GC-MS analysis and antioxidant activity of volatiles from *Pluchea dioscoridis* (L.) DC

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**Abstract**

The essential oil of the aerial parts of *Pluchea dioscoridis* (L.) DC, an endemic medicinal plant growing in Egypt, was obtained by hydrodistillation and analyzed by GC-MS. The TIC chromatogram of the oil exhibited more than 80 components; their percentages (as determined by normalization method) ranged from 0.1 – 18.3 %; fifteen of which are considered major (the percentages of these components ranged from 1.34-18.3 %) and identified by library search of their MS data as well as by literature survey. Our results are remarkably different from the previously reported oil constituents. Eight of the major components are reported for the first time in the oil, while four components have been previously reported in oil samples. *In-vitro* testing for its antioxidant activity by measuring the reducing power and DPPH free radical scavenging activity were determined to be 22.77 mg ascorbic acid equivalent (AAe)/mL of oil, and 1.65 mg AAe/mL of oil, respectively. The reducing power of the oil was compared with values determined for clove oil and eucalyptus oil.

**Keywords**

*Pluchea dioscoridis* (L.) DC

Essential oils

GC-MS

Antioxidant activity

Clove and Eucalyptus oils

1. Introduction

*Pluchea dioscoridis* (L.) DC. (Asteraceae) is a common wild evergreen shrub, attaining a height of one to three meters, richly branched and hairy. The plant is widely distributed in the Middle Eastern and surrounding African countries. In Egypt, the plant is known by the Arabic name “Barnoof”, and occurs mainly in Nile region, Oases of the Western Desert, Mediterranean coastal strip, Eastern Desert and Sinai Peninsula (Shaltout and Slima, 2007; Boulos, 2002).

*Pluchea dioscoridis* possesses a good reputation in folk medicine for treating rheumatic pains (Boulos and El-Hadidi, 1989), epilepsy in children, colic (Batanouny et al. 1999), and as a remedy for cold (Batanouny et al. 1999; Watt and Breyer-Brandwijk 1962). The oil is effective for controlling myiasis caused by the common green bottle fly (*Lucilia sericata*) larvae feeding on the host’s necrotic or living tissue. Myiasis is one of the most important parasitic diseases affecting the human welfare (Mazyad, 1999). *Pluchea dioscoridis* oil also showed a marked mosquito larvicidal activity against *Culex pipiens* which is a carrier of several diseases including malaria, yellow fever, dengue, filariasis and encephalitis (Grace, 2002).

The literature pointed out at the existence of two previous GC-MS studies of the essential oil prepared from plants growing in Cairo (Egypt). These studies resulted in ambiguous results (Grace, 2002; El-Hamouly and Ibrahim 2003). It was striking to observe that the top 5 major components in one study (Grace, 2002) are completely absent in the oil of the other study (El-Hamouly and Ibrahim 2003), meanwhile, 4 of the top 5 major components in the oil of the second study (El-Hamouly and Ibrahim 2003) are absent in the other oil. Consequently, the aim of the present study is to determine the composition of the plant essential oil applying standardized and reliable methodologies making use of recent computer-search programs. Moreover, and owing to the reported...
uses of the plant in folk medicine, it was essential to evaluate the antioxidant activity of the essential oil by measuring the reducing power (Oyaizu 1986) and DPPH free radical scavenging activity (Mărgitaş 2009).

2. Materials and Methods

2.1. Plant Material and chemicals

The aerial parts of *P. dioscoridis* were collected in January 2010 around noon time from Abee’s Farm in Alexandria. The identity of the collected plant was established by Prof. Dr. Aly M. Metwally. Department of Pharmacognosy, University of Alexandria, Egypt.

Solvents and chemicals were purchased from the following suppliers: rutin (Merck, Dramstadt, Germany), ascorbic acid B.P. (Amoli organics Pvt. Ltd., India), clove and eucalyptus oils (Oxford Chemicals Ltd, UK), methanol (Lab-Scan Ltd., Dublin, Ireland), hexane (SDS, France), ethanol 95% (El-Nasr Pharm. Chem. Co., Egypt), DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical (Sigma-Aldrich, Germany), potassium ferricyanide L.R. (Nice-Chem. Pvt. Ltd., India), trichloroacetic acid (SD Fine-Chem. Ltd., India), ferric chloride (Loba Chemie Pvt. Ltd., India).

2.2. Instruments

The following instruments were used: Helios-α UV/visible spectrophotometer supported with Vision 32 software (Thermospectronic, UK); 80-2 Electric centrifuge, China; Clevenger-type apparatus for isolation of oils lighter than water; Clarus 600 Gas Chromatograph/Mass spectrometer provided with TurboMass™ 5.03 GC/MS software (Perkin Elmer).

2.3. Isolation of the essential oil

The oil was prepared from fresh aerial parts of plant by hydrodistillation using the USP method (USP/NF 2007) and Clevenger-type apparatus. Accurately weighed fresh aerial parts of the plant (200 g) were cut and introduced into a round-bottom, short neck, 2-liter flask. One liter of distilled water (just covering the plant material) was added to the flask, then a volatile oil trap (for oils lighter than water) and a cold-finger condenser were attached to the flask. The contents of the flask were then boiled for 2.5 hours; the rate of condensation was about 90 drops/min. After 2.5 hours no more oil was collected in the graduated tube of the oil trap. The distilled oil in the graduated tube (0.2 mL) was extracted using 5 mL of HPLC-grade hexane. The hexane extract was dried over 0.2 g of anhydrous sodium sulfate, transferred quantitatively into 5-mL volumetric flask and its volume was completed using the same solvent to prepare 4% stock solution of essential oil.

2.4. Gas Chromatography-Mass Spectrometry

The qualitative and quantitative analyses of the essential oil were performed using Clarus 600 Gas Chromatograph/Mass spectrometer (Perkin Elmer) provided with TurboMass™ 5.03 GC/MS software. Rtx®-5MS (a fused silica, low-polarity phase; 5% diphenyl/95% dimethyl polysiloxane from Restek) column 30 m x 0.32 mm i.d. and 0.25 µm film thickness. Injection volume 1.0 µL, sampling rate 12.50 pts/s. Helium flow rate 1.4 mL/min. Injector temperature is 250 °C. Oven temperature program: initial temperature 30 °C for 2.0 min, ramp 2.0 °C/min to 250 °C, hold for 5.0 min. MS source temperature is 200 °C, electron energy is 70 eV. EI spectra were scanned from 43.00 to 600 m/z. Identification of peaks through NIST mass data search libraries and the highest REV and FOR similarity indicators hits. Further identification was achieved by comparing the obtained data with those of the literature. Components relative percentages were obtained by normalization without using correction factors.

2.5. Antioxidant activity of the essential oil

2.5.1. Determination of the reducing power of *P. dioscoridis* essential oil

The reducing power assay was determined according to the method of (Oyaizu 1986) with minor modification. The obtained result was compared with that of two famous essential oils well reputed for extremely high and moderately high reducing power; oil of clove and oil of eucalyptus, respectively. The stock solution of *P. dioscoridis* oil (4%) was subjected to serial dilutions using methanol to prepare 0.02% oil solution. Clove oil was subjected to serial dilutions using methanol to prepare 0.1% oil solution. Eucalyptus oil was subjected to serial dilutions using methanol to prepare 0.1% oil solution. The stock solution of *P. dioscoridis* oil (4%) was subjected to serial dilutions using methanol to prepare 0.02% oil solution. Clove oil was subjected to serial dilutions using methanol to prepare 0.1% oil solution. Eucalyptus oil was subjected to serial dilutions using methanol to prepare 0.1% oil solution. Sample solutions (2.5 mL) were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 mL, 1%) solution and the mixture was incubated at 50°C for 20 min. Trichloroacetic acid (2.5 mL, 10%) solution was added to the reaction mixture to stop the reaction, then centrifuged at 3000 revolution/min for 10 min. The upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and ferric chloride (0.5 mL, 0.1%) solution and the absorbance was measured.
3. Results and Discussion

The essential oil yield by hydrodistillation of the fresh *P. dioscoridis* aerial parts (moisture content 69% w/w, determined by the toluene azeotropic method USP/NF 2007) was 0.1 % v/w. Gas chromatography-mass spectrometry (GC-MS) study of the oil disclosed the presence of more than 80 components (Figure 1).

The arbitrary units of the peak areas ranged from 7 - 10 digits. It was decided to consider only those peaks whose areas consist of 9 - 10 digits as the major components of the oil and constitute 73.19% of the oil. For their quantitative determination the normalization method was followed. The identification of these 15 components was accomplished by matching their mass spectra with those present in the National Institute of Standards and Technology (NIST) main library, as well as by comparison with published data (Adams, 2007; NIST 05. Mass spectral library; United States National Library of Medicine; Sigma-Aldrich Technical Service; The PubChem Project, PubChem Structure Search).

The hit list window gives for every component a text-listing of the best 20 hits resulting from the Turbomass library search. It includes hit number, compound name, forward (FOR) fit value, reverse (REV) fit value (Milman et al. 1999), chemical formula, molecular weight and Chemical Abstract Service (CAS) registry number. The chemical composition of the major components of the essential oil is summarized in Table 1.

2.5.2. In-vitro determination of free radical scavenging activity of *P. dioscoridis* essential oil by DPPH method

The stock solution of *P. dioscoridis* essential oil (4%) was diluted 10 folds using methanol to prepare 0.4% sample solution of essential oil. 400-µL aliquots of the previously described standard and sample solutions were mixed with 2 mL of DPPH solution (0.02 mg/mL). The mixtures were kept in the dark at room temperature for 15 min and then the absorbance was measured at 517 nm (Mărgitaș 2009). Control samples containing the same amount of solvent and DPPH solution were concomitantly prepared and their absorbance measured (A control). The percentage of absorbance inhibition at 517 nm was calculated using the following expression:

\[
\% \text{ inhibition} = \left[ \frac{(A \text{ control} - A \text{ sample})}{A \text{ control}} \right] \times 100
\]

The extent of decolorization is calculated as percentage reduction of absorbance, and this is determined as a function of concentration and calculated relative to ascorbic acid concentration. The radical scavenging activity is expressed in mg ascorbic acid equivalent (mg A Ae) per mL of essential oil.
Moreover, the present study reveals that only one of the top 5 components (δ-Cadinene) is reported in (Grace, 2002) and (El-Hamouly and Ibrahim 2003) but not among the top components. Consequently, it would be highly recommended to carry out a further study of the major components of the essential oil to reveal whether this variation in the chemical composition is attributed to ecological factors, age of the collected plant samples, season and time of collection, etc., or to probable existence of chemo-types among *P. dioscoridis*.

The essential oil exhibited moderately high reducing power (22.77 mg AAE/mL of oil), and moderate DPPH free radical scavenging activity (1.65 mg AAE/mL of oil).

<table>
<thead>
<tr>
<th>C_{15}H_{24}</th>
<th>Oil prepared in present study</th>
<th>Oil prepared in (Grace, 2002)</th>
<th>Oil prepared in (El-Hamouly and Ibrahim 2003)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Guaiene</td>
<td>2.49%</td>
<td>2.73%</td>
<td>2.52%</td>
</tr>
<tr>
<td>β-Caryophyllene</td>
<td>2.70%</td>
<td>3.27%</td>
<td>1.25%</td>
</tr>
<tr>
<td>Alloaromadendrene</td>
<td>1.98%</td>
<td>3.53%</td>
<td>2.79%</td>
</tr>
<tr>
<td>δ-Cadinene</td>
<td>7.68%</td>
<td>3.75%</td>
<td>3.52%</td>
</tr>
</tbody>
</table>

The obtained results disclosed that eight of the major components of the essential oil are reported for the first time in *P. dioscoridis*. These are: aristolene; β-cadinene; 2-(3-isopropyl-4-methylpent-3-en-1-ynyl)-2-methylcyclobutanone; 2-methyl-3-(3-methylbut-2-enyl)-2-(4-methylpent-3-enyloxetane; γ-muurolene; 1-methylene-2b-hydroxymethyl-3,3-dimethyl-4b-(3-methylbut-2-enyl)-cyclohexane; (-)-alloisolongifolene; and 2,4,4-trimethyl-3-hydroxymethyl-5a-(3-methylbut-2-enyl)-cyclohexene. Meanwhile, only four components are of common occurrence in the studied oil samples. These belong to sesquiterpene hydrocarbons (C15H24) and their percentages ranged from 7.68% to 1.25% (Table 2). Concerning the top 5 components in the studied oil samples (Table 3), it was astonishing to notice that these components are totally different in the three oil samples.

Moreover, the present study reveals that only one of the top 5 components (δ-Cadinene) is reported in (Grace, 2002) and (El-Hamouly and Ibrahim 2003) but not among the top components.

Consequently, it would be highly recommended to carry out a further study of the major components of the essential oil to reveal whether this variation in the chemical composition is attributed to ecological factors, age of the collected plant samples, season and time of collection, etc., or to probable existence of chemo-types among *P. dioscoridis*.

The essential oil exhibited moderately high reducing power (22.77 mg AAE/mL of oil), and moderate DPPH free radical scavenging activity (1.65 mg AAE/mL of oil).
On comparing the obtained result of the reducing power with those of the two chosen medicinal oils, it could be inferred that oil of *P. dioscoridis* exhibited slightly higher reducing power than that of oil of eucalyptus, but distinctly lower than that of oil of clove. This result is highly expected as oil of clove is the most powerful antioxidant essential oil. The ORAC scores reported for oils of clove and eucalyptus are 1,078,700 and 2,410 micromole trolox equivalent per 100 g oil (µTE/100g), respectively (USDA). The ORAC is an antioxidant scale developed at Tufts University in Boston, Massachusetts by which the oxygen radical absorption capacity is estimated and expressed as micromole trolox equivalent per 100 g. The reducing powers of *P. dioscoridis*, clove and eucalyptus oils are given in Table 4 and the bar chart Figure 2.

### Table 3: The top 5 components in the studied *P. dioscoridis* oil samples

<table>
<thead>
<tr>
<th>Oil prepared in present study</th>
<th>Oil prepared in (Grace, 2002)</th>
<th>Oil prepared in (El-Hamouly and Ibrahim, 2003)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,4,4-trimethyl-3-hydroxymethyl-5a-</td>
<td><em>cis,trans</em>-Farnesol</td>
<td>Sesquiterpene oxide</td>
</tr>
<tr>
<td>(3-methyl-but-2-enyl)- cyclohexene (18.3%)</td>
<td>(16.5%)</td>
<td>(14.41%)</td>
</tr>
<tr>
<td>(-)-Alloisolongifolene (9.16%)</td>
<td>Isobutyl benzene</td>
<td>α-Cadinol (10.15%)</td>
</tr>
<tr>
<td>δ-Cadinene (7.68%)</td>
<td>Longifolene (7.46%)</td>
<td>Muurolool (9.19%)</td>
</tr>
<tr>
<td>β-Cadinene (6.87%)</td>
<td>Germacrene D-4-ol (6.2%)</td>
<td>1,2-Benzene dicarboxylic acid dioctylester (7.8%)</td>
</tr>
<tr>
<td>2-(3-Isopropyl-4-methylpent-3-en-1-ynyl)-2-</td>
<td>Torreyol (4%)</td>
<td>β-Maaline (7.49%)</td>
</tr>
<tr>
<td>methyl-cyclo-butane (5.57%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 4: Reducing power of clove, *P. dioscoridis* and eucalyptus essential oils.

<table>
<thead>
<tr>
<th>Essential oils</th>
<th>Reducing power (mg AAe/ml oil)*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. dioscoridis</em> oil</td>
<td>22.77±1.9</td>
</tr>
<tr>
<td>Clove oil</td>
<td>162.55 ± 0.7</td>
</tr>
<tr>
<td>Eucalyptus oil</td>
<td>17.02 ± 2.1</td>
</tr>
</tbody>
</table>

*The results are mean of three determinations ± SD.

4. Conclusion

The obtained results from GC-MS analysis of the volatiles isolated from *P. dioscoridis* remarkably different from the previously reported ones. Eight of the major components are reported for the first time in the oil, while four components have been previously reported in oil samples. In-vitro testing for its antioxidant activity by measuring the reducing power and DPPH free radical scavenging activity were determined to be 22.77 mg ascorbic acid equivalent (AAe)/mL of oil and 1.65 mg AAe/mL of...
The reducing power of the oil was compared with values determined for clove oil and eucalyptus oil.

5. Conflict of interest

The authors report no declaration of conflict of interest.

6. References


USDA Researchers at Tufts University. Oxygen radical absorbance capacity (ORAC Scale). http://www.therapeutic-grade.com/refs/orac.html
