anju Rao conceived and designed the analysis. All the authors read and approved the manuscript.

**References**


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