Phytochemical screening, GC-MS analysis of Decalepis hamiltonii Wight & Arn. An endangered medicinal plant

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Abstract
The present paper reports the phytochemical and GC-MS analysis studies of an endemic endangered, climbing shrub, Decalepis hamiltonii. D. hamiltonii which belongs to the family Asclepiadaceae is a perennial slow growing medicinal shrub commonly called as “Maredu kommulu, Nannari kommu”. It is generally considered as a tubers root food mostly in the southern part of India. There is a growing demand for roots of D. hamiltonii in the pharmaceutical trade due to its use as an anti-inflammatory, degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, neurodegenerative diseases and others. In the present study the qualitative and analysis confirmed the presence of various phytochemicals like alkaloids, flavonoids, phenols, steroids and terpenoids. Quantitative estimation of flavonoids and phenols was also carried out and further analysis of the components present in it by GC-MS analysis. The roots were sequentially extracted by methanol. The extract showed the presence of all phytoconstituents studied. The GC-MS analysis of the methanolic extract revealed the presence of ten major compounds. This study forms a basis for the biological characterization and importance of the compounds identified.

Keywords: Phytochemical analysis, root extract, qualitative and quantitative analysis, GC-MS, Decalepis hamiltonii

1. Introduction
Indian traditional system of medicine is based on various systems of medicine such as Ayurveda, Siddha, Unani and Homoeopathy. During the last few years the graph of standardization of medicinal plants of potential therapeutic significance has been increased. The evaluation of all medicinal plants is based on phytochemical and pharmacological approaches which lead to drug discovery and it is referred to as “natural product screening” [1]. Secondary products from the plants are responsible for its action or pharmacological activity [2, 3]. Decalepis hamiltonii Wight & Arn. The species is endemic and endangered to peninsular India geographical distribution of D. hamiltonii is in southern India and rare in evergreen forests of Western Ghats and commonly called as maredu kommulu or barre sugandhi or maradu gaddalu or makali beru belonging to the family Asclepiadaceae [4, 5]. It has been recorded in the dry and moist deciduous forests of Karnataka, Andhra Pradesh and Tamil Nadu. Its roots have been used in Ayurveda, the ancient Indian traditional systems of medicine to stimulate appetite, skin diseases, diaphoretic, hemorrhoids, rheumatism, asthma, bronchitis, somatic and antiviral and as a general tonic [6]. It is also useful as a blood purifier, preservative, diarrhoea, respiratory disorders, fever, bronchitis, asthma, eye diseases, urinary disorders, loss of appetite, burning sensation and rheumatism and especially for epileptic fits in children and as a source of bio insecticide for stored food grains [7]. Earlier studies have shown that roots contains aldehyde, inositols, amyrins and lupeols [8] as well as volatile compounds such as 2-hydroxy-4-methoxy benzaldehyde, vanillin, 2-phenylethyl alcohol, benzaldehyde and others [9]. The plant has a use in many medicinal preparations and due to this there is a heavy demand for it. As the whole plant is uprooted from its natural habitat for the use of the herbal drug industry its numbers are decreasing drastically in the natural population. To meet the huge demand for its supply, there is a need to develop a specific technology for production of D. hamiltonii in a large scale. The maintenance of genetic purity is a limitation for large scale cultivation. Decalepis is one of the most important medicinally important medicinal plants whose potential medicinal properties or related information of all the species of Decalepis over its range of distribution, current status and the role of biotechnology in the conservation of this important genus. The roots of D. hamiltonii are little bitter and then sweet.
It is so characteristic with a familiar lingering after taste and smell of vanillin, the substance that is in *Vanilla planifolia*, an orchid used in ice-creams, chocolates, drinks etc. Although vanillin has been synthesized since 1874 natural source of this flavoring are still in demand and the roots of Decalepis species can be used as substitute for vanillin \[^{10}\]. The present study of GC-MS analysis and phytochemical investigation of an endangered medicinal plant *D. hamiltonii* has been taken up to carry out gas chromatography and mass spectra analysis of root extract and qualitative phytochemical analysis for alkaloids, flavonoids, tannins, saponins, phenols, steroids, terpenoids and glycosides present in roots, stem and leaves.

**Materials and methods**

**Material**

*Decalepis hamiltonii* plants collected from Botanical garden Department of Botany, University College of Science, Saifabad, *D. hamiltonii* is a slow growing, perennial woody climber of tropical and subtropical regions with a twining woody stem and opposite petiolate leaves, entire, smooth shiny, varying in shape. Flowers are small, in axillary sessile racemes. The root is long, rigid and cylindrical. These plants were subjected to phytochemical investigation studies (Qualitative and Quantitative) and GC-MS analysis and for the presence of important secondary metabolite compounds.

**Preparation of extracts**

Plant samples root, leaves and stem were washed with distilled water and air-dried at room temperature for 7-10 days, then oven-dried at 40 °C to remove the residual moisture. The dried plant parts were pulverized and stored in air-tight containers at 4 °C for future use. 50 g of powdered samples of gum were extracted with methanol by soxhlation method at 60 to 80 °C. The three filtrates were separately concentrated in water bath at 40 °C and evaporated under reduced pressure.

**Phytochemical analysis**

The extracts obtained from the powdered root, leaves and stem of *D. hamiltonii* were subjected to phytochemical tests to determine the presence of active secondary metabolites using standard procedures. This extract was filtered through a fine mesh into a test tube. This crude extract was used for the phytochemical investigation of secondary metabolites, GC-MS tests given below and the tests were carried out in triplicate.

**Qualitative analysis**

**Test for identification of Alkaloids**

About 0.5 gm of methanol extract was taken in a test tube and was diluted and homogenized with 10 ml distilled water, dissolved in 20 ml dilute HCl solution and clarified by filtration. The filtrate was tested with Dragendorff's and Mayer's reagent. The treated solution was observed for precipitation of white or creamy colour.

**Test for identification of Flavonoids**

About 0.5 gm of extract was introduced into 10 ml of ethyl acetate in a test tube and heated in boiling water for 1 min. The mixture was then filtered. About 4 ml of the filtrate was shaken with 1 ml 1% aluminium chloride solution and incubated for 10 min. Formation of yellow colour in the presence of 1 ml dilute ammonia solution indicated the presence of flavonoids.

**Test for identification of Phenols**

About 0.5 gm of extract was taken in a test tube, mixed with 100ml distilled water and heated gently. To this, 2 ml of ferric chloride solution was added and observed for the formation of green or blue colour.

**Test for identification of Saponins**

About 0.5 gm of methanol extract was taken in a test tube and 5 ml distilled water was added to it. The solution was shaken vigorously and observed for persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

**Test for identification of Steroids**

About 0.5 gm of methanol extract was taken in a test tube and 2 ml of acetic anhydride was added to it and 2 ml of sulphuric acid was added by the sides of the test tube and observed for the colour change to violet or blue green.

**Test for identification of Tannins**

Five grams of the ground powder was extracted with 10 ml ammoniacal chloroform and 5 ml chloroform. The mixture was filtered and the filtrate was shaken with 10 drops of 0.5 M sulphuric acid. Creamish white precipitate was observed for the presence of tannins.

**Test for identification of Terpenoids**

5 ml of the methanol extract was mixed with 2 ml of chloroform and 2ml concentrated sulphuric acid to form a layer. A reddish brown coloration of the interface showed the presence of Terpenoids.

**Quantitative analysis**

Quantitative analysis was carried out to estimate total flavonoids and total phenols.

**Determination of total flavonoids**

Aluminium chloride - colorimetric method \[^{11}\] with some modifications was used to determine flavonoid content. 1.0 ml root extract was mixed with 1.0 ml methanol, 0.5 ml aluminium chloride (1.2%) and 0.5 ml potassium acetate (0.1176%). The mixture was allowed to stand for 30 min at room temperature. Later the absorbance was measured at 415 nm. Quercetin was used as standard. Flavonoid content is expressed in terms of quercetin equivalent (mg/g of extracted compound).

**Determination of total phenols**

Total phenolic content of the extracts was determined by Folin Ciocalteu reagent method \[^{12}\] with some modifications. The root extract (1.0 ml) was mixed with Ciocalteu reagent and allowed to stand for 15 min and 5 ml of saturated Na2CO3 was added. The mixture was allowed to stand for 30 min at room temperature and the total phenols were determined spectrophotometrically at 760 nm. Gallic acid was used as a standard. Total phenol values are expressed in terms of gallic acid equivalent (mg/ g of extracted compound).

**GC-MS Analysis**

GC-MS analyses of methanol extract were performed using a Shimadzu QP2010 Gas-Chromatography–Mass spectroscopy. It employed a fused silica column packed with Elite -5 ms [5% Diphenyl 95% Dimethyl poly siloxane, 30 mm × 0.25 mm × 0.25 μm df] and the components were separated using

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helium as carrier gas at a constant flow of 1 ml / min. The 2 µl sample extract injected to the instrument. It was detected by the turbo gold mass detector with aid of Turbo mass 5.2 software. During the GC Process the oven was maintained at temperature of 110 °C with 2 min holding. The injector temperature was set at 250 °C. The inlet line temperature was 200 °C and source temperature was 200 °C. Mass spectra were taken at 70 eV, a scan period of 0.5 S and fragment from 45 - 450 Da. The MS detection was completed in 36 min. Interpretation on mass spectrum GC-MS was conducted using the database of National Institute standard and technology (NIST and WILEY) having more than 62,000 patterns. The spectrum of unknown components stored in the NIST and WILEY library.

Results and discussion
The present study contributes valuable information of bioactive compounds in *Decalepis hamiltonii*. Qualitative analysis of plant extract was carried out for Alkaloids, Flavonoids, Phenols, Steroids, Tannins, Terpenoids, Saponins and Glycosides. All of the phytochemicals like Alkaloids, Flavonoids, Phenols, Steroids, Tannins, Terpenoids, Saponins and Glycosides were present in root extract and Alkaloids, Flavonoids, Phenols, Steroids and Terpenoids were present in leaves and stem extract of *D. hamiltonii* except Saponins, Glycosides and Tannins (Fig-3, Table-1) which is similar to the reports of Asclepiadaceae family [13, 14, 15]. The plant extracts were quantitatively analyzed for Flavonoids and Phenol (Table-2). Whereas, our study reports the absence of Saponins [16]. Indicated that Saponins were present in *D. hamiltonii* in the methanol extract. Several medicinal properties have been attributed to Saponins [16] but surprisingly, Saponins were not found in the present study. Flavonoids and Phenol are however reported in the present study which agrees with the findings of [16, 17] who has attributed anti-diabetic, anti-aging anti-inflammation and bactericidal effects.

Gas chromatography coupled mass spectrometry (GC-MS) is an analytical method that combines the features of gas chromatography and mass spectrometry to identify different substances within a test sample. The GC-MS analysis of methanol extracts was performed using a Shimadzu QP-2010 Gas- Chromatography –Mass spectroscopy. Analysis on GCMS was carried out with reference to NIST and WILEY library at Central analytical facility University College of Technology, Osmania University, Hyderabad, containing more than 62000 patterns. The spectra of unknown compounds were compared with spectra of known compounds stored in identification of compounds was confirmed based on the active principle, Molecular Weight (MW), Concentration (%), Retention Time (RT), Molecular Formula (MF) and Peak Area (PA) is presented in (Fig-4 and Table-3). More than ten major compounds were identified in the extract being Furfural, Methyl-2-Furoate, 2-hydroxy-4-methoxy benzaldehyde, Vanillin, Tetradecane, Diethyl Phthalate, Hexadecane, Carbromal, Lupeol, Norolean-12-En respectively along with other minor constituents. The identified compounds in the roots of methanolic extract of *D. hamiltonii*. These similar studies was conducted on different parts of *D. hamiltonii* [13, 18]. The identified compounds in the roots of methanolic extract of *Decalepis hamiltonii* possess many biological properties. Among the identified phytochemicals, phenolic compound have the property of antioxidant and antifungal activity study as situation has forced to search new antimicrobial substances in various sources like medicinal plants [19].

![Fig 3: Test results of qualitative analysis of phytochemical constituents of the methanol extract of *D. hamiltoni*](image)

A: Test results roots extract, B: Test results stem extract and C: Test results leaves extract.

### Table 1: Qualitative analysis of phytochemical constituents of the methanol extract of roots, leaves and stem from *D. hamiltoni*.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Test for Phytochemicals</th>
<th>Test results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>+ve</td>
</tr>
<tr>
<td>2</td>
<td>Flavonoids</td>
<td>+ve</td>
</tr>
<tr>
<td>3</td>
<td>Phenols</td>
<td>+ve</td>
</tr>
<tr>
<td>4</td>
<td>Steroids</td>
<td>+ve</td>
</tr>
<tr>
<td>5</td>
<td>Tannins</td>
<td>+ve</td>
</tr>
<tr>
<td>6</td>
<td>Terpenoids</td>
<td>+ve</td>
</tr>
<tr>
<td>7</td>
<td>Saponins</td>
<td>+ve</td>
</tr>
<tr>
<td>8</td>
<td>Glycosides</td>
<td>+ve</td>
</tr>
</tbody>
</table>

+ ve Presence of the compound.
- ve Absence of the compound.
Table 2: Quantitative analysis of the methanol root extracts of *D. hamiltoni* for estimation of Flavonoids and Phenols.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Plant extract</th>
<th>Phytochemicals</th>
<th>Average Estimated value (mg/gm) (Mean±S.E)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Root</td>
<td>Flavonoids</td>
<td>7.95±0.85</td>
</tr>
<tr>
<td>2.</td>
<td>Root</td>
<td>Phenols</td>
<td>14.22±0.96</td>
</tr>
</tbody>
</table>

* Phenols are expressed as Gallic acid equivalent (GAE) and Flavonoids are expressed as Quercetin equivalents (QE) in mg/100 gm.

Table 3: Components detected in the root of methanol extract of *Decalepis hamiltonii*.

<table>
<thead>
<tr>
<th>S. No</th>
<th>RT</th>
<th>Name of the component</th>
<th>Molecular formula</th>
<th>MW</th>
<th>Peak of Area %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.800</td>
<td>Furfural</td>
<td>C₅H₄O₂</td>
<td>96</td>
<td>6.47</td>
</tr>
<tr>
<td>2</td>
<td>4.611</td>
<td>Methyl-2-Furoate</td>
<td>C₆H₆O₂</td>
<td>101</td>
<td>0.22</td>
</tr>
<tr>
<td>3</td>
<td>8.047</td>
<td>2-hydroxy-4-methoxy benzaledhyde</td>
<td>C₈H₁₀O₃</td>
<td>152</td>
<td>21.48</td>
</tr>
<tr>
<td>4</td>
<td>9.777</td>
<td>Vanillin</td>
<td>C₅H₁₀</td>
<td>152</td>
<td>2.68</td>
</tr>
<tr>
<td>5</td>
<td>10.487</td>
<td>Tetradecane</td>
<td>C₁₀H₂₀</td>
<td>198</td>
<td>0.36</td>
</tr>
<tr>
<td>6</td>
<td>11.505</td>
<td>Diethyl Phthalate</td>
<td>C₁₂H₁₄O₄</td>
<td>222</td>
<td>1.18</td>
</tr>
<tr>
<td>7</td>
<td>11.658</td>
<td>Hexadecane</td>
<td>C₁₆H₃₂</td>
<td>226</td>
<td>0.59</td>
</tr>
<tr>
<td>8</td>
<td>40.858</td>
<td>Carbromal</td>
<td>C₆H₇BrN₂O₂</td>
<td>236</td>
<td>0.53</td>
</tr>
<tr>
<td>9</td>
<td>41.706</td>
<td>Luteol</td>
<td>C₁₃H₂₀O₂</td>
<td>468</td>
<td>0.56</td>
</tr>
<tr>
<td>10</td>
<td>42.359</td>
<td>Norolean-12-Ene</td>
<td>C₁₀H₁₆</td>
<td>396</td>
<td>29.23</td>
</tr>
</tbody>
</table>

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Conclusion
It is concluded that *Decalepis hamiltonii* is a plant with a variety of ethnic medicinal uses. The qualitative and quantitative analysis of *Decalepis hamiltonii* shows the presence of bioactive compounds such as Alkaloids, Flavonoids, Phenols, Tannins, Terpenoids and Glycosides. This is valuable information for preparation of drugs in pharmaceutical industry and stress the need for more intensive research since they play a great role in healthcare. The present study describes of GC-MS analysis of methanol extract of root of *D. hamiltonii* showed the presence of 10 bioactive components Furfural, Methyl-2-Furoate, 2-hydroxy-4-methoxy benzaldehyde, Vanillin, Tetradecane, Diethyl Phthalate, Hexadecane, Carbromal, Lupeol, Norolean-12-En. which suggests the contribution of these compounds on pharmacological activity. These active principles provide inspiration for further investigation in the discovery of novel herbal drugs. Hence, the roots, of *D. hamiltonii* might be utilized for the development of traditional medicines and further investigation is in need to elute novel active compounds which may create the new way to treat many incurable diseases.

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Reference
