Preliminary phytochemical and gas chromatography–mass spectrometry study of one medicinal plant *Carissa carandas*

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**INTRODUCTION**

Plants are major sources of medicines in organized (Ayurveda, Siddha, and Unani, Allopathy) and unorganized (folk, tribal, and native) forms of medicinal practice. The knowledge of the biomolecules present in the plants with medicinal properties is ever growing.¹⁻⁴ Numerous reports on the phytochemical and gas chromatography–mass spectrometry (GC–MS) analysis studies of many plants and plant parts are available indicating the presence of such biomolecules.⁵⁻¹⁵ The present study deals with the phytochemical and GC–MS analysis of the various leaf extracts of one such medicinal plant, *Carissa carandas* L.

*Carissa carandas* L. is a thorny bush with small edible berries belonging to the family Apocynaceae.

**ABSTRACT**

**Objective:** The present study deals with the preliminary phytochemical and gas chromatography–mass spectrometry (GC–MS) analysis of different leaf extracts of one medicinal *Carissa carandas*. **Materials and Methods:** The phytochemical and GC–MS study of one medicinal plant, *C. carandas* was done as per standard protocols to correlate its medicinal activity with the biomolecules present in it. **Results:** The methanol extract of *C. carandas* indicated the presence of flavonoids, tannins, saponins, and steroids, whereas flavonoids, alkaloids, saponins, and steroids were present in hexane fraction. Alkaloids, saponins, proteins, and triterpenoids were present in aqueous extract. The GC–MS analysis of hexane and aqueous leaf extracts indicated the presence of some important biomolecules such as pentadecanoic acid, 14-methyl-, methyl ester, 9,12,15-octadecatrienoic acid, (Z, Z, Z)-Phytol, Bis(2-ethylhexyl) phthalate, Squalene, Octasiloxyane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl, beta-Sitosterol, 9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyloxy)-1-[(trimethylsilyloxy)methyl] ethyl ester, (Z, Z, Z)-Acetamide, N-methyl-N-[4-(3-hydroxyprpyrolidinyl)-2-butynyl]-, 5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z) and 6-Octadecenoic acid, methyl ester, (Z). **Conclusions:** The presence of these molecules augurs well with the medicinal properties attributed to this plant ethnobotanical. Further work is in progress toward understanding this plant’s role as a medicine.

**KEY WORDS:** Alkaloid, *Carissa carandas*, Flavonoid, Gas chromatography–mass spectrometry, Phytochemical, Phytol, Squalene, Tannin
MATERIALS AND METHODS

Collection of Samples

Fresh leaves of *C. carandas* were collected from the hilly areas near Chengalpattu, Tamil Nadu, India. The leaves were thoroughly washed to remove any dust and impurities and shade dried. The dried leaves were ground to fine powder.

Preparation of Extracts

About 500 g of dried powder of *C. carandas* was packed in separate round bottom flask for sample extraction using hexane, methanol, and distilled water as solvents. The extraction was conducted by 750 ml of the solvent for a period of 72 h. At the end of the extraction, the solvent was concentrated under reduced pressure and the crude extracts were stored in refrigerator till further analysis.

Phytochemical Analysis

The extracts prepared were analyzed for the presence of alkaloids, saponins, tannins, steroids, flavonoids, anthraquinones, triple sugars, amino acids, proteins, glycosides, and reducing sugars as per standard protocols.\(^{[30-32]}\)

**Test for Alkaloids**

About 0.5 g of the prepared residue was dissolved in 2 N hydrochloric acids. The mixture was filtered and the filtrate was divided into two equal portions. One portion was treated with a few drops of Mayer’s reagent and the other was treated with equal amount of Dragendorff’s reagent, respectively. The appearance of creamish precipitate and orange precipitate, respectively, indicated the presence of alkaloids.

**Test for Saponins**

About 0.5 g of the plant leaf extract was vigorously shaken with water in a test tube and then heated to boil. Frothing was observed which was taken as evidence for the presence of the saponins.

**Test for Tannins**

About 0.5 g of plant leaf extract was added in 10 ml of water in a test tube and filtered. A few drops of 0.1% ferric chloride were added and observed for brownish-green or blue-black coloration.

**Test for Steroids**

About 2 ml of acetic anhydride was added to 2 ml of plant leaf extract along with 2 ml conc. sulfuric acid. The color change from violet to blue or green is observed for the presence of steroids.

**Test for Flavonoids**

About 2 ml of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, few drops of conc. hydrochloric acid were added and the red color was observed for flavonoids and orange color for flavones.

**Test for Anthraquinones**

About 0.5 g of extract was taken in a dry test tube and 5 ml of chloroform was added and shaken for 5 min. The extract was filtered and the filtrate shaken with equal volume of 10% of ammonia solution. A pink violet or red color in the ammonical layer was observed for the presence of anthraquinones.

**Test for Cardiac Glycosides**

About 0.2 g of extract was dissolved in 1 ml of glacial acetic acid containing 1 drop of ferric chloride solution. This was then under layered with 1 ml of concentrated sulfuric acid. A brown ring obtained at the interface indicated the presence of cardiac glycosides.

**Test for Amino Acids**

To 2 ml of sample was added to 2 ml of Ninhydrin reagent and kept in water bath for 20 min. Appearance of purple color indicated the presence of amino acids in the sample.

**Test for Proteins**

To 2 ml of the extract solution, 1 ml of 40% NaOH solution and 1–2 drops of 1% CuSO\(_4\) solution were added. A violet color indicated the presence of peptide linkage of the molecule.

**Test for Triterpenoids**

About 5 ml of each extract was added to 2 ml of chloroform and 3 ml of con. H\(_2\)SO\(_4\) to form a monolayer of reddish-brown coloration of the interface was showed to form positive result for the triterpenoids.

**Test for Triple Sugar**

To 2 ml of extract, 2 drops of Molisch’s reagent were added and shaken well. About 2 ml of con. H\(_2\)SO\(_4\) was added on the sides of the test tube. A reddish-violet ring appeared at the junction of two layers immediately indicated the presence of triple sugars.

The GC–MS analysis was performed by standard procedures. The results obtained were tabulated and the medicinal values of the important compounds present were collected from various sources such as Dr. Duke’s Phytochemical and Ethnobotanical Database, NIST Chemical Library and available literature.

RESULTS

The phytochemical analysis results of methanol, hexane, and water extracts of the leaves of *C. carandas* are tabulated in Table 1. GC–MS graph of *C. carandas*
(hexane fraction-AC-1) is shown in Figure 1 and that of aqueous extract is shown in Figure 2. The results of GC–MS are tabulated in Table 2, indicating the retention time, mol. formula, peak percentage value, and reported medicinal roles for the hexane fraction. The GC–MS report is shown in Figure 2. The results of GC–MS are tabulated in Table 3, indicating the retention time, mol. formula, peak percentage value, and reported medicinal roles for the aqueous fraction of C. carandas leaf extracts.

**DISCUSSION**

The phytochemical analysis of methanol, hexane, and aqueous leaf extracts C. carandas indicated the following results. Flavonoids, tannins, saponins, and steroids were present in methanolic extract. Flavonoids, alkaloids, saponins, and steroids were present in hexane fraction, whereas alkaloids, saponins, proteins, and triterpenoids were present in aqueous extract.

The compounds present in the hexane and aqueous extracts of as found in the GC–MS analysis are tabulated in Table 1:

<table>
<thead>
<tr>
<th>Phytochemical</th>
<th>Methanol</th>
<th>Hexane</th>
<th>Aqueous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Triple sugar</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Amino acid</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Anthraquinone</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Proteins</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Triterpenoids</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
</tbody>
</table>

+: Present, −: Absent

**Table 1: Preliminary phytochemical constituents of methanol, hexane, and aqueous extracts of Carissa carandas**

**Figure 1:** Gas chromatography–mass spectrometry graph of Carissa carandas (hexane fraction-AC-1)

**Figure 2:** Gas chromatography–mass spectrometry graph of Carissa carandas (aqueous fraction-AC-2 charged with methanol)
Table 2: The retention time, mol. formula, peak percentage value, and reported medicinal roles of various compounds present in the aqueous extracts of the leaves of *Carissa carandas*

<table>
<thead>
<tr>
<th>Retention time</th>
<th>Compound</th>
<th>Mol. formula</th>
<th>% peak value</th>
<th>Medicinal role</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.54625</td>
<td>Cyclohexanone, 2-cyclohexylidenez</td>
<td>C₆H₁₂O₂</td>
<td>14.27</td>
<td>Not known</td>
</tr>
<tr>
<td>5.68375</td>
<td>Cyclohexanone, 2-cyclohexylidenez</td>
<td>C₆H₁₂O₂</td>
<td>14.27</td>
<td>Not known</td>
</tr>
<tr>
<td>5.84591</td>
<td>Cyclohexanone</td>
<td>C₆H₁₂O₂</td>
<td>14.27</td>
<td>Not known</td>
</tr>
<tr>
<td>6.0551</td>
<td>1,1'-Bicyclohexyl[2]-one, 1'-hydroxyl</td>
<td>C₆H₁₂O₂</td>
<td>27.78</td>
<td>Not known</td>
</tr>
<tr>
<td>8.12066</td>
<td>Pentadecanoic acid, 14-methyl-, methyl ester</td>
<td>C₁₉H₃₈O₂</td>
<td>1.46</td>
<td>Antioxidant</td>
</tr>
<tr>
<td>9.57341</td>
<td>9,12,15-Octadecatrienoic acid, (Z, Z, Z)-</td>
<td>C₁₉H₃₈O₂</td>
<td>1.82</td>
<td>Anti-inflammatory, hypocholesteremic, cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic</td>
</tr>
<tr>
<td>9.6601</td>
<td>Phytol</td>
<td>C₂₀H₄₀O</td>
<td>13.72</td>
<td>Antimicrobial</td>
</tr>
<tr>
<td>13.02591</td>
<td>Bis(2-ethylhexyl) phthalate</td>
<td>C₈H₁₆(CO)₂C₆H₁₂</td>
<td>11.76</td>
<td>Used for plastic medical instruments, Monoxygenase inhibitor, biochemical precursor in the preparation of steroids, natural moisturizer, used in cosmetics</td>
</tr>
<tr>
<td>15.09325</td>
<td>Squalene</td>
<td>C₂₆H₅₀</td>
<td>7.85</td>
<td>Skin ointments, steroid precursor</td>
</tr>
<tr>
<td>17.77878</td>
<td>Octasiloxane, 1,1,3,3,5,5,7,7,7,9,11,11,13,13,15,15-hexadecamethyl-</td>
<td>C₁₅H₃₀O₇Si₅</td>
<td>1.17</td>
<td>Antimicrobial</td>
</tr>
<tr>
<td>17.93521</td>
<td>Octasiloxane, 1,1,3,3,5,5,7,7,7,7,9,11,11,13,13,15,15-hexadecamethyl-</td>
<td>C₁₅H₃₀O₇Si₅</td>
<td>1.14</td>
<td>Antimicrobial</td>
</tr>
<tr>
<td>18.32533</td>
<td>Octasiloxane, 1,1,3,3,5,5,7,7,7,7,9,11,11,13,13,15,15-hexadecamethyl-</td>
<td>C₁₅H₃₀O₇Si₅</td>
<td>7.41</td>
<td></td>
</tr>
<tr>
<td>18.87755</td>
<td>Octasiloxane, 1,1,3,3,5,5,7,7,9,11,11,13,13,15,15-hexadecamethyl-</td>
<td>C₁₅H₃₀O₇Si₅</td>
<td>1.47</td>
<td>Antimicrobial</td>
</tr>
</tbody>
</table>

Table 3: The retention time, mol. formula, peak percentage value, and reported medicinal roles of various compounds present in the aqueous extracts of the leaves of *Carissa carandas*

<table>
<thead>
<tr>
<th>Retention time</th>
<th>Compound</th>
<th>Mol. formula</th>
<th>% peak value</th>
<th>Medicinal role</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.4984333</td>
<td>9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl) oxy]-1-[(trimethylsilyl) oxy] methyl ethyl ester, (Z, Z, Z)-</td>
<td>C₁₅H₃₄O₂Si₂</td>
<td>1.76</td>
<td>Antioxidant, antiabetic, anti-inflammatory</td>
</tr>
<tr>
<td>4.6831333</td>
<td>9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl) oxy]-1-[(trimethylsilyl) oxy] methyl ethyl ester, (Z, Z, Z)-</td>
<td>C₁₅H₃₄O₂Si₂</td>
<td>6.74</td>
<td>Antioxidant, antiabetic, anti-inflammatory</td>
</tr>
<tr>
<td>5.09775</td>
<td>9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl) oxy]-1-[(trimethylsilyl) oxy] methyl ethyl ester, (Z, Z, Z)-</td>
<td>C₁₅H₃₄O₂Si₂</td>
<td>2.07</td>
<td>Antioxidant, antiabetic, anti-inflammatory</td>
</tr>
<tr>
<td>5.5444</td>
<td>Pyrazole[4,5-b] imidazole, 1-formyl-3-ethyl-6-, beta.-d-ribofuranosylz</td>
<td>C₁₅H₂₉O₅Si₂</td>
<td>2.26</td>
<td>Not known</td>
</tr>
<tr>
<td>5.9835</td>
<td>9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl) oxy]-1-[(trimethylsilyl) oxy] methyl ethyl ester, (Z, Z, Z)-</td>
<td>C₁₅H₃₄O₂Si₂</td>
<td>3.76</td>
<td>Antioxidant, antiabetic, anti-inflammatory</td>
</tr>
<tr>
<td>6.0419333</td>
<td>N-[4-(4-Chlorophenyl)-isothiazol-5-yl]-1-methylpiperidin-2-imine</td>
<td>C₁₅H₁₆ClNS</td>
<td>3.76</td>
<td>Not known</td>
</tr>
<tr>
<td>7.1424833</td>
<td>N-methyl-N-[4-(3-hydroxypropylidinyl)-2-butynyl]-5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-</td>
<td>C₁₅H₁₄N₂O₂</td>
<td>7.21</td>
<td>Anti-inflammatory activities</td>
</tr>
<tr>
<td>8.0263</td>
<td>Undecanoic acid, 11-bromo-, methyl ester</td>
<td>C₁₅H₂₃O₂Br</td>
<td>4.45</td>
<td>Not known</td>
</tr>
<tr>
<td>8.1506667</td>
<td>Undecanoic acid, 11-bromo-, methyl ester</td>
<td>C₁₅H₂₃O₂Br</td>
<td>11.88</td>
<td>Ingredient of skin protection, antifungal, Antihyperlipidemia and antithrombosis</td>
</tr>
<tr>
<td>9.523833</td>
<td>9,12-Octadecadienoic acid (Z, Z)-</td>
<td>C₁₅H₂₈O₂</td>
<td>28.92</td>
<td>Not known</td>
</tr>
<tr>
<td>9.5790</td>
<td>6-Octadecenoic acid, methyl ester, (Z)-</td>
<td>C₁₅H₂₈O₂</td>
<td>18.85</td>
<td>Anti-inflammatory, antihyperlipidemia</td>
</tr>
<tr>
<td>9.7939</td>
<td>9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl) oxy]-1-[(trimethylsilyl) oxy] methyl ethyl ester, (Z, Z, Z)-</td>
<td>C₁₅H₃₄O₂Si₂</td>
<td>2.72</td>
<td>Antioxidant, antiabetic, anti-inflammatory</td>
</tr>
</tbody>
</table>
were not same. The important compounds with medicinal values present in hexane extract were Pentadecanoic acid, 14-methyl-, methyl ester, 9,12,15-Octadecatrienoic acid, (Z, Z, Z)-, Phytol, Bis(2-ethylhexyl) phthalate, Squalene, Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl, and beta-Sitosterol as shown in Table 2.

The various biomolecules present in aqueous extracts were 9,12,15-Octadecatrienoic acid, 2-[(3-methylisilyloxy)-1-[(3-methylisilyloxy)methyl]ethyl ester, (Z, Z, Z)-, Acetamide, N-methyl-N-[4-(3-hydroxyprrolidinyl)-2-butynyl]-, 5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-, and 6-Octadecenoic acid, methyl ester, (Z)- as shown in Table 3.

The medicinal properties of some of the compounds are elaborated below.

9,12,15-Octadecatrienoic acid, (Z, Z, Z)-, anticancer, antioxidant, anti-inflammatory, antitumor, antimicrobial, diuretic, and chemopreventive properties used in vaccine formulations. Squalene is an antihyperlipidemia and antiatherosclerosis. Squalene is a monooxygenase inhibitor and is commonly used as a biochemical precursor in the preparation of steroids. Squalene is also a natural moisturizer with low acute toxicity and is not significant human skin irritants or sensitizers. It is a skin protective compound used in cosmetics. Phytol, acetate is a phytol compound with antimicrobial, anti-inflammatory, anticancer, and antiinflammatory properties. Hexadecanoic acid, methyl ester is a fatty acid ester having antioxidant, hypcholesterolemic, and 5-alpha-reductase inhibitor activity. Beta-sitosterol is used for heart disease and high cholesterol. It is also used for boosting the immune system and for preventing colon cancer, as well as for gallstones, the common cold and flu (influenza), HIV/AIDS, rheumatoid arthritis, tuberculosis, psoriasis, allergies, cervical cancer, fibromyalgia, systemic lupus erythematosus, asthma, hair loss, bronchitis, migraine headache, and chronic fatigue syndrome.

The medicinal roles of some compounds such as Pyrazole[4,5-b]imidazole, 1-formyl-3-ethyl-6-, beta-d-, Cyclohexanone, 2-cyclohexylidenes, N-[4-(4-Chlorophenyl)isothiazol-5-yl]-1-methylpyriderin-2-imine, 5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-, and 6-Octadecenoic acid, methyl ester, (Z)- are not known, although they were present in large amounts. Further study on the medicinal properties of these molecules is warranted.

The medicinal roles of various compounds present in the leaf extracts of C. carandas augurs well with its use for the treatment for various diseases ethnobotanical. Further work is in progress in this regard.

CONCLUSIONS

From the above discussion, it is clear that the medicinal roles of various compounds present in the leaf extracts of C. carandas augurs well with its use for the treatment for various diseases ethnobotanical. Further work is in progress in this regard.

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REFERENCES


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