Phytochemical Analysis of Bioactive Compounds from *Calophyllum inophyllum* L., Leaf Extract Using GC-MS Analysis.


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**ABSTRACT**

Phytoconstituents are the natural bioactive compounds found in plants. These phytoconstituents work with nutrients and fiber to form an integrated part of defense system against various diseases and stress conditions. The present study involves phytochemical analysis of *Calophyllum inophyllum* leaf extract obtained using methanol as solvent by Gas Chromatography and Mass Spectrometry (GC-MS) method. The GC-MS analysis showed peaks of nine different phytochemical compounds namely Linoleic acid (32.25%), Methyl Oleate (32.25%), Phytol (17.82%), Methylstearate (11.96%), Diphenylmethane (8%), 2-Phenazinamine (8%), Adenanthis (1.47%), Carbazole (1.45%), 5-Aminomethyl-dibenzosuberane (0.28%). These different phytochemicals have been found to possess a wide range of activities, which may help in the protection against incurable diseases.

**Keywords: Calophyllum inophyllum, GC-MS analysis, phytoconstituents, Methanol, Linoleic acid.**

**INTRODUCTION**

The plant kingdom represents a rich source of organic compounds, many of which have been used for medicinal and other purposes. Higher plants as sources of bioactive compounds continue to play a dominant role in the maintenance of human health. Plants are rich sources in secondary metabolites with interesting biological activities. In fact, many of the currently available drugs were derived either directly or indirectly from the plants. The genus *Calophyllum* belongs to the family Clusiaceae and is composed of about 180-200 different species confined to the warm humid tropics of the world. *Calophyllum inophyllum* L. is a tree that can grow 8-20 meter tall with a broad spreading crown of irregular branches which exudes white latex when bruised. The leaves have opposite arrangements, and are petiolate, thick and shiny with numerous parallel secondary veins. The flower is 25 mm wide and occurs in racemose or paniculate inflorescences consisting of 4 - 15 flowers. The fresh fruit and its oil were used externally against rheumatism, in topical infection and seborrhea in human adult. The dried leaf and its decoction used to cure rheumatism, skin infections, cuts and sores. The fresh leaf's infusion are used to cure bacterial infection, fungal infection and as vermifuge/pecidiulicide. Kashman and Patil reported that (+)- calanolide A and inophyll B isolated from *Calophyllum lanigerum* Miq. and *Calophyllum inophyllum* L. showed strong activity against Human Immuno Deficiency virus type 1 [HIV-1]. Five bioactive compounds isolated from *Calophyllum inophyllum* L., leaves namely calophyllic acid and isocalophyllic acid mixture, 3-oxofriedelin-28-oic acid, canophyllic acid, amentoflavone and shikimic acid showed dose dependent lipid lowering activity in *in vivo* experiments. Mass spectrometry, coupled with chromatographic separations such as Gas chromatography is normally used for direct analysis of components existing in traditional medicines and medicinal plants. Phytochemicals are responsible for medicinal activity of plants. In recent years GC-MS studies have been increasingly applied for the analysis of chinese medicinal plants as this technique has proved to be a valuable method for the analysis of non polar components and volatile essential oil, fatty acids and lipids. The present study is to identify the bioactive compounds from the methanol leaf extract of *Calophyllum inophyllum* using GC-MS analysis.

**MATERIALS AND METHODS**

Collection of Plant Material: The leaves of *Calophyllum inophyllum* were collected during month of September 2013, from Rajiv Gandhi Salai (OMR), in Chennai, Tamil nadu, India. The plant material was identified and authenticated by Department of Botany, Ramakrishna Mission Vivekananda College, Chennai, India. Herbarium voucher submitted.

Preparation of Leaf Extract: The fresh leaves were collected and washed with running tap water, chopped into small pieces and then kept in shade dry for 30 days and then grounded using electric blender. 50 gm of powdered leaves were extracted with 300ml of methanol in soxhelet apparatus for 6 hours. The extract was then concentrated at reduced pressure using rotary evaporator and stored in vials at 4°C until further analysis. GC-MS Analysis: GC-MS analysis was carried out on a
RESULTS

GC-MS is one of the significant methods used to identify the constituents of volatile matter, long and branched chain hydrocarbons, alcohols, acids, esters etc. The GC-MS analysis of *Calophyllum inophyllum* leaves showed the presence of nine compounds namely Methyl isostearate (RT-19.2), Phytol (RT-19.12), Methyl Oleate (RT-18.8), Linoleic acid (RT-18.72), 5-Aminomethyl-dibenzosuberane (RT-17.27), 2-Phenazinamine (RT-16.48), Diphenylmethane (RT-16.25), Carbazole (RT-15.65), Adenanthin (RT-15.17). The identification of the compounds was confirmed based on the peak area, retention time and molecular formula. The active principle with their Retention time (RT), Molecular formula, Molecular weight (MW) and Peak area in percentage are presented in Table 1, and Fig 1. The individual fragmentations of the components are illustrated in Fig 2A-21.

**DISCUSSION**

Traditional medicines are prepared from a single plant or combination of more than one plant. Indian contribution to herbal market and emphasis on novel research is continuously increasing. Phytochemical constituents are responsible for pharmacological activities of plant species. The phytochemical analysis of leaf extract of *Calophyllum inophyllum* showed presence of secondary metabolites such as Alkaloids, Carbohydrates, Glycosides,

<table>
<thead>
<tr>
<th>S.NO.</th>
<th>Retention Time(RT)</th>
<th>Name of the compounds</th>
<th>Molecular formula</th>
<th>Molecular weight</th>
<th>Peak area%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>15.17</td>
<td>Adenanthin</td>
<td>C_{26}H_{34}O_{9}</td>
<td>490.54</td>
<td>1.47%</td>
</tr>
<tr>
<td>2.</td>
<td>15.65</td>
<td>Carbazole</td>
<td>C_{12}H_{3}N</td>
<td>167.20</td>
<td>1.45%</td>
</tr>
<tr>
<td>3.</td>
<td>16.25</td>
<td>Diphenyl methane</td>
<td>C_{12}H_{12}</td>
<td>168.23</td>
<td>8.0%</td>
</tr>
<tr>
<td>4.</td>
<td>16.48</td>
<td>2-Phenazinamine</td>
<td>C_{12}H_{9}N_{3}</td>
<td>195.21</td>
<td>8.0%</td>
</tr>
<tr>
<td>5.</td>
<td>17.27</td>
<td>5-Aminomethyl-dibenzosuberane</td>
<td>C_{16}H_{17}N</td>
<td>223.32</td>
<td>0.28%</td>
</tr>
<tr>
<td>6.</td>
<td>18.72</td>
<td>Linoleic acid</td>
<td>C_{19}H_{36}O_{2}</td>
<td>294.47</td>
<td>32.25%</td>
</tr>
<tr>
<td>7.</td>
<td>18.80</td>
<td>Methyl Oleate</td>
<td>C_{19}H_{36}O_{2}</td>
<td>296.48</td>
<td>32.25%</td>
</tr>
<tr>
<td>8.</td>
<td>19.12</td>
<td>Phytol</td>
<td>C_{20}H_{36}O</td>
<td>296</td>
<td>17.82%</td>
</tr>
<tr>
<td>9.</td>
<td>19.20</td>
<td>Methyl isostearate</td>
<td>C_{15}H_{38}O_{2}</td>
<td>298.50</td>
<td>11.96%</td>
</tr>
</tbody>
</table>

Table 1: GC-MS profile of methanol leaf extract of *Calophyllum inophyllum*.
Saponins, Phenols, Tannins, Flavanoids, Proteins, Steroids and Coumarins. These secondary metabolites possess antioxidant and anticancer activities\(^\text{12}\).

Earlier reports of *Calophyllum inophyllum* leaf extract using alcoholic solvents by Malarvizhi 2011 yielded seventeen compounds\(^\text{13}\), in which two compounds are found similar to present study namely Linoleic acid, Phytol and seven different compounds were observed namely Adenanthin, Carbazole, Diphenylmethane, 2-Phenazinamine, 5-Aminomethyl-dibenzosuberane,
Methyl Oleate, Methyl isostearate. The difference in plant components from previous study might arise from solvent variation whereas, the present study of methanolic leaf extract exhibited important bioactive compounds which possess various biological activities.

Phytol is one among the nine compounds of the present study. Nanadagopalan 2015 reported the presence of Phytol in the leaves of Kigelia reticulata aerial parts, which was found to be effective in different stages of arthritis. The compound Linoleic acid is used against Antiinflammatory, Nematicide, Insecticide, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Antihistaminic, Antiacne, Antiarthritic, Antieczemic. Potterat 2005 has reported that a new carbazole alkaloid, named clausine Z, has been isolated from the stems and leaves of Calophyllum excavata.

From the present study it is concluded that the bioactive compounds thus identified from GC-MS analysis of the methanol leaf extract of Calophyllum inophyllum has justified the use of leaf extract for various ailments by traditional practitioners, further studies on isolation and identification of individual constituents are recommended.

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REFERENCES