Antioxidant and antibacterial activities of Camptotheca acuminata D. seed oil

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This study was designed to explore the in vitro antioxidant and antibacterial activities of Camptotheca acuminata D. seed oil, which were extracted by supercritical fluid extraction (SFE) or petroleum ether extraction methods. The major constituent of the oil were described as (Z,Z,Z)-9,12,15-Octadecatrien-1-ol (54.92%) and 2-[(trimethylsilyl)oxy]-3-[4-[(trimethylsilyl)oxy]phenyl]-trimethylsilyl ester (26.53%) in supercritical fluid and petroleum ether extracts. The oil and the components were subjected to screen for their possible antioxidant activity by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay and β-carotene bleaching test. In the DPPH test system, free radical scavenging activities of supercritical fluid extracts and petroleum ether extracts were determined to be 7.55 ± 0.11% and 4.38 ± 0.08% (v/v), respectively. As to the β-carotene bleaching test system, the two values were 15.93 ± 0.11% and 6.87 ± 0.15% (v/v), respectively. The activities of antioxidant and antibacterial in components of petroleum ether were more efficient than in components of supercritical fluid extraction. As to the antimicrobial activities of the essential oil against 8 species bacterium, C. acuminata D. seed oil had remarkable antibacterial activity, especially to staphylococcus aureus (ATCC 6538). Thus, C. acuminata D. seed oil could be judged as a kind of patent drug which has antioxidant and antibacterial activity effectively.

Key words: Camptotheca acuminata D. seed oil, antioxidant activities, antibacterial activities.

INTRODUCTION

Camptotheca acuminata Decaisne belongs to the family of Nyssaceae. It is a kind of deciduous and medicinal tree from south China and listed as an endangered species in China (Cheng et al., 2008; Li et al., 2002; (Yang et al., 2011) DOI: 10.1002/qua.23046). The main active ingredients of C. acuminata D. extracts are quinoline and indole alkaloids (Wall et al., 1966). The camptothecin (CPT) (a terpenoid quinoline alkaloid) and its analogues are the potent topoisomerase I inhibitors, which have been used as the anticancer drugs to treat ovarian, lung and colorectal cancers or the antiviral agents (Li and Adair, 1994; Oberlies and Kroll, 2004).

Some studies on anticancer activities of C. acuminata D. have been reported. However, the antioxidant activity of its seed oil has not yet been studied. The seed oil of C. acuminata D. possesses the potential as high-quality edible oil that is beneficial to health and valuable natural antioxidants in cosmetic and pharmaceutical industries. Industrial seed oils are generally obtained with the aid of mechanical process and organic solvent extraction (mainly hexane). The oil prepared by mechanical separation is of high quality, but in most cases the yield is low. Hexane extraction can achieve almost complete recovery of the oil, but the solvent is quite harmful to human health and environment, embarrassing the use in food, cosmetic and pharmaceutical industries. Supercritical fluid extraction (SFE) with supercritical carbon dioxide (SC-CO₂) is an alternative method to
extract the oils from natural products and has received considerable attention (Gomes et al., 2007; Lu et al., 2007; SalgIn, 2007). The oil obtained by SC-CO\textsubscript{2} extraction is of high quality, and the yield is comparable with those of organic solvent extractions (Friedrich et al., 1982; Molero Gómez et al., 1996). In fact, CO\textsubscript{2} extracts have been generally considered as safety in food applications (Gerard and May, 2002), and SFE has been served as a very potential technology in food and pharmaceutical operations (King, 2000).

To the best of our knowledge, the antioxidant and antibacterial activities of C. acuminate D. seed oil in vitro have not yet been evaluated. Therefore, in this work, the oil will be separately extracted by SFE and petroleum ether extraction methods, and the activities will be evaluated by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay, β-carotene bleaching test, as well as minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) determinations. We anticipate that the investigation will be of value in the development of antioxidant and antibacterial agents.

MATERIALS AND METHODS

Preparation of extract

**Isolation of C. acumina seed oil by petroleum ether**

C. acuminate D. seeds were air dried at room temperature and ground, and 100 g were subjected to the distillation with a British-type clevenger apparatus at 100°C for 3 h. Then, the extracts were filtered and concentrated in vacuum at 45°C, yielding the seed oil in yellow.

**Supercritical carbon dioxide (SC-CO\textsubscript{2}) extraction**

C. acuminate D. seeds were air dried at room temperature and ground in a grinder with a mesh of 2 mm in diameter. Then, they were passed through a 0.5 mm sieve to obtain a fine powder. The extraction temperature was 45°C and the pressure was 5.5 MPa. The flow rate was determined using a watch. The flow rate of CO\textsubscript{2} was 10 kg/h; and the extraction time was 2 h. Liquid CO\textsubscript{2} was supplied from a gas cylinder. Before passing into the extraction vessel filled with the samples by pump, the liquid CO\textsubscript{2} was adjusted to the desired pressure and heated to a specified temperature to reach the supercritical state. Finally, the extracts were lyophilized and kept in dark at 4°C until the next step.

**Gas chromatography**

The sample was diluted by ethyl acetate (1:100) and mixed. The analysis of the essential oil was performed using a VG platform II Gas chromatography-Mass spectroscopy (GC–MS) system equipped with an Rtx-5MS capillary column (30 x 0.25 mm; film thickness 0.25 mm). For the Rtx-5MS detection, the injector equipped with an Rtx-5MS capillary column (30 × 0.25 mm; film thickness 0.25 mm). For the Rtx-5MS detection, the injector and detector MS transfer line temperatures were set at 200 and 280°C, respectively, and the sample collection time was 3 min (m/z = 40 to 500).

**Antioxidant activity**

**General description**

Antioxidant activity was assessed by DPPH assay and β-carotene bleaching test. All data collected for each assay were the average of the measurements of three independent experiments.

**DPPH radical scavenging assay and the oil obtained by SC-CO\textsubscript{2} extraction**

We measured the bleaching of purple-colored ethanol solution of DPPH. This spectrophotometric assay uses the stable radical DPPH as a reagent (Wu et al., 2010). An aliquot of the sample (100 μl) was mixed with 1.4 ml of ethanol and then added to 1 ml of 0.004% DPPH (Sigma–Aldrich) in ethanol. The mixture was vigorously shaken and then immediately placed in a UV–Vis spectrophotometer (WARENESS) to monitor the decrease in absorbance at 517 nm. Monitoring was continued for 70 min until the reaction reached a plateau. Ascorbic acid (Sigma–Aldrich), a stable antioxidant, was used as a synthetic reference. The radical scavenging activities of samples were calculated as the inhibition percentage of DPPH according to the formula:

$$\text{Inhibition percentage (Ip)} = \left[\frac{(AB-AA)}{AB}\right] \times 100 \quad (\text{Yen and Duh, 1994})$$

where AB and AA are the absorbance values of the blank sample and the tested samples examined after 70 min, respectively.

**β-Carotene-linoleic acid bleaching assay**

Antioxidant activity of the samples was determined with β-carotene bleaching test (Wu et al., 2010). Approximately 10 mg of β-carotene (type I synthetic, Sigma–Aldrich) was dissolved in 10 ml chloroform. The carotene–chloroform solution of 0.2 ml was pipetted into a boiling flask containing 20 mg linoleic acid (Sigma–Aldrich) and 200 mg Tween 40 (Sigma–Aldrich). Chloroform was removed by a rotary evaporator (RE-52AA) at 40°C for 5 min, and then the residue, 50 ml of distilled water was added, slowly with vigorous agitation, to form an emulsion. The emulsion (5 ml) was added to a tube containing 0.2 ml of the sample solution and the absorbance was immediately measured at 470 nm against a blank consisting of an emulsion without β-carotene. The tubes were placed in a water bath at 50°C, and the oxidation of the emulsion was monitored by a spectrophotometer at 470 nm over a period of 60 min. Control samples contained 10 ml of water instead. Butylated hydroxyltoluene (BHT) (Sigma–Aldrich), a stable antioxidant, was used as a synthetic reference. The antioxidant activity was expressed as the inhibition percentage relative to the control after 60 min incubation with the following equation:

$$\text{AA} = \frac{100(DRC - RS)}{DRC}$$

Where AA, antioxidant activity; DRC, degradation rate of the control [ln(a/b)/60]; a, absorbance at time 0; b, absorbance at 60 min.

**Antimicrobial activity**

The microorganisms used for testing antimicrobial sensitivity included Bacillus subtilis 6633, Staphylococcus aureus ATCC 6538...
Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) determination of *Camptotheca acuminate* D. seed oil

The MIC and MBC were measured by the broth micro-dilution method (NCCLS, 2002). The essential oils were individually dissolved in sterilized physiological saline solution (0.9% w/v) supplemented with Tween 80 (Sigma) at a final concentration of 0.5% (v/v). Serial doubling dilutions of the oils were prepared in a 96-well microtiter (µL) plate in the range of 0.156 to 4.000% (v/v). Each essential oil dilution (100 µL) was dispensed into the wells of a microtiter plate, and each well was then inoculated with 100 µL of the suspension. The obtained suspensions were mixed with a micro-pipette. The final concentration of each strain was adjusted to 10^5 to 10^6 CFU/mL. All microtiter plates against all microorganisms were incubated at 37°C for 24 h, except for *A. niger* that was incubated at 25°C for 5 days. After incubation, the wells were examined for the microorganism growth, and the MICs were determined. The MIC was defined as the lowest concentration of the essential oil at which the microorganism did not show visible growth. The MBCs were confirmed by reinoculating on agar plates with 10 µL of each culture medium from the microplates. The MBCs were defined as the lowest concentration of the essential oil at which incubated microorganisms were completely killed. *Streptomycin* and *Amphotericin B* were served as the positive controls. Each experiment was repeated for three times.

RESULTS AND DISCUSSION

Chemical composition

We identified 50 components in the *C. acuminate* D. seed oil obtained by SC-CO₂ extraction (Table 1). Fifty-three (53) components were identified in the oil obtained by petroleum ether (Table 2). The major components were (Z,Z,Z) - 9, 12, 15 - Octadecatrien- 1- oil (54.92%), octadecanoic acid (13.46%), n-hexadecanoic acid (11.63%) in the seed oil (Table 1) obtained by SC-CO₂. While the major components were [4-[[trimethylsilyl]oxy][phenyl]-2-[[trimethylsilyl]oxy]-3-2-propenopropionic acid trimethylsilyl ester (26.53%), gammalositosterol (23.49%), (Z,Z,Z)-9,12,15-octadecatrienoic acid, and methyl ester (19.19%) in the seed oil obtained by petroleum ether.

Antioxidant activity

The antioxidant activities of the essential *C. acuminate* D. seed oil obtained by SC-CO₂ extraction or petroleum ether were determined by two complementary test systems: DPPH assay and β-carotene bleaching tests. The results of antioxidant activity in these test systems were collected and shown in Figures 1 to 3. In the DPPH test system, free radical-scavenging activity of *C. acuminate* D. seed oil obtained by SC-CO₂ extraction was determined to be 18.13 ± 0.92%; whereas the oil obtained by petroleum ether was 87.13 ± 1.81% (Figure 1). As for the lipid peroxidation inhibitory activity of the essential oil by the β-carotene bleaching test, the results were consistent with the data obtained from the DPPH test (Figure 2). Compared with BHT, the effects of *C. acuminate* D. seed oil obtained by SC-CO₂ extraction or petroleum ether were 80.82 ± 0.32% and 85.47 ± 0.54%, respectively. The concentration of 50% inhibition (IC₅₀) values of BHT, *C. acuminate* D. seed oil obtained by SC-CO₂ or petroleum ether were 3.24 ± 0.12%, 7.55 ± 0.11% and 4.20 ± 0.08%, respectively (Figure 3). It seemed that the antioxidant activities of all the tested samples were mostly related to their concentrations, and the IC₅₀ values of these two types of seed oil were both higher than that of the synthetic antioxidant BHT (Figures 2 and 3).

Antimicrobial activity

Minimal inhibitory concentration (MICs) and minimal bactericidal concentration (MBCs) of *Camptotheca acuminate* D. seed oil

As shown in Tables 3 and 4, the essential oils exhibited inhibitory effects of all the testing organisms. The oil obtained by SC-CO₂ exhibited somewhat higher antimicrobial activity on *S. epidermidis* ATCC 49134 rather than other microorganisms; whereas the oil obtained by petroleum ether showed more potent on *S. aureus* ATCC 6538, *P. aeruginosa* and *C. albicans*. The antimicrobial activities of the seed oil obtained by SC-CO₂ against *B. subtilis* 6633, *P. vulgaris* and *A. niger* V. Tiegh were less than those of other microorganisms; whereas the oil obtained by petroleum ether showed less inhibitory effects on *B. subtilis* 6633 and *P. vulgaris*. The MICs of the oil obtained by SC-CO₂ extraction ranged from 0.625% (v/v) to more than 5.000% (v/v) for all testing microorganisms; while as to petroleum ether, the values ranged from 0.625% (v/v) to more than 5.000% (v/v). The MBCs were similar or even higher than the corresponding MIC values.

The activity components of *Camptotheca acuminate* D. seed oil

The essential oil of *C. sativum* obtained on hydrodistillation was analyzed by GC–MS. We identified 24 components, representing 92.7% of the total oil. Table 1 summarized the constituents identified by GC–MS analysis, their retention indices and area percentages (concentrations). The oil was dominated by aldehydes and alcohols, which accounted for 55.5 and 36.3%, respectively. The major aldehydes were 2E-decenal (15.9%) and decanal (14.3%), while the alcohols mainly consisted of 2E-decen-1-ol (14.2%) and n-decanol.
Table 1. The chemical compositions of *C. acuminate* D. seed oil obtained by supercritical carbon dioxide extraction (SC-CO₂).

<table>
<thead>
<tr>
<th>No.</th>
<th>RT</th>
<th>Compounds</th>
<th>Molecular formula</th>
<th>MW</th>
<th>Relative</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>3.246</td>
<td>Nonane</td>
<td>C₉H₁₈</td>
<td>128</td>
<td>0.25</td>
</tr>
<tr>
<td>2.</td>
<td>3.652</td>
<td>propyl-cyclohexane,</td>
<td>C₅H₁₁O</td>
<td>126</td>
<td>0.10</td>
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<tr>
<td>3.</td>
<td>4.097</td>
<td>Hexanoic acid</td>
<td>C₆H₁₂O₂</td>
<td>116</td>
<td>0.19</td>
</tr>
<tr>
<td>4.</td>
<td>4.443</td>
<td>(E,E)-2,4-Heptadienal,</td>
<td>C₇H₁₄O</td>
<td>110</td>
<td>0.14</td>
</tr>
<tr>
<td>5.</td>
<td>4.627</td>
<td>1-ethyl-Cyclohexene,</td>
<td>C₆H₁₄</td>
<td>110</td>
<td>0.05</td>
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<tr>
<td>6.</td>
<td>6.079</td>
<td>Phenylethyl Alcohol</td>
<td>C₉H₁₈O</td>
<td>122</td>
<td>0.06</td>
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<tr>
<td>7.</td>
<td>8.510</td>
<td>8-Methylene-3 oxatricycle[5,2.0.0(2.4)]nonane</td>
<td>C₈H₁₆O</td>
<td>136</td>
<td>0.13</td>
</tr>
<tr>
<td>8.</td>
<td>8.552</td>
<td>(E,E)-2,4-Decadienal,</td>
<td>C₁₀H₁₈O</td>
<td>152</td>
<td>0.09</td>
</tr>
<tr>
<td>9.</td>
<td>8.872</td>
<td>2,7-Dimethyl-1,3,7-Octatriene,</td>
<td>C₁₀H₁₈</td>
<td>136</td>
<td>0.33</td>
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<tr>
<td>10.</td>
<td>12.737</td>
<td>7-Bromomethyl-Pentadec-7-ene,</td>
<td>C₁₆H₃₅Br</td>
<td>302</td>
<td>0.06</td>
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<tr>
<td>11.</td>
<td>14.169</td>
<td>Tetradecanoic acid</td>
<td>C₁₄H₂₈O₂</td>
<td>228</td>
<td>0.07</td>
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<tr>
<td>12.</td>
<td>14.574</td>
<td>Heneicosane</td>
<td>C₂₀H₄₁O</td>
<td>296</td>
<td>0.09</td>
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<td>13.</td>
<td>15.015</td>
<td>3,7,11,15-Tetramethyl-2-hexadecen-1-ol</td>
<td>C₂₀H₄₂O</td>
<td>296</td>
<td>0.07</td>
</tr>
<tr>
<td>14.</td>
<td>15.090</td>
<td>6,10,14-Trimethyl-2-Pentadecanone,</td>
<td>C₁₈H₃₈O</td>
<td>268</td>
<td>0.09</td>
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<td>15.</td>
<td>15.225</td>
<td>Pentadecanoic acid</td>
<td>C₁₅H₃₀O</td>
<td>242</td>
<td>0.06</td>
</tr>
<tr>
<td>16.</td>
<td>15.417</td>
<td>1,2-Benzenedicarboxylic acid, bis(2-methyl)propyl) ester</td>
<td>C₁₅H₃₂O₄</td>
<td>278</td>
<td>0.79</td>
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<td>17.</td>
<td>15.888</td>
<td>1,2-Benzenedicarboxylic acid, butyl octyl ester</td>
<td>C₂₀H₃₆O₄</td>
<td>334</td>
<td>0.09</td>
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<tr>
<td>18.</td>
<td>16.077</td>
<td>9-Hexadecenoic acid</td>
<td>C₁₆H₃₂O₂</td>
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<td>0.14</td>
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<td>19.</td>
<td>16.329</td>
<td>n-Hexadecanoic acid</td>
<td>C₁₆H₃₂O₂</td>
<td>256</td>
<td>11.63</td>
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<td>20.</td>
<td>16.595</td>
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<td>0.28</td>
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<td>21.</td>
<td>17.217</td>
<td>Heptadecanoic acid</td>
<td>C₁₇H₃₄O</td>
<td>270</td>
<td>0.07</td>
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<tr>
<td>22.</td>
<td>17.424</td>
<td>1-Octadecanol</td>
<td>C₁₈H₃₈O</td>
<td>270</td>
<td>0.14</td>
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<td>23.</td>
<td>17.537</td>
<td>2,6,10,15-Tetramethyl-Heptadecane,</td>
<td>C₂₁H₄₄O</td>
<td>296</td>
<td>0.12</td>
</tr>
<tr>
<td>24.</td>
<td>17.618</td>
<td>(Z,Z,Z)-9,12,15-Octadecatrienoic acid, methyl ester,</td>
<td>C₁₉H₃₂O₂</td>
<td>292</td>
<td>0.10</td>
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<td>25.</td>
<td>17.714</td>
<td>Phytol</td>
<td>C₂₀H₄₄O</td>
<td>296</td>
<td>0.15</td>
</tr>
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<td>26.</td>
<td>18.195</td>
<td>(Z,Z,Z)-9,12,15-Octadecatrien-1-ol,</td>
<td>C₁₉H₃₂O₂</td>
<td>264</td>
<td>54.92</td>
</tr>
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<td>27.</td>
<td>18.265</td>
<td>Octadecanoic acid</td>
<td>C₁₈H₃₂O₂</td>
<td>284</td>
<td>13.46</td>
</tr>
<tr>
<td>28.</td>
<td>18.917</td>
<td>cis,cis,cis-7,10,13-Hexadecatrien</td>
<td>C₁₈H₃₈O</td>
<td>234</td>
<td>0.55</td>
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<tr>
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<td>352</td>
<td>0.15</td>
</tr>
<tr>
<td>30.</td>
<td>19.542</td>
<td>11,14,17-Eicosatrienoic acid, methyl ester</td>
<td>C₂₁H₄₂O₂</td>
<td>320</td>
<td>0.29</td>
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<td>31.</td>
<td>19.882</td>
<td>E-8-Methyl-7-dodecen-1-ol acetate,</td>
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<td>240</td>
<td>0.24</td>
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<tr>
<td>32.</td>
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<td>Tetratetracontane</td>
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<td>33.</td>
<td>21.084</td>
<td>2-Mono-Palmitin</td>
<td>C₁₉H₃₂O₄</td>
<td>330</td>
<td>0.99</td>
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<td>34.</td>
<td>21.672</td>
<td>Pentatriacontane</td>
<td>C₂₅H₅₂O</td>
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<td>35.</td>
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<td>Octadecanal</td>
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<td>36.</td>
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<td>(Z,Z)-9,12-Octadecadienoic acid, trimethylsilyl ester</td>
<td>C₂₁H₄₂O₅Si</td>
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<td>0.18</td>
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<td>37.</td>
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<td>(all-Z)-4,7,10,13,16,19-Docosahexaenoic acid, methyl ester,</td>
<td>C₂₃H₄₂O₂</td>
<td>342</td>
<td>0.14</td>
</tr>
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<td>38.</td>
<td>22.502</td>
<td>E,Z-1,3,12-Nonadecatriene</td>
<td>C₁₉H₃₄</td>
<td>262</td>
<td>3.94</td>
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<td>39.</td>
<td>22.585</td>
<td>Methyl(Z)-5,11,14,17-eicosatetraenoate</td>
<td>C₂₁H₄₂O₂</td>
<td>318</td>
<td>4.30</td>
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<td>0.63</td>
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<td>1-Hentetracontanol</td>
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<td>0.66</td>
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<td>42.</td>
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<td>(all-E)-2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22 Tetracosahexaene,</td>
<td>C₃₀H₅₀</td>
<td>410</td>
<td>1.39</td>
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<td>43.</td>
<td>24.183</td>
<td>6,6-Dimethyl-Bicyclo[3.1.1] hept-2-ene-2-ethanol,</td>
<td>C₁₁H₁₈O</td>
<td>166</td>
<td>0.06</td>
</tr>
<tr>
<td>44.</td>
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<td>Octadecanal</td>
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<td>0.10</td>
</tr>
<tr>
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<td>26.519</td>
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<td>C₂₈H₄₆O₂</td>
<td>416</td>
<td>0.28</td>
</tr>
<tr>
<td>50.</td>
<td>26.862</td>
<td>Tetratriacontane</td>
<td>C₃₄H₇₀</td>
<td>478</td>
<td>0.15</td>
</tr>
</tbody>
</table>
Table 2. The chemical compositions of *C. acuminata* D. seed oil obtained by petroleum ether.

<table>
<thead>
<tr>
<th>No.</th>
<th>RT</th>
<th>Compounds</th>
<th>Molecular formula</th>
<th>MW</th>
<th>Relative</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>3.235</td>
<td>1,3,5,7-Cyclooctatetraene</td>
<td>C₇H₁₄</td>
<td>104</td>
<td>0.10</td>
</tr>
<tr>
<td>2.</td>
<td>5.199</td>
<td>3,6-dimethyl-Decane</td>
<td>C₁₈H₃₆</td>
<td>170</td>
<td>0.06</td>
</tr>
<tr>
<td>3.</td>
<td>6.183</td>
<td>1,2,4,5-tetramethyl-Benzene</td>
<td>C₁₉H₂₀</td>
<td>134</td>
<td>0.07</td>
</tr>
<tr>
<td>4.</td>
<td>8.014</td>
<td>Pentadecane</td>
<td>C₂₀H₄₀</td>
<td>212</td>
<td>0.07</td>
</tr>
<tr>
<td>5.</td>
<td>8.301</td>
<td>4,6-dimethyl-Dodecane</td>
<td>C₂₁H₄₀</td>
<td>188</td>
<td>0.15</td>
</tr>
<tr>
<td>6.</td>
<td>8.923</td>
<td>Heptadecane</td>
<td>C₂₂H₄₄</td>
<td>240</td>
<td>0.04</td>
</tr>
<tr>
<td>7.</td>
<td>11.008</td>
<td>Acetamidocyclohexane</td>
<td>C₁₉H₂₇NO</td>
<td>141</td>
<td>0.07</td>
</tr>
<tr>
<td>8.</td>
<td>11.125</td>
<td>Cetyl iodide</td>
<td>C₁₈H₃₉I</td>
<td>352</td>
<td>0.12</td>
</tr>
<tr>
<td>9.</td>
<td>11.158</td>
<td>Heptadecane</td>
<td>C₂₂H₄₄</td>
<td>240</td>
<td>0.06</td>
</tr>
<tr>
<td>10.</td>
<td>11.393</td>
<td>2,4-bis(1,1-dimethylethyl)-Phenol,</td>
<td>C₂₁H₂₉</td>
<td>206</td>
<td>0.07</td>
</tr>
<tr>
<td>11.</td>
<td>11.668</td>
<td>Hexadecane</td>
<td>C₂₀H₄₀</td>
<td>296</td>
<td>0.04</td>
</tr>
<tr>
<td>12.</td>
<td>13.629</td>
<td>Eicosane</td>
<td>C₂₂H₄₄</td>
<td>282</td>
<td>0.08</td>
</tr>
<tr>
<td>13.</td>
<td>14.103</td>
<td>Hexadecane</td>
<td>C₂₀H₄₀</td>
<td>226</td>
<td>0.04</td>
</tr>
<tr>
<td>14.</td>
<td>14.177</td>
<td>Tetradecanoic acid</td>
<td>C₁₈H₃₈</td>
<td>228</td>
<td>0.11</td>
</tr>
<tr>
<td>15.</td>
<td>15.008</td>
<td>5-Isopropyl-1-methyl-1-cyclohexene</td>
<td>C₁₈H₃₀</td>
<td>138</td>
<td>0.07</td>
</tr>
<tr>
<td>16.</td>
<td>15.095</td>
<td>6,10,14-trimethyl-2-Pentadecane,</td>
<td>C₂₀H₄₀</td>
<td>268</td>
<td>0.06</td>
</tr>
<tr>
<td>17.</td>
<td>15.419</td>
<td>1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester</td>
<td>C₂₅H₃₀O₂</td>
<td>278</td>
<td>0.16</td>
</tr>
<tr>
<td>18.</td>
<td>15.600</td>
<td>diethyl-Boronic acid</td>
<td>C₁₈H₃₈O₂</td>
<td>86</td>
<td>0.05</td>
</tr>
<tr>
<td>19.</td>
<td>15.676</td>
<td>2,6,10,14-tetramethyl-Hexadecane</td>
<td>C₂₄H₄₀</td>
<td>282</td>
<td>0.06</td>
</tr>
<tr>
<td>20.</td>
<td>16.270</td>
<td>n-Hexadecanoic acid</td>
<td>C₂₄H₄₀</td>
<td>256</td>
<td>3.19</td>
</tr>
<tr>
<td>21.</td>
<td>17.424</td>
<td>1-Octadecanol</td>
<td>C₂₄H₄₀</td>
<td>270</td>
<td>0.06</td>
</tr>
<tr>
<td>22.</td>
<td>17.538</td>
<td>Hexadecane</td>
<td>C₂₄H₄₀</td>
<td>296</td>
<td>0.08</td>
</tr>
<tr>
<td>23.</td>
<td>17.714</td>
<td>Phytol</td>
<td>C₁₈H₃₈</td>
<td>296</td>
<td>0.13</td>
</tr>
<tr>
<td>24.</td>
<td>18.037</td>
<td>(Z,Z,Z)-9,12,15-Octadecatrienoic acid, methyl ester,</td>
<td>C₂₅H₄₀O₂</td>
<td>292</td>
<td>19.19</td>
</tr>
<tr>
<td>25.</td>
<td>18.162</td>
<td>(Z,Z)-9,12-Octadecadienoic acid</td>
<td>C₂₅H₄₀O₂</td>
<td>280</td>
<td>3.35</td>
</tr>
<tr>
<td>26.</td>
<td>18.442</td>
<td>1,54-Dibromotetrapentacontane</td>
<td>C₃₀H₄₄Br₂</td>
<td>914</td>
<td>0.60</td>
</tr>
<tr>
<td>27.</td>
<td>19.292</td>
<td>Pentacosane</td>
<td>C₃₀H₆₀</td>
<td>352</td>
<td>0.11</td>
</tr>
<tr>
<td>28.</td>
<td>20.118</td>
<td>Tetracosane</td>
<td>C₂₈H₅₂</td>
<td>338</td>
<td>0.08</td>
</tr>
<tr>
<td>29.</td>
<td>20.792</td>
<td>trans-9-Octadecan-1-ol</td>
<td>C₂₈H₄₄</td>
<td>268</td>
<td>0.09</td>
</tr>
<tr>
<td>30.</td>
<td>20.910</td>
<td>Tetratetracontane</td>
<td>C₃₀H₆₄</td>
<td>618</td>
<td>0.37</td>
</tr>
<tr>
<td>31.</td>
<td>21.078</td>
<td>2-mono-Phalmitin</td>
<td>C₂₈H₄₄O₂</td>
<td>330</td>
<td>0.14</td>
</tr>
<tr>
<td>32.</td>
<td>21.673</td>
<td>Pentatracontane</td>
<td>C₃₀H₆₄</td>
<td>492</td>
<td>0.07</td>
</tr>
<tr>
<td>33.</td>
<td>21.906</td>
<td>(3, beta,)-Ergost-5-en-3-ol</td>
<td>C₂₃H₄₀O₂</td>
<td>400</td>
<td>1.98</td>
</tr>
<tr>
<td>34.</td>
<td>22.142</td>
<td>3-Fluorobenzoic acid, 4-hexadecyl ester</td>
<td>C₂₃H₂₈O₂</td>
<td>364</td>
<td>0.23</td>
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<tr>
<td>35.</td>
<td>22.342</td>
<td>1-Pent-3-ynylcyclopta-1,3-diene</td>
<td>C₁₀H₁₀</td>
<td>132</td>
<td>0.10</td>
</tr>
<tr>
<td>36.</td>
<td>22.441</td>
<td>1,54-dibromo-Tetrapentacontane</td>
<td>C₃₂H₅₂Br₂</td>
<td>914</td>
<td>0.46</td>
</tr>
<tr>
<td>37.</td>
<td>22.489</td>
<td>9-Octadecanoic acid, (E,E,E)-1,2,3-propanetriyl ester,</td>
<td>C₃₀H₄₄O₂</td>
<td>884</td>
<td>0.31</td>
</tr>
<tr>
<td>38.</td>
<td>22.569</td>
<td>(Z,Z,Z)-9,12,15-Octadecatrienoic acid, ethyl ester,</td>
<td>C₃₀H₄₄O₂</td>
<td>306</td>
<td>0.34</td>
</tr>
<tr>
<td>39.</td>
<td>22.608</td>
<td>2-Nonadecanone</td>
<td>C₂₄H₄₀O₂</td>
<td>282</td>
<td>0.29</td>
</tr>
<tr>
<td>40.</td>
<td>22.675</td>
<td>Stigmastane-3,6-dione</td>
<td>C₂₈H₄₄O₂</td>
<td>428</td>
<td>0.18</td>
</tr>
<tr>
<td>41.</td>
<td>23.250</td>
<td>Pentfluoropropionic acid, heptadecyl ester</td>
<td>C₂₅H₄₄O₂</td>
<td>402</td>
<td>0.28</td>
</tr>
<tr>
<td>42.</td>
<td>23.483</td>
<td>1-Hexadecanesulfonfyl chloride</td>
<td>C₂₅H₄₄O₄S</td>
<td>324</td>
<td>0.46</td>
</tr>
<tr>
<td>43.</td>
<td>23.654</td>
<td>All-trans-Squalene</td>
<td>C₃₀H₄₄O₂</td>
<td>410</td>
<td>0.85</td>
</tr>
<tr>
<td>44.</td>
<td>24.167</td>
<td>[4-[[trimethylsilyl]oxy]phenyl]-2-[[trimethylsilyl]oxy]-3-2-Propanoic acid, trimethylsilyl ester</td>
<td>C₃₃H₅₄O₆Si₃</td>
<td>396</td>
<td>26.53</td>
</tr>
<tr>
<td>45.</td>
<td>24.265</td>
<td>3-Gamma-Sitosterol</td>
<td>C₃₀H₄₄O₂</td>
<td>414</td>
<td>23.49</td>
</tr>
<tr>
<td>46.</td>
<td>24.578</td>
<td>Fucosterol</td>
<td>C₃₀H₄₄O₂</td>
<td>412</td>
<td>1.38</td>
</tr>
<tr>
<td>47.</td>
<td>25.034</td>
<td>[3S-(3,alpa.,5a.alpa.,7a.alpa.,11a.beta.,11b.alpa.,)]-dodecadihydro-3,8,8,11a-tetramethyl-3,5a-Epoxyxaphill[2,1-d]oxepin</td>
<td>C₃₀H₄₄O₂</td>
<td>278</td>
<td>1.39</td>
</tr>
<tr>
<td>48.</td>
<td>25.318</td>
<td>Betulin</td>
<td>C₁₈H₃₀O₂</td>
<td>442</td>
<td>0.43</td>
</tr>
<tr>
<td>49.</td>
<td>25.560</td>
<td>Cedran-8-yl acetate</td>
<td>C₁₈H₃₀O₂</td>
<td>264</td>
<td>3.32</td>
</tr>
<tr>
<td>50.</td>
<td>25.725</td>
<td>Lup-20(29)-en-3-one</td>
<td>C₁₈H₃₀O₂</td>
<td>424</td>
<td>4.30</td>
</tr>
<tr>
<td>51.</td>
<td>26.081</td>
<td>cis-1-Chloro-9-octadecane</td>
<td>C₁₈H₃₈Cl</td>
<td>286</td>
<td>0.52</td>
</tr>
<tr>
<td>52.</td>
<td>26.323</td>
<td>Lupenyl acetate</td>
<td>C₁₉H₄₀O₂</td>
<td>468</td>
<td>3.79</td>
</tr>
<tr>
<td>53.</td>
<td>26.514</td>
<td>Beta-Tocopherol</td>
<td>C₁₉H₃₈O₂</td>
<td>416</td>
<td>0.41</td>
</tr>
</tbody>
</table>
(13.6%). Other aldehydes in appreciable amounts were 2E-tridecen-1-al (6.75%), 2E-dodecenal (6.23%), dodecanal (4.36%) and undecanal (3.23%). The alcohol undecanol (3.37%) was also in fairly good amount. The monoterpenes apinene (0.04%) and linalool (0.32%) were in trace amounts. However, the chemical composition of the essential oil was different from that observed from Tunisian plant materials (Msaada et al., 2007). Indeed, in the Tunisia study, the predominant aldehyde was 2E-dodecanal, while in our study, it was 2E-decenal. The essential oil was evaluated for antimicrobial activity against pathogenic strains of Gram positive (S. aureus and Bacillus spp.) and Gram negative (E. coli, P. aeruginosa, S. typhi, Klebsiella pneumoniae, and Proteus mirabilis) bacteria. It was active against all the bacterial strains except P. aeruginosa.

The oil also showed an obvious antifungal activity against C. albicans and P. aeruginosa, which also been

Figure 1. The rate of DPPH elimination. Values of each curve are means ± SD (n, 3). *p < 0.01.

Figure 2. β-Carotene bleaching test. Values of each curve are means ± SD (n, 3). *p < 0.01.
Figure 3. The concentration of 50% inhibition (IC\textsubscript{50}) values of C. acuminate D. seed oil obtained by SC-CO\textsubscript{2} or petroleum ether.

Table 3. The inhibitory effects of C. acuminate D. seed oil obtained by SC-CO\textsubscript{2} against all the testing organisms.

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>MIC (%)</th>
<th>MBC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. subtilis 6633</td>
<td>&gt;5</td>
<td>&gt;5</td>
</tr>
<tr>
<td>S. aureus ATCC 6538</td>
<td>1.25</td>
<td>1.25</td>
</tr>
<tr>
<td>S. epidermidis ATCC 49134</td>
<td>0.625</td>
<td>2.5</td>
</tr>
<tr>
<td>E. coli ATCC 11229</td>
<td>2.5</td>
<td>&gt;5</td>
</tr>
<tr>
<td>P. vulgaris</td>
<td>&gt;5</td>
<td>&gt;5</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>2.5</td>
<td>&lt;5</td>
</tr>
<tr>
<td>C. albicans</td>
<td>1.25</td>
<td>5</td>
</tr>
<tr>
<td>A. niger V. Tiegh</td>
<td>&gt;5</td>
<td>&gt;5</td>
</tr>
</tbody>
</table>

Table 4. The inhibitory effects of C. acuminate D. seed oil obtained by petroleum ether against all the testing organisms.

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>MIC (%)</th>
<th>MBC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. subtilis 6633</td>
<td>&gt;5</td>
<td>&gt;5</td>
</tr>
<tr>
<td>S. aureus ATCC 6538</td>
<td>0.625</td>
<td>1.25</td>
</tr>
<tr>
<td>S. epidermidis ATCC 49134</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>E. coli ATCC 11229</td>
<td>5</td>
<td>&gt;5</td>
</tr>
<tr>
<td>P. vulgaris</td>
<td>&gt;5</td>
<td>&gt;5</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>0.625</td>
<td>1.25</td>
</tr>
<tr>
<td>C. albicans</td>
<td>0.625</td>
<td>2.5</td>
</tr>
<tr>
<td>A. niger V. Tiegh</td>
<td>1.25</td>
<td>5</td>
</tr>
</tbody>
</table>

observed to be resistant to the essential oils from other plants, such as Achillea holoserica (Magiatis et al., 1999) and Stachys species (Skaltsa et al., 2003). This microorganism is less susceptible to the anti-microbial properties of essential oils than others, and its tolerance is thought to result from its outer membrane (Cox and Radolf, 2001). And the ability of essential oil to disrupt the permeability barrier of cell membrane structures and the accompanying loss of chemiosmotic control are the most likely reason for its lethal action (Cox and Radolf, 2001). This antimicrobial activity against bacteria and fungi has also been demonstrated in essential oils extracted from C. sativum seeds (Lo Cantore et al., 2004). Although the concentrations of the oil were generally about 100 times more than those of the standard antibiotics (chloramphenicol), they showed marked antibacterial and antifungal activities, as demonstrated by their zones of inhibition (Tables 3 to 6). This concentration difference between the essential oil and the standard antibiotic can be explained by the fact that the active components in the oil comprise only a fraction of the oil. Therefore, the concentration of the active components could be much lower than the standard antibiotics we used. Importantly, if the active components were isolated and purified, they would probably show higher antimicrobial activities than those observed here. Among the Gram negative bacteria, the oil was very active against K. pneumoniae and P. mirabilis. The best activity was observed for the Gram positive bacteria. In general, the oil showed greater
antibacterial activity than antifungal activity (Tables 3 to 6). Aldehydes and alcohols are known to be active but with different specificity and activity levels, which is related not only to the functional group but also to hydrogen bonding parameters (Skaltsa et al., 2003). As a minor component in this study, linalool has been found to have antimicrobial activity against various microbes, except for *P. aeruginosae* (Carson and Riley, 1995), which is also known to inhibit spore germination and fungal growth. The inhibition of sporelation appeared to arise from respiratory suppression of aerial mycelia (Lahlou and Berrada, 2001).

### Conclusions

Our study showed that *C. acuminate D.* seed oils had extraordinary antioxidant and antibacterial activity *in vitro*. Due to its virulence, this seed oil can work as natural antioxidants and antimicrobial, which is a promising alternative to the use of synthetic antioxidants in food supplement or in pharmaceutical and cosmetic industry. But there have been few studies on the activity of *C. acuminate D.* seed oil. In this study, we evaluated its inhibitory activity in several common bacteria and estimated its antioxidant effectiveness by β-carotene bleaching and DPPH tests. Our results further demonstrated that *C. acuminate D.* seed oil had remarkable antioxidant and antibacterial activity, especially *S. aureus* ATCC 6538. The seed oil possesses various biological functions, notably antibacterial and Antioxidant properties that can be widely used as alternative to synthetic antioxidant or bacterial. Therefore, we hope our study provides a foundation for future research of extracting ingredients from plants or herbs as natural antioxidant and antibacterial.

### ACKNOWLEDGEMENT

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### REFERENCES


