Phytochemical and biological activity of Algerian *Centaurea melitensis*

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Abstract: The aerial parts of *Centaurea melitensis* (Asteraceae) yielded the germacranolides 8α-O-Isobutyroylsalonitenolide (1), Actiopicrin (2) and Onopordopicrin (6), the elimanolides Dehydromelitensin-β-hydroxyisobutyrate (4) 8α-O-(4-hydroxymethacrylate) melitensine (5), together with two flavonoids Hispidulin (3), Ne-petin (7) and a phenolic acid protocatechuic acid (8), the structures of these compounds were determined by direct comparison of the spectral data with published data. The antioxidant property of the extract was determined by using the DPPH method.

Keywords: Asteraceae; *Centaurea melitensis*; Elimanolides; Flavonoids; Germacranolides; Sesquiterpene lactones.

Introduction

The family Asteraceae is one of the largest angiosperm family and comprises about 1300 genera and 25000 spices distributed over three subfamilies and 17 tribes (Bremer 1994). The large genus *Centaurea* (Asteraceae) comprises about 500 spices which are predominately distributed around the Mediterranean area and Western Asia (Mabberlay 1997). Amongst the biological effects exerted by *Centaurea* species, it is noteworthy that several species were particularly recommended against inflammatory conditions such as abscesses (*Centaurea iberica* Trev. ex Sprengel), asthma (*Centaurea iberica* Trev.ex Sprengel), hemorrhoids (*Centaurea drahifolia* Sm), wound healing (*Centaurea iberica* Trev.ex Sprengel, *Centaurea virgata* Lam., *Centaurea pterocaula* Trautv.), to reduce fever (*Centaurea calcitrapa* L., *Centaurea jacea* L., *Centaurea iberica* Trev.ex Sprengel, *Centaurea solstitialis* ssp. *solstitialis*), and headache (*Centaurea solstitialis* L. ssp. *solstitialis*) (Yesilada 2002). A wide range of therapeutic effects have also been attributed to *Centaurea* species in traditional medicines worldwide including endocrine diseases (diabetes), inflammatory disorders (rheumatic pain, antipyretic), gastrointestinal symptoms (diarrhea, indigestions, and stomachic), urogenital ailments (diuretic, to induce menstruation), cardiovascular problems (hypotensive), parasitic and microbial infections (antibacterial, antimalarial…), etc. (Kaij-A-Kamb et al.1992 ; Farrag et al.1993 ; Barrero et al.1997 ; Orallo et al.1998). *Centaurea melitensis* L. is a perennial plant belonging to the Asteraceae family that abundantly grows in Algeria (Quezel and Santa 1963), this spices is used in folk medicine for treating hypoglycemia (Kamanzi et al. 1983). In the present study, we investigated the phytochemical composition and the antioxidant activity of *C. melitensis*.

Materials and methods

Plant material

Aerial parts of *Centaurea melitensis* L. were collected from Boussaâda (Algeria) in June 2008, and authenticated by Dr Gérard De Belair (Department of biology, Annaba University, Algeria). A voucher specimen is deposited in the Herbarium of our laboratory.
Centaurea melitensis as a source of secondary metabolites

Extraction and isolation and structures elucidation

Dried and ground aerial parts (1070g) of plant were extracted with MeOH (3times) at room temperature, and the combined extracts were evaporated under reduced pressure. Methanolic extract (150ml) was suspended in H2O and then partitioned with CH2Cl2. Ethyl acetate and n-butanol successively (each 300ml×3times). The combined CH2Cl2 and Ethyl acetate fractions were separately concentrated using a rotary evaporator at a maximum temperature of 45°C, which afforded 11g, 13g extracts, respectively. CH2Cl2 fraction (9g) was subjected to silica gel column chromatography, starting the elution with CH2Cl2 and gradually increasing the polarity of the solvent by the addition of Acetone, and finally with 100% Acetone. One hundred and twenty four fractions (124 Frs) were collected and monitored by TLC. Based on TLC profiles, four fractions; F1 (Frs : 7→53, 400mg), F2 (Frs : 57→59 ; 40mg), F3 (Frs : 66→76, 75.8 mg), F4 (Frs : 80→83 ; 36.7mg) were selected for further purification. F1 was chromatographed over silica gel column to afford a mixture of sesquiterpene lactones, purification of the intermediate fractions by means of preparative TLC (CH2Cl2-Acetone: 90:10), gave compounds (1) and (2). F2 was chromatographed over sephadex LH-20 (100% MeOH) to afford compound (3). F3 was chromatographed on preparative TLC (CH2Cl2: Acetone: 80:20) gave two compounds (4), (5). F4 contained considerable amount of compound (6) which precipitated in crystalline form. Ethyl acetate fraction (8g) was chromatographed over silica gel column to afford a mixture of sesquiterpene lactones, purification of the intermediate fractions by means of preparative TLC (CH2Cl2-Acetone: 90:10), gave compounds (1) and (2). F2 was chromatographed over sephadex LH-20 (100% MeOH) to afford compound (3). F3 was chromatographed on preparative TLC (CH2Cl2: Acetone: 80:20) gave two compounds (4), (5). F4 contained considerable amount of compound (6) which precipitated in crystalline form. Ethyl acetate fraction (8g) was chromatographed over silica gel column to afford a mixture of sesquiterpene lactones, purification of the intermediate fractions by means of preparative TLC (CH2Cl2-Acetone: 90:10), gave compounds (1) and (2). F2 was chromatographed over sephadex LH-20 (100% MeOH) to afford compound (3). F3 was chromatographed on preparative TLC (CH2Cl2: Acetone: 80:20) gave two compounds (4), (5). F4 contained considerable amount of compound (6) which precipitated in crystalline form.

Biological material

Antioxidant activity

DPPH: 2, 2-diphenyl-1-picrylhydrazyl (Molecular formula C15H11N3O5) solution in MeOH (100µg/ml) was used in this assay (Takao et al. 1994) to assess the free radical scavenging (antioxidant) property of the extract. Quercetin, a well known natural oxidant was used as reference.

Determination of Antioxidant activity

Qualitative assay

Test extract of C.melitensis (5mg/ml) and Quercetin were dissolved in MeOH and applied on a TLC plate (Merck, Germany).after this plate was sprayed with DPPH solution using an atomizer and allowed to develop for 30 min. The white spots against a pink background indicated the antioxidant activity.

Quantitative assay

Stock solutions of the test extract of C. melitensis were prepared to obtain different concentrations (0.0012, 0.0025, 0.005, 0.01, 0.02, 0.03, 0.05, 0.1, 0.2, 0.4, 0.5, 0.7 mg/ml). Prepared solutions (15µl each) were mixed with DPPH (1.5ml) and allowed to stand 15min for any reaction to occurs. The UV absorbance of these solutions was recorded at 517nm. The experiment was performed in triplicate and the average absorption was noted for each concentration. The same procedure was followed for the Quercetin (used as reference). The percentage (I %) of inhibition of DPPH by the methanolic extract of C. melitensis was calculated from the equation:

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I% = \[\text{AC} - \text{AE}\] / \text{AC} \times 100.

AC: Absorption in the absence of the inhibited control (negative control).

AE: Absorption in the presence of the inhibited sample (extract).

Results and discussion

The methanolic extract of *C. melitensis* exhibited a very important antioxidant in the DPPH assay with a maximal inhibition activity of 89.02% at the high concentration of 0.7mg/ml.

Table 1: Antioxidant activity of the methanolic extract of *C. melitensis* at different concentrations (mg/ml).

<table>
<thead>
<tr>
<th>Concentrations of extract (mg/ml)</th>
<th>1% of DPPH radical by extract (C. melitensis)</th>
<th>1% of DPPH radical by Quercetin</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0012</td>
<td>01.95%</td>
<td>46.45±1.78%</td>
</tr>
<tr>
<td>0.0025</td>
<td>05.32%</td>
<td>94.72±0.30%</td>
</tr>
<tr>
<td>0.005</td>
<td>11.41%</td>
<td>95.81±0.13%</td>
</tr>
<tr>
<td>0.01</td>
<td>13.04%</td>
<td>95.91±0.00%</td>
</tr>
<tr>
<td>0.02</td>
<td>17.71%</td>
<td>96.06±0.04%</td>
</tr>
<tr>
<td>0.03</td>
<td>22.82%</td>
<td>ND</td>
</tr>
<tr>
<td>0.05</td>
<td>34.13%</td>
<td>ND</td>
</tr>
<tr>
<td>0.10</td>
<td>55.10%</td>
<td>ND</td>
</tr>
<tr>
<td>0.20</td>
<td>87.71%</td>
<td>ND</td>
</tr>
<tr>
<td>0.40</td>
<td>88.80%</td>
<td>ND</td>
</tr>
<tr>
<td>0.50</td>
<td>88.91%</td>
<td>ND</td>
</tr>
<tr>
<td>0.7</td>
<td>89.02%</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND: not done; Concentrations values are in mg/ml; %: Percentage of inhibition

The results summarized in Table 1 showed that the percentage of inhibition of DPPH radical increase proportionally with the increase of extract concentration, the obtained inhibition (I%) varied from 1.95% to 89.02%; these results were compared with those of the Quercetin.

It is generally assumed that the radical scavenging or antioxidant activity of plants is a consequence of the presence of the natural phenolic antioxidants, like: lignans, flavonoids and phenolic acids. The antioxidant activity of phenolic natural products is predominantly due to their redox properties, i.e. the ability to act as reducing agents, hydrogen donors and singlet oxygen quenchers, and to some extent, could also be due to their metal chelation potential (Shoeb et al. 2006).

Relationship between antioxidant activity and concentration of the extract of *C. melitensis*

The correlation between antioxidant activity and concentrations of the extract of *C. melitensis* is shown (Figure 1). The R² value was 0.74. The present study shows that highly positive relationship exists between the antioxidant activity and the increasing in concentrations of the extract. Previous work (Cai et al. 2004; Shan et al. 2005) showed that there were highly positive linear correlation between antioxidant capacity and total phenolic content of a large number of spices and herbs.

Conclusion

In conclusion, the present study addressed the phytochemical and biological investigation of *C. melitensis* leading to the identification of eight (8) different compounds (sesquiterpene lactones, flavonoids and a phenolic acid). The important antioxidant activity of the extract, suggests the potential presence of phenolic compounds in *C. melitensis*.

References


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