INTRODUCTION

Medicinal plants are important sources of herbal medicines like Ayurvedic, Allopathy, Siddha, Unani, and folk medicine. Traditional medicine is available in the form of powder, paste, infusion, etc., for the treatment of several diseases [1-4]. Most of the plants are naturally producing a widespread amount of biomolecules present in the parts of the plants with medicinal properties that are always developing [5-8].

C. carandas Linn. It belongs to the family R. apocynaceae and is found to be widely used as a medicinal plant by tribals throughout India. The fruit-bearing plant that grows as a tiny shrub is known as karonda [9]. The fruits, leaves, barks, and roots of C. carandas have been used for the treatment of many human diseases, such as diarrhea, stomachaches, anorexia, intermittent fever, mouth ulcers, sore throats, syphilitic pain, burning sensations, scabies, epilepsy [10]. C. carandas fruit has antioxidant, antidiabetic, antimicrobial, cytotoxicity, anticonvulsant, hepatoprotective, antihyperlipidemic, cardiac depressant, analgesic, anti-inflammatory, antipyretic, antiviral properties, antitumor activity, and lipase conditioning [1, 11, 12]. In C. carandas fruits, leaves have been shown to have high antioxidant and DNA damage-inhibiting potential [13]. Chemical constituents include steroids, terpenes, tannins, flavonoids, benzenoids, phenylpropanoids, lignans, sesquiterpenes, and coumarins [14].

However, the bioactive compound responsible for the antioxidant activity of C. carandas fruit extracts from different solvent extractions varies among the varieties. Liquid Chromatography-Mass Spectrometry (LC-MS/MS) analysis was used to profile the phenolic, flavonoid, and anthocyanin components. Gas Chromatography-Mass Spectrometry (GC-MS) analysis to assess the fatty acids and organic acids. Additional analysis of this substance leads to the conclusion that C. carandas fruits may have medicinal uses.

MATERIALS AND METHODS

Collection of plant materials

The fruits of C. carandas were collected from Jawadhi Hills (Tiruppur, and Thiruvanmalai district, Latitude 12 34'N Longitude 78 49'E) as shown in fig. 1, in March 2019. All plant materials and Fruits were identified and authenticated by Prof. P. Jayaraman, a botanist at the Herbarium of Plant Anatomy Research Center. A voucher specimen number PARC/2022/4685 of C. carandas has been deposited in the Plant Anatomy Research Center's herbarium, Chennai, Tamil Nadu, India. The fruits were gathered and washed thoroughly under running tap water, then oven-dried for one week at 40-60 °C. The dried fruit pulp was uniformly ground using an electric grinder.

Preparation of the fruit extract

100g of fruit powder was homogenized with 500 ml of water, ethanol, and methanol, filtered through Whatman No. 1 filter paper, and the filter was evaporated to dryness using a rotary evaporator. The fruit extracts of different solvents to LCMS and GCMS analysis. Store the residue at 4 °C for further use [16].

Wash the fruits with water and oven-dry for one week at 40-50 °C. Grind the fruit pulp into powder form, and then 100g of fruit powder was homogenized with 500 ml of water, ethanol, and methanol, filtered through whatman no. 1 filter paper, and the filter was evaporated to dryness using a rotary evaporator. The fruit extracts of different solvents to LCMS and GCMS analysis. Store the residue at 4 °C for further use [16].
Sample preparation

The phenolic acids and flavonoids for LC-MS/MS analysis are isolated from 80% methanol as described in [17, 18]. Homogenize a 10g fruit in methanol (80%), centrifuge, and make up to 50 ml. Evaporate 20 ml of extract under vacuum at 45 °C and then dilute to 5 ml with water. Extract thrice with petroleum ether in 40 ml of ethyl acetate using a separating funnel. The aqueous layer is essential for extracting phenolic acid, as the ethyl acetate extract evaporates to dryness under vacuum at room temperature. The dry residue was added with 4 ml of 2N NaOH and allowed to hydrolyze overnight. Then, this was acidified to pH 2 using 5 ml of 2N HCl and re-extracted with 50 ml of ethyl acetate. The ethyl acetate layer was again re-extracted twice with 25 ml of 0.1N NaHCO₃. The ethyl acetate layer, containing flavonoids, was dried under vacuum. This residue was mixed in 2 ml of MS-grade methanol and filtered through a 0.2μm nylon filter before injection into LCMS. The mobile phase consists of solvent A: aqueous formic acid (0.1% v/v) and solvent B: methanol.

LC and MS/MS conditions

The phenolic acids and flavonoids are resolved in the analytical column BEH-C18 (2.1 x 50 mm, 1.7μm) from Waters India Ltd., protected by a Vanguard BEH-C18 (Waters, USA) with a gradient flow of organic and aqueous phases at a flow rate of 1 ml/min. During analysis, column temperature was maintained at 25 °C, and the sample injection volume was 2 μl. The mobile phase consists of (solvent A) aqueous formic acid (0.1% v/v) and (solvent B) Acetonitrile [18, 19]. A ratio of Solvent 95:5 (A: B) was maintained for 1 min. 95:5 (A: B) for 13-15 min, 95:5 (A: B) for 20 min. The eluted phenolic acids and flavonoids were monitored by a PDA detector at 210-400 nm. Mass spectra of compounds were recorded in the scanning range from M/z 50-450. ESI source in both positive and negative ion modes, 600 °C Qprobe Temp, 10 ml/min flow rate, 45PSI nebulizer gas at 125V. The UPLC column effluent was pumped directly without any split into the LCMS/MS system optimized for the phenolic acids and flavonoid analysis.

Anthocyanins

Anthocyanins were extracted from the fruit by following the method described [20]. Grind 5g of fruit sample in a mortar pestle using 1% acidified methanol under dark conditions. Using acidified methanol, make up the volume to 50 ml, take 5 ml of the extract from this, dry it under a vacuum evaporator, and dissolve the residue in the mobile phase. Filter it through a 0.2μm nylon filter before injection into the LCMS.

Organic acids

Extraction procedure

Organic acids were extracted as follows, homogenized 5g of sample with 10 ml of 80% methanol, filtered, and dried at reduced pressure [21, 22]. The extract is then allowed to cool and centrifuged at 10000 rpm for 15 min. The supernatant was collected after removing methanol traces using a vacuum evaporator. Filter and extract with 25 ml of ethyl acetate 3-4 times. Remove traces of organic solvent and collect the lower concentrated aqueous phase to reconstitute with water for 3PE purification.

Make up the volume with acidified methanol. Dry the elution under nitrogen. Reconstitute (Solvent A and B; 50:50) in the mobile phase and filter it. 4μl filtrate is injected into the LC-MS/MS for analysis.

LC and MS-MS conditions

The initial gradient is composed of 100% aqueous phase (A) and 0% organic phase (B). The mobile phase is composed of 100% aqueous phase (A) and 0% organic solvent and collect the lower concentrated aqueous phase to reconstitute with water for SPE purification. The sample injection volume was 4μl. The eluted organic acids are monitored using a PDA detector, and the UPLC column effluent is pumped directly, without any split, into the TQD-MS/MS (Waters, USA) system, optimized for the identification and quantification of anthocyanin and organic acids.

Fatty acids

GC-FID analysis

GC-FID analysis of fatty acid methyl esters was done using a Varian-3800 gas chromatography system with a flame ionization detector (FID) on a fused silica capillary column (VF-5 Factor, USA), 30 m x 0.25 mm i.d. and 0.25μm film thickness. Helium (99.9%) was used as carrier gas at a constant flow rate of 1 ml/min. The flow rates of H2 and air are maintained at 20 ml/min and 250 ml/min, respectively. The temperature of the column is: initial temperature of the oven at 100 °C for 4 min, then increased by 3 °C per minute up to 220 °C and held for 4 min. Increase the temperature to 260 °C at a rate of 5 °C per minute and hold for 10 min. Injector and detector temperatures are maintained at 250 °C and 260 °C, respectively. Initially, the injection is completed in split-less mode, followed by split mode (1:30) after 1.5 min.

GC-MS

By applying the same temperature program described above for GC-FID analysis, GC-MS analysis was performed on a Varian-3800 gas chromatograph and a Varian 4000 GC-MS ion trap mass selective detector. Helium was accustomed as a carrier gas at a flow rate of 1 ml/min. The temperature is: injector: 260 °C; ion source: 220 °C; trap: 200 °C; transfer line: 260 °C; Detector: 260 °C; and injector: 220 °C. The mass scan range was (m/z): 50-450 Atomic Mass Units (amu), Electron Impact (EI): 70. Fatty acids are separated on a VF-5MS fused silica capillary column (Varian, USA) (30 m x 0.25 mm id with 0.25 μm film thickness). Identification of FA was completed by comparing the relative retention times of FAME peaks with a reference standard (Sigma-Aldrich, USA). Spectral data can be found in the Wiley and NIST-2007 libraries [23].

RESULTS

Table 1. Shows the results of C. carandas fruit sample varieties (green, pink, sweet) extracted by ethanol, methanol, and water. The green fruits had a higher concentration of phenolic acids (1889.15 mg/100g) compared to sweet (1249.82 mg/100g) and pink
Table 1: Profiling of phenolic acids in different extracts of green, pink, and sweet varieties of *Carissa carandas* fruits

<table>
<thead>
<tr>
<th>Phenolic acids</th>
<th>Green (mg/100g FW)</th>
<th>Pink (mg/100g FW)</th>
<th>Sweet (mg/100g FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aqueous</td>
<td>Ethanol</td>
<td>Methanol</td>
</tr>
<tr>
<td>p-Hydroxybenzoic acid</td>
<td>25.55</td>
<td>30.1</td>
<td>28.88</td>
</tr>
<tr>
<td>Salicylic acid</td>
<td>20.37</td>
<td>40.6</td>
<td>35.35</td>
</tr>
<tr>
<td>2,4-Dihydroxy benzoic acid</td>
<td>0.22</td>
<td>0.75</td>
<td>0.49</td>
</tr>
<tr>
<td>Gentisic acid</td>
<td>2.52</td>
<td>4.277</td>
<td>25.34</td>
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<tr>
<td>Protocatechuic acid</td>
<td>0.63</td>
<td>2.38</td>
<td>1.7</td>
</tr>
<tr>
<td>p-Coumaric acid</td>
<td>15.89</td>
<td>64.89</td>
<td>62.58</td>
</tr>
<tr>
<td>o-Coumaric acid</td>
<td>4.9</td>
<td>8.12</td>
<td>12.74</td>
</tr>
<tr>
<td>Vanillic acid</td>
<td>33.11</td>
<td>431.06</td>
<td>182.49</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>1.96</td>
<td>33.04</td>
<td>6.51</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>0.17</td>
<td>0.78</td>
<td>1.96</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>230.72</td>
<td>920.01</td>
<td>411.32</td>
</tr>
<tr>
<td>Syringic acid</td>
<td>109.91</td>
<td>124.01</td>
<td>138.78</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>0.23</td>
<td>0.38</td>
<td>0.85</td>
</tr>
<tr>
<td>t-Cinnamic acid</td>
<td>94.08</td>
<td>190.26</td>
<td>104.09</td>
</tr>
<tr>
<td>Total</td>
<td>540.26</td>
<td>1899.15</td>
<td>1013.08</td>
</tr>
</tbody>
</table>

The MRM details of phenolic acid analysis are depicted in Table 2.

Table 2: MRM details for phenolic acids

<table>
<thead>
<tr>
<th>Compound</th>
<th>Formula/mass</th>
<th>Parent m/z</th>
<th>Cone voltage</th>
<th>Daughters</th>
<th>Collision energy</th>
<th>Ion mode</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caffeic acid</td>
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<td>178.90</td>
<td>30</td>
<td>135.05</td>
<td>16</td>
<td>ES-</td>
</tr>
<tr>
<td>2,4-Dihydroxy benzoic acid</td>
<td>154</td>
<td>152.90</td>
<td>28</td>
<td>65.02</td>
<td>18</td>
<td>ES-</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>354</td>
<td>352.97</td>
<td>22</td>
<td>191.10</td>
<td>18</td>
<td>ES-</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>194</td>
<td>192.90</td>
<td>26</td>
<td>134.02</td>
<td>14</td>
<td>ES-</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>170</td>
<td>168.90</td>
<td>28</td>
<td>125.03</td>
<td>12</td>
<td>ES-</td>
</tr>
<tr>
<td>Gentisic acid</td>
<td>154</td>
<td>152.90</td>
<td>24</td>
<td>108.98</td>
<td>12</td>
<td>ES-</td>
</tr>
<tr>
<td>o-Coumaric acid</td>
<td>164</td>
<td>162.90</td>
<td>22</td>
<td>119.06</td>
<td>12</td>
<td>ES-</td>
</tr>
<tr>
<td>p-Coumaric acid</td>
<td>164</td>
<td>162.90</td>
<td>24</td>
<td>119.05</td>
<td>14</td>
<td>ES-</td>
</tr>
<tr>
<td>p-Hydroxybenzoic acid</td>
<td>138</td>
<td>136.90</td>
<td>26</td>
<td>93.01</td>
<td>12</td>
<td>ES-</td>
</tr>
<tr>
<td>Protocatechuic acid</td>
<td>154</td>
<td>152.90</td>
<td>26</td>
<td>109.05</td>
<td>16</td>
<td>ES-</td>
</tr>
<tr>
<td>Salicylic acid</td>
<td>138</td>
<td>136.90</td>
<td>28</td>
<td>93.10</td>
<td>14</td>
<td>ES-</td>
</tr>
<tr>
<td>Syringic acid</td>
<td>198</td>
<td>196.97</td>
<td>26</td>
<td>182.07</td>
<td>10</td>
<td>ES-</td>
</tr>
<tr>
<td>t-Cinnamic acid</td>
<td>148</td>
<td>146.90</td>
<td>26</td>
<td>103.05</td>
<td>10</td>
<td>ES-</td>
</tr>
<tr>
<td>Vanillic acid</td>
<td>168</td>
<td>166.97</td>
<td>26</td>
<td>108.01</td>
<td>20</td>
<td>ES-</td>
</tr>
</tbody>
</table>

Fig. 2: Chromatogram details of phenolic acids present in *C. carandas* fruit extract analyzed by LCMS. A, B and C indicates samples of sour green aqueous, methanol and ethanol extracts. D, E and F showed the results of sour pink aqueous, methanol and ethanol extracts. G, H and I indicate the sweet variety aqueous, methanol and ethanol extracts.
Table 3: Profiling of flavonoids in different extracts of green, pink, and sweet varieties of *C. caranda* fruits

<table>
<thead>
<tr>
<th>Flavonoids</th>
<th>Green</th>
<th>Aqueous</th>
<th>Ethanol</th>
<th>Methanol</th>
<th>Pink</th>
<th>Aqueous</th>
<th>Ethanol</th>
<th>Methanol</th>
<th>Sweet</th>
<th>Aqueous</th>
<th>Ethanol</th>
<th>Methanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Umbelliferone</td>
<td>15.22</td>
<td>321.75</td>
<td>292.06</td>
<td>119.44</td>
<td>286.51</td>
<td>231.77</td>
<td>8.46</td>
<td>235.77</td>
<td>227.84</td>
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</tr>
<tr>
<td>Apigenin</td>
<td>7.34</td>
<td>12.87</td>
<td>13.47</td>
<td>3.78</td>
<td>19.87</td>
<td>8.36</td>
<td>2.14</td>
<td>15.67</td>
<td>9.32</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Naringenin</td>
<td>33.12</td>
<td>117.44</td>
<td>102.75</td>
<td>19.12</td>
<td>53.87</td>
<td>48.11</td>
<td>23.07</td>
<td>27.18</td>
<td>23.42</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Catechin</td>
<td>125.43</td>
<td>204.32</td>
<td>179.49</td>
<td>109.24</td>
<td>132.87</td>
<td>124.33</td>
<td>79.39</td>
<td>108.57</td>
<td>87.39</td>
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</tr>
<tr>
<td>Hesperetin</td>
<td>43.01</td>
<td>64.28</td>
<td>55.22</td>
<td>38.79</td>
<td>56.33</td>
<td>38.21</td>
<td>41.02</td>
<td>27.85</td>
<td>17.05</td>
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</tr>
<tr>
<td>Quercetin</td>
<td>113.07</td>
<td>155.81</td>
<td>97.15</td>
<td>135.1</td>
<td>72.41</td>
<td>134.08</td>
<td>73.42</td>
<td>62.79</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Rutin</td>
<td>1204.42</td>
<td>2845.57</td>
<td>2732.11</td>
<td>908.79</td>
<td>1814.08</td>
<td>1675.23</td>
<td>987.49</td>
<td>1124.37</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Kaempferol</td>
<td>5125.44</td>
<td>9264.24</td>
<td>8997.37</td>
<td>3734.28</td>
<td>6784.34</td>
<td>3897.11</td>
<td>5358.65</td>
<td>4879.34</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>7078.57</td>
<td>13387.35</td>
<td>12861.28</td>
<td>5279.23</td>
<td>9317.83</td>
<td>5237.14</td>
<td>7184.54</td>
<td>6725.25</td>
<td></td>
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</tbody>
</table>

Table 4 displays the result of MRM details of different flavonoid compounds. Fig. 3. Represent the chromatogram details of flavonoids present in the fruit extract analyzed by LCMS.

**Fig. 3:** Chromatogram details of flavonoids present in *C. carandas* fruit extract analyzed by LC-MS. A, B and C indicates samples of sour green aqueous, methanol and ethanol extracts. D, E and F showed the results of sour pink aqueous, methanol and ethanol extracts. G, H and I indicate the sweet variety aqueous, methanol and ethanol extracts.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Formula/Mass</th>
<th>Parent m/z</th>
<th>Cone voltage</th>
<th>Daughters</th>
<th>Collision energy</th>
<th>Ion mode</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apigenin</td>
<td>270</td>
<td>268.97</td>
<td>46</td>
<td>107.04</td>
<td>30</td>
<td>ES</td>
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<tr>
<td>Catechin</td>
<td>302</td>
<td>300.97</td>
<td>42</td>
<td>286.15</td>
<td>16</td>
<td>ES</td>
</tr>
<tr>
<td>Hesperetin</td>
<td>286</td>
<td>284.97</td>
<td>54</td>
<td>145.5</td>
<td>36</td>
<td>ES</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>302</td>
<td>301.03</td>
<td>36</td>
<td>151.12</td>
<td>20</td>
<td>ES</td>
</tr>
<tr>
<td>Rutin</td>
<td>610</td>
<td>609.1</td>
<td>60</td>
<td>300.2</td>
<td>42</td>
<td>ES</td>
</tr>
<tr>
<td>Epicatechin</td>
<td>290.27</td>
<td>289.15</td>
<td>20</td>
<td>245.15</td>
<td>15</td>
<td>ES</td>
</tr>
<tr>
<td>Epigallo catechin</td>
<td>306.27</td>
<td>305.05</td>
<td>20</td>
<td>219.05</td>
<td>15</td>
<td>ES</td>
</tr>
</tbody>
</table>

Table 4: MRM details for flavonoids
The sweet C. carandas variety recorded the highest concentration of fatty acids (147.2 mg/100g FW) compared to the sour varieties of pink and green (94.9 mg/100 g FW) and 72.79 mg/100 g FW, respectively. Oleic acid was the main monounsaturated fatty acid presence of organic acids like oxalic acid, maleic acid, citric acid, tartaric acid, malic acid, ascorbic acid, shikimic acid, and fumaric acid. Among these, maleic acid was the major component in all samples. The aqueous extract of all samples recorded the highest concentration of organic acids compared to the ethanol and methanol extracts. The aqueous extract of the green variety showed higher levels (3045.86 mg/100 g FW) compared to the pink (2665.07 mg/100g FW) and sweet (1227.55 mg/100g FW) varieties. Evidence suggests that organic acids such as maleic or citric acids may have a positive health benefit as antioxidants.

Table 5: Profiling of anthocyanins in the different extract of green, pink and sweet variety of C. carandas fruits

<table>
<thead>
<tr>
<th>Anthocyanins (mg/g FW)</th>
<th>Green Aqueous</th>
<th>Ethanol</th>
<th>Methanol</th>
<th>Pink Aqueous</th>
<th>Ethanol</th>
<th>Methanol</th>
<th>Sweet Aqueous</th>
<th>Ethanol</th>
<th>Methanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyanidin 3-glucoside</td>
<td>0.78</td>
<td>1.88</td>
<td>1.74</td>
<td>0.35</td>
<td>1.51</td>
<td>1.48</td>
<td>0.24</td>
<td>1.09</td>
<td>1.12</td>
</tr>
<tr>
<td>Delphinidin 3-glucoside</td>
<td>0.31</td>
<td>0.44</td>
<td>0.37</td>
<td>0.09</td>
<td>0.157</td>
<td>0.11</td>
<td>0.063</td>
<td>0.104</td>
<td>0.12</td>
</tr>
<tr>
<td>Pelargonidin 3-glucoside</td>
<td>0.031</td>
<td>0.073</td>
<td>0.056</td>
<td>0.024</td>
<td>0.040</td>
<td>0.037</td>
<td>0.021</td>
<td>0.052</td>
<td>0.029</td>
</tr>
<tr>
<td>Malvidin 3-glucoside</td>
<td>0.028</td>
<td>0.091</td>
<td>0.083</td>
<td>0.038</td>
<td>0.057</td>
<td>0.065</td>
<td>0.018</td>
<td>0.039</td>
<td>0.025</td>
</tr>
<tr>
<td>Total</td>
<td>0.059</td>
<td>2.485</td>
<td>2.249</td>
<td>0.502</td>
<td>1.564</td>
<td>1.692</td>
<td>0.342</td>
<td>1.285</td>
<td>1.294</td>
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</table>

Table 6: Profiling of organic acids in different extracts of green, pink, and sweet varieties of C. carandas fruits

<table>
<thead>
<tr>
<th>Organic acids (mg/100g FW)</th>
<th>Green Aqueous</th>
<th>Ethanol</th>
<th>Methanol</th>
<th>Pink Aqueous</th>
<th>Ethanol</th>
<th>Methanol</th>
<th>Sweet Aqueous</th>
<th>Ethanol</th>
<th>Methanol</th>
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<tbody>
<tr>
<td>Oxalic acid</td>
<td>2.97</td>
<td>2.45</td>
<td>1.18</td>
<td>2.14</td>
<td>1.87</td>
<td>0.94</td>
<td>2.56</td>
<td>2.22</td>
<td>0.87</td>
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<tr>
<td>Maleic acid</td>
<td>0.55</td>
<td>0.39</td>
<td>0.19</td>
<td>0.37</td>
<td>0.25</td>
<td>0.13</td>
<td>0.3</td>
<td>0.24</td>
<td>0.12</td>
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<tr>
<td>Citric acid</td>
<td>345.03</td>
<td>308.21</td>
<td>189.79</td>
<td>227.51</td>
<td>203.14</td>
<td>112.32</td>
<td>162.65</td>
<td>143.57</td>
<td>69.55</td>
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<tr>
<td>Tartaric acid</td>
<td>16.04</td>
<td>13.87</td>
<td>8.97</td>
<td>10.26</td>
<td>8.97</td>
<td>5.89</td>
<td>14.2</td>
<td>12.39</td>
<td>4.87</td>
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<tr>
<td>Malic acid</td>
<td>2612.43</td>
<td>1874.13</td>
<td>745.27</td>
<td>2373.07</td>
<td>1758.25</td>
<td>677.84</td>
<td>1003.1</td>
<td>945.87</td>
<td>389.72</td>
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<tr>
<td>Ascorbic acid</td>
<td>61.19</td>
<td>45.98</td>
<td>28.77</td>
<td>47.73</td>
<td>33.97</td>
<td>22.98</td>
<td>43.05</td>
<td>36.91</td>
<td>24.22</td>
</tr>
<tr>
<td>Shikimic acid</td>
<td>6.94</td>
<td>5.32</td>
<td>1.48</td>
<td>3.71</td>
<td>3.24</td>
<td>1.85</td>
<td>1.34</td>
<td>1.08</td>
<td>0.37</td>
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<tr>
<td>Fumaric acid</td>
<td>0.71</td>
<td>0.48</td>
<td>0.087</td>
<td>0.28</td>
<td>0.15</td>
<td>0.079</td>
<td>0.35</td>
<td>0.21</td>
<td>0.08</td>
</tr>
<tr>
<td>Total</td>
<td>3045.86</td>
<td>2250.83</td>
<td>975.73</td>
<td>2665.07</td>
<td>1227.55</td>
<td>697.73</td>
<td>1142.49</td>
<td>489.83</td>
<td></td>
</tr>
</tbody>
</table>

The essential fatty acid profiling in all the sample extracts was analyzed by GCMS. The result is presented in table 7.

Table 7: Profiling of fatty acids in green, pink and sweet varieties of C. carandas fruits

<table>
<thead>
<tr>
<th>Profiling</th>
<th>Fatty acids (mg/100g FW)</th>
<th>Green</th>
<th>Pink</th>
<th>Sweet</th>
</tr>
</thead>
<tbody>
<tr>
<td>C8:0</td>
<td>Caprylic</td>
<td>0.055</td>
<td>0.043</td>
<td>0.074</td>
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<tr>
<td>C9:0</td>
<td>Nonanoic</td>
<td>0.014</td>
<td>0.027</td>
<td>0.043</td>
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<tr>
<td>C10:0</td>
<td>Capric</td>
<td>0.380</td>
<td>0.090</td>
<td>0.170</td>
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<tr>
<td>C11:0</td>
<td>Undecanoic</td>
<td>0.057</td>
<td>0.145</td>
<td>0.217</td>
</tr>
<tr>
<td>C12:0</td>
<td>Lauric</td>
<td>0.017</td>
<td>0.015</td>
<td>0.065</td>
</tr>
<tr>
<td>C13:0</td>
<td>Tridecanoic</td>
<td>0.381</td>
<td>0.742</td>
<td>1.160</td>
</tr>
<tr>
<td>C14:0</td>
<td>Myristic</td>
<td>0.039</td>
<td>0.075</td>
<td>0.152</td>
</tr>
<tr>
<td>C15:0</td>
<td>Pentadecanoic</td>
<td>0.652</td>
<td>1.290</td>
<td>1.747</td>
</tr>
<tr>
<td>C16:0</td>
<td>Palmitic</td>
<td>33.871</td>
<td>37.801</td>
<td>52.841</td>
</tr>
<tr>
<td>C16:1</td>
<td>Palmitoleic</td>
<td>0.336</td>
<td>0.588</td>
<td>1.385</td>
</tr>
<tr>
<td>C17:0</td>
<td>Heptadecanoic</td>
<td>8.282</td>
<td>11.173</td>
<td>26.931</td>
</tr>
<tr>
<td>C18:0</td>
<td>Stearic</td>
<td>13.745</td>
<td>10.910</td>
<td>9.000</td>
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<tr>
<td>C18:1</td>
<td>Oleic</td>
<td>6.656</td>
<td>24.921</td>
<td>34.351</td>
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<td>C18:2</td>
<td>Linoleic</td>
<td>1.655</td>
<td>1.154</td>
<td>2.214</td>
</tr>
<tr>
<td>C18:3</td>
<td>Linolenic</td>
<td>0.035</td>
<td>0.321</td>
<td>0.123</td>
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<tr>
<td>C19:0</td>
<td>Nonadecanoic</td>
<td>0.017</td>
<td>0.023</td>
<td>0.088</td>
</tr>
<tr>
<td>C20:0</td>
<td>Arachidic</td>
<td>0.125</td>
<td>0.252</td>
<td>0.612</td>
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<tr>
<td>C20:1</td>
<td>Eicosanoic</td>
<td>0.041</td>
<td>0.175</td>
<td>0.246</td>
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<tr>
<td>C21:0</td>
<td>Heneicosanoic</td>
<td>0.076</td>
<td>0.176</td>
<td>0.082</td>
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<tr>
<td>C22:0</td>
<td>Behenic</td>
<td>1.658</td>
<td>0.016</td>
<td>1.967</td>
</tr>
<tr>
<td>C22:1</td>
<td>Erucic</td>
<td>0.285</td>
<td>0.492</td>
<td>2.326</td>
</tr>
<tr>
<td>C23:0</td>
<td>Tricosanoic</td>
<td>0.360</td>
<td>0.392</td>
<td>1.143</td>
</tr>
<tr>
<td>C24:0</td>
<td>Tetracosanoic</td>
<td>0.739</td>
<td>1.098</td>
<td>3.466</td>
</tr>
<tr>
<td>C26:0</td>
<td>Hexacosanoic</td>
<td>2.928</td>
<td>2.255</td>
<td>5.654</td>
</tr>
<tr>
<td>C28:0</td>
<td>Octacosanoic</td>
<td>0.391</td>
<td>0.782</td>
<td>1.722</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>72.795</td>
<td>94.946</td>
<td>147.206</td>
</tr>
</tbody>
</table>
present at the highest concentration in the sweet variety (343.5 mg/100g FW) compared to the sour varieties (24.92 mg/100g FW in pink and 6.66 mg/100g FW in green). The main component observed was the saturated fatty acid (SFA) palmitic acid, which had a significantly higher concentration (52.84 mg/100g FW) in the sweet variety compared to the sour variety. Essential fatty acids are important to cells and good health; they must be consumed via diet, as the human body cannot synthesize them. The key lipid units present in fatty acids are necessary for human nutrition as a source of energy and for physiological and structural functions.

**DISCUSSION**

The present study found that *C. carandas* fruits of green, pink, and sweet varieties were extracted in different solvents. The green variety showed the highest phytochemical content. The ethanol extract from the green variety had a higher concentration of phenolic acids when compared to the sweet and pink varieties. The ethanol extract from the green variety had higher concentrations of ferulic acid, gallic acid, 2, 4-dihydroxybenzoic acid, t-cinnamic acid, vanillic acid, and salicylic acid when compared with methanol and aqueous extracts. Reports suggest that many phytochemicals from plants were extracted by ethanol [24]. The results of various phenolic compounds in *C. carandas*, such as vanillic acid, ellagic acid, and gallic acid [25, 26] were similar to the present study. Higher phenolic acids in green fruits demonstrate that ethanolic extract is a potent source of novel bioactive compounds and an alternative for managing oxidative stress-induced diabetes.

Ethanol extracts of the green variety show higher concentrations of flavonoids (13387.35 µg/100g) compared to methanol (12861.28 µg/100g) and aqueous (7078.57 µg/100g) extracts. Flavonoids, known as scavengers, strongly eliminate oxidative radicals. Rutin is the principal flavonoid compound in *C. carandas* fruits. The high concentration of flavonoids in ethanolic extract may account for the highest antioxidant activity of the green fruits in our study. Flavonoids are most widely present in plants, such as quercetin, catechin, and kaempferol. The radical-scavenging activity of a flavonoid compound may depend on the number of hydroxyl groups present in the molecule. Quercetin was found to protect DNA damage in human lymphocytes induced by hydrogen peroxide. This protection might be due to the dihydroxy structure of both flavonoids being essential for the inhibition of DNA damage [27-29], which demonstrated that, in addition to Quercetin, kaempferol could also inhibit H2O2-induced DNA strand breaks in human lymphocytes. The quercetin decreased lipid peroxidation and protein oxidation against dimethoate-induced oxidative stress by increasing superoxide dismutase and catalase enzyme activities in human lymphocytes [30]. Ethanol extract of green fruit in our study may increase antioxidant enzyme activities to protect DNA from oxidative stress. Anthocyanin contents in different solvent extracts of *C. caranda* fruit varieties were observed to be varied in each extract. Among the tested samples, the maximum concentration was present in the ethanol extract of the green variety (2.485 mg/g FW) compared to the pink variety (1.564 mg/g FW) and the sweet variety (1.285 mg/g FW), respectively. Aqueous extracts of all the test samples recorded a lower concentration of anthocyanins when compared to ethanol and methanol extracts. Cyanidin-3-glucoside was the major anthocyanin in *C. carandas* fruit extract. Anthocyanin plays a significant role in antioxidant activity against free radical-induced oxidative stress. The conjugated structure of anthocyanin evaluated maximum antioxidant activity because of its better electron delocalization ability [31]. The green variety with a higher anthocyanin content may act as a free radical scavenger. The results were similar to those of other studies where different berries were evaluated for their antioxidant activity [32-34]. Organic acids like oxalic acid, maleic acid, citric acid, tartaric acid, malic acid, ascorbic acid, shikimic acid, and fumaric acid are present in the examined fruit. The aqueous extract of the green variety showed higher levels of malic and citric acid when compared with the pink and sweet varieties of *C. carandas* [35, 36]. Evidence suggests that organic acids such as malic or citric acids act as antioxidants and esters, with an increasing number of commercial applications as organic compounds [37]. The fatty acid constituents of *C. carandas* was evaluated by GCMS. The sweet variety shows the highest concentration of fatty acids (147.2 mg/100g FW) when compared with the pink and green varieties (94.9 mg/100 g FW, 72.79 mg/100 g FW, respectively). The sweet variety contains palmitic acid (saturated fatty acid), Oleic acid (mono-unsaturated fatty acid) [38, 39], and Octadecenoic acid (unsaturated fatty acid) in higher concentrations when compared with other varieties. Essential fatty acids are important to cell structure and good health.

**CONCLUSION**

The present study found that *C. carandas* fruits of green, pink, and sweet varieties were extracted in different solvents. The green variety showed the highest phytochemical content with LC-MS profiling when compared with other varieties. Ethanol extract of all varieties showed the highest phenolic and flavonoid content. The Anthocyanin content of the green variety was several times higher than other varieties, which makes it appropriate for preventing lifestyle diseases. Aqueous extract of green variety showed higher levels of organic acids. Sweet variety shows the presence of elevated levels of fatty acids by GCMS analysis. The differences in phytochemical value among *C. carandas* varieties have a great role as an antidiabetic fruit.

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Nil

**ABBREVIATION**

LCMS/MS—Liquid chromatography-mass spectrometry, GCMS—Gas chromatography-Mass spectrometry, FW—Fruit weight, DNA—Deoxyribonucleic acid, NaOH—Sodium hydroxide, NaHCO3—Sodium bicarbonate, PDA—Photodiode array, GC-FID—Gas chromatography Flame ionization detector

**AUTHORS CONTRIBUTIONS**

Sudha completed the research work plan and Manuscript writing. Malarkodi did the review of the literature collection, Gokulakrishnan did the work plan and Manuscript corrections. All authors have read and agree to the manuscript’s published version.

**CONFLICTS OF INTERESTS**

The authors declared no conflicts of interest.

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3.1.1. In vitro studies

3.1.2. In vivo studies

3.2. Phytochemical analysis

3.3. Biological activities

3.3.1. Anti-inflammatory activity

3.3.2. Antimicrobial activity

3.3.3. Antioxidant activity

3.3.4. Antihyperglycemic activity

3.3.5. Antihypertensive activity

3.3.6. Anticancer activity

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