INTRODUCTION

Canthium parviflorum (Syn.: Canthium coromandelicum) (Rubiaceae family) is a bushy thorny herb, found throughout the Western Ghats, and coast of the coromandel region of India. Canthium herbal medicine is used for the treatment of diabetes among major tribal groups in South India [1,2]. The leaves were used for wound healing and diuretic activity in animals [3] and gastrointestinal disorders such as diarrhea and constipation [4]. The root and leaves were used as diuretic, diarrhea, strangury, fever, leucorrhoea, and intestinal worm in children [5]. The bark is made into paste along with turmeric and lime and applied on the forehead to cure headache. The whole plant is used against diabetes, controls high blood pressure, reduce unwanted fats in the body, and as a blood purifier. C. parviflorum leaves have been reported to exhibit significant antimicrobial, anti-HIV activity [6], hypocholesterolemic activity [7], oral hypoglycemic activity, wound healing and diuretic activities, antioxidant properties, and antibacterial activity [8]. However, complete information on the phytochemical composition and its antioxidant stability is not very well studied. In the present experiment, the phytochemical composition by gas chromatography and mass spectrometry (GC-MS) of the C. parviflorum was reported.

METHODS

Plant materials

Leaves of C. parviflorum were collected from Siluvinippatti village, Sivagangai, Tamil Nadu, during the month of June - August 2016.

Preparation of extract

The leaves were washed thoroughly with tap water and in distilled water and then dried the leaves at room temperature. The dried leaves were ground to a fine powder in a mechanic grinder. About 2.0 g of powdered plant extracted with 200 ml of different solvents such as methanol, ethanol, and hexane. The extracts were filtered through Whatmann No.1 filter paper, and the solvent was removed by evaporating in a water bath, which gave rise to a solid mass of the extract.

Phytochemical screening

Phytochemical screening chemical tests were carried out on the various extracts using standard procedures to identify the phytoconstituents [9,10].

GC-MS

The ethanolic extract was subjected to GC-MS analysis on the instrument GC and MS JEOL GC mate equipped with secondary electron multiplier. JEOL GCmate II GC-MS with data system is a high resolution, double-focusing instrument. The column (HP5) was fused silica 50 m×0.25 mm i.d. Analysis conditions were 20 minutes at 100°C, 3 minutes at 235°C for column temperature and 240°C for injector temperature, helium was the carrier gas, and split ratio was 5:4. The sample (1 μl) was evaporated in a splitless injector at 300°C. Run time was 22 minutes. The components were identified by GC coupled with MS.

Identification of components

Interpretation on mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST08 and Wiley08 library. Identification of components was based on comparison of their mass spectra. As the compounds separated, on elution through the column, were detected in electronic signals. As individual compounds eluted from the gas chromatographic column, they entered the electron ionization detector where they were bombarded with a stream of electrons causing them to break apart into fragments. The fragments were actually charged ions with a certain mass. The m/z ratio obtained was calibrated from the graph obtained which was called as the mass spectrum graph which is the fingerprint of the molecule.

RESULTS AND DISCUSSION

The preliminary phytochemical analysis of C. parviflorum leaves containing bioactive chemicals such as alkaloids, flavonoids, steroids, terpenoids, and phenolic compounds (Table 1).

GC-MS analysis

GC-MS analysis was carried out on the ethanolic extract of C. parviflorum, and 8 compounds were identified. The chromatogram shows 8 prominent peaks and given in Fig. 1.

The identified chemical constituents and biological activities of identified chemical constituents are shown in Table 2.
The largest peak (RT 19.13) is due to the presence of phytol. The molecular formula and molecular weight of this compound are C_{20}H_{40}O and 296, respectively. Phytol is known to be antimicrobial, anticancer, anti-inflammatory and hepatoprotective [11]. Mass spectrum of phytol was shown in Fig. 2.

The second less prominent peak (RT 19.35) is due to the presence of octadecanoic acid, methyl ester. Mass spectrum of octadecanoic acid, methyl ester was shown in Fig. 3.

### Table 1: Phytochemical screening of *Canthium parviflorum* leaves

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Methanol extract</th>
<th>Ethanol extract</th>
<th>Hexane extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Present</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>Phenols</td>
<td>Present</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>Steroids</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
</tbody>
</table>

The third less significant peak (RT 17.3) is due to 4',5,7-trihydroxyisoflavone, known as genistein. Genistein is an angiogenesis inhibitor and a phytoestrogen and belongs to the category of isoflavones. Genistein was first isolated in 1899 from the dyer’s broom, Genista tinctoria; hence, the chemical name. The compound structure was established in 1926 when it was found to be identical with prunetol. It was chemically synthesized in 1928. Besides functioning as antioxidant and anthelmintic, genistein was, among other flavonoids, found to be a strong topoisomerase inhibitor, similarly to some chemotherapeutic anticancer drugs, for example, etoposide and doxorubicin [12,13]. In high doses, it was found to be strongly toxic to normal cells [14]. This effect may be responsible for both anticarcinogenic and carcinogenic potential of the substance [15,16]. Mass spectrum of 4',5,7-trihydroxyisoflavone was shown in Fig. 4.

The steroidal compound estra-1,3,5(10)-trien-17α'-ol is found to be responsible for the peak at RT 18.3. The mass spectrum of the compound showed the molecular ion peak at m/z 256 (Fig. 5). Molecular formula is C_{19}H_{26}O_{2} and molecular weight is 256.4085. This compound is found to show medicinal activity such as androgenic alopecia (hair loss).

Peak height 19.98 is responsible for (E)-9-octadecenoic acid, ethyl ester. Its molecular formula is C_{20}H_{38}O_{2} and molecular weight is 310. The mass spectrum of the compound showed the molecular ion peak at m/z 310 (Fig. 6).

Peak height 21.17 is responsible for eicosanoic acid, methyl ester. Molecular formula is C_{21}H_{42}O_{2} and molecular weight is 326. 9-octadecenoic acid (Z)-methyl ester found to be effective against fungi *Aspergillus flavus* [17]. The mass spectrum of the compound showed the molecular ion peak at m/z 326 (Fig. 7).

The peak value, i.e., RT 22.62 corresponds to (Z)-9-octadecenoic acid, butyl ester other name oleic acid, butyl ester. The mass spectrum of the compound showed the molecular ion peak at m/z 338.3965 (Fig. 8). Molecular formula is C_{22}H_{42}O_{2} and molecular weight is 338.5677.

The peak value, i.e., RT 24.87 corresponds to corynan-17-ol, 18,19-didehydro-10-methoxy-acetate. The mass spectrum of the compound showed the molecular ion peak at m/z 367.7373 (Fig. 9); molecular formula is C_{22}H_{28}N_{2}O_{3} and molecular weight is 368.47.
CONCLUSION

In the present study, 8 phytoconstituents were identified from ethanol extract of the *C. parviflorum* by GC-MS analysis. Phytochemical analysis of *C. parviflorum* leaves extracts revealed the presence of various biochemical compounds such as flavonoids, alkaloids, terpenoids, steroids, and phenolic compounds. This plant-derived bioactive compounds used as source of antibiotic, antioxidant, anti-inflammatory, anticancer properties, and pharmaceutical industries used for drug formulation [18]. This plant is widely used in traditional medicinal system of India. Due to medicinal properties, there is enormous scope for future research on *C. parviflorum*, and further clinical and pharmacological investigation should be conducted to investigate unexploited potential of this plant.

Table 2: Phytocomponents identified in the ethanolic extract of *C. parviflorum* by GC-MS

<table>
<thead>
<tr>
<th>S. No.</th>
<th>RT</th>
<th>Name of the phytocompounds</th>
<th>Biological activity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17.4</td>
<td>4’,5,7-trihydroxyisoflavone</td>
<td>Antioxidant, anthelmintic, anticancer, atherosclerosis</td>
</tr>
<tr>
<td>2</td>
<td>18.3</td>
<td>Estra-1,3,5(10)-trien-17a’-ol</td>
<td>Androgenic alopecia (hair loss)</td>
</tr>
<tr>
<td>3</td>
<td>19.13</td>
<td>Phytol</td>
<td>Antimicrobial, anticancer, anti-inflammatory, hepatoprotective</td>
</tr>
<tr>
<td>4</td>
<td>19.35</td>
<td>Octadecanoic acid, methyl ester</td>
<td>Antioxidant, Antimicrobial</td>
</tr>
<tr>
<td>5</td>
<td>19.98</td>
<td>(E)-9-octadecenoic acid, ethyl ester</td>
<td>Antioxidant, anti-inflammatory</td>
</tr>
<tr>
<td>6</td>
<td>21.17</td>
<td>Eicosanoic acid, methyl ester</td>
<td>Alpha-glucosidase inhibitors</td>
</tr>
<tr>
<td>7</td>
<td>22.62</td>
<td>(Z)-9-octadecenoic acid, butyl ester</td>
<td>Antimicrobial</td>
</tr>
<tr>
<td>8</td>
<td>24.87</td>
<td>Corynan-17-ol, 18,19-didehydro-10-methoxy-acetate (ester)</td>
<td>Antacids, anti-inflammatory</td>
</tr>
</tbody>
</table>

*Activity source: Dr. Duke’s Phytochemical and Ethnobotanical Database
Fig. 5: Mass spectrum of estra-1,3,5(10)-trien-17α'-ol

Fig. 6: Mass spectrum of (E)-9-octadecenoic acid, ethyl ester

Fig. 7: Mass spectrum of eicosanoic acid, methyl ester
ACKNOWLEDGMENT

The authors wish to thank Sophisticated Analytical Instrument Facility (SAIF), Indian Institute of Technology Madras (IITM), Chennai, for GC-MS analysis.

REFERENCES


