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Sesquiterpene lactones with antinociceptive and antipyretic activity from two *Centaurea* species

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ABSTRACT

Ethnopharmacological relevance: Several *Centaurea* species are used to alleviate pain and inflammatory symptoms in rheumatoid arthritis, high fever, and head ache in Turkish folk medicine.
Aim of the study: The effectiveness of extracts, fractions and subfractions from dried *Centaurea solstitialis* L. subsp. *solstitialis* (*CSS*) (Asteraceae) roots and aerial parts were studied on mice.
Materials and methods: The antinociceptive and antipyretic effects of *Centaurea solstitialis* L. subsp. *solstitialis* have been investigated by using p-benzoquinone-induced writhing reflex for antinociceptive activity and Freund's Complete Adjuvant-induced pyrexia model for antipyretic activity assessment in mice.
Results: The ethanolic extract from the aerial parts of the plant was shown to possess significant antinociceptive ($p < 0.01$) and antipyretic activities ($p < 0.01$). The extract was then submitted to subsequent solvent extractions and chromatographic processes. Through bioassay-guided fractionation and isolation procedures two sesquiterpene lactones, solstitialin A and acetyl solstitialin, were isolated and defined as the active components of *CSS*. On the other hand, a comparative study was conducted on another species, *Centaurea depressa* Bieb., which has no similar folkloric utilization. Following the same fractionation chart same compounds were defined as the active ingredients.
Conclusion: Results of the present study proved that aerial part of *CSS* possesses antinociceptive and antipyretic activities supporting the folkloric assertion in Turkish folk medicine. However, these effects seem not limited to *CSS*, some other *Centaurea* species, in fact, having no folkloric use might be equally active.

1. Introduction

The genus *Centaurea* (Asteraceae) has a widespread distribution in Turkey. Among 179 species grown in Turkey, 109 are endemic (Wagenitz, 1975). Particularly the aerial parts with flowers or only flowers of some *Centaurea* species are used in Turkish folk medicine to alleviate a wide range of symptoms. For example, the flowers of *Centaurea iberica* Trev. ex Sprengel and *Centaurea solstitialis* L. ssp. *solstitialis* (*CSS*), and the aerial parts of *Centaurea virgata* Lam. are used against stomach ache and previously antiulcerogenic activity of CSS was evaluated thoroughly (Yesilada et al., 2004; Gürbüz and Yesilada, 2007). 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 iberica* Trev. ex Sprengel, *Centaurea jacea* L., *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). In Chinese traditional medicine, *Centaurea uniflora* has been used against fever and for detoxification and ethyl acetate extract of this species inhibited membrane lipid peroxidation and showed anti-atherosclerotic effect (Wei et al., 1997). Aqueous extract of *Centaurea chilensis* has been used to reduce fever and rheumatic pain in folk medicine (Negrete et al., 1984, 1993; Sepulveda et al., 1994). *Centaurea ornate* is also used against rheumatic pain (Bastos et al., 1994; Vazquez et al., 1997) and *Centaurea sinaica* is used to reduce fever (Al-Easa et al., 1992). Tea prepared from the aerial parts of

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several *Centaurea* species are also used as hypoglycaemic, such as *Centaurea ornato* (Bastos et al., 1994; Vazquez et al., 1997), *Centaurea aspera, Centaurea seridis* var. *maritima* and *Centaurea melitensis* (Kamanzi et al., 1983; Chucla et al., 1988). Due to the bitter taste, *Centaurea* species are also used as digestive tonic or stomachic, i.e., *Centaurea melitensis* (Kamanzi et al., 1983), *Centaurea pallascens* in Egypt (Ali et al., 1987). *Centaurea melitensis* and *Centaurea pallascens* (Kamanzi et al., 1983; Ali et al., 1987), as well as *Centaurea sinaica* (Al-Easa et al., 1992) are also used as diuretic. In Spain, aerial part of *Centaurea ornato* is used as depurative and cholagogue, while the underground part as antispasmodic (Bastos et al., 1994; Vazquez et al., 1997). For *Centaurea sinaica* cytostatic, diuretic, antimalarial, astringent, antineoplastic, allergenic, stomachic, tonic and emmenagogue properties are attributed (Al-Easa et al., 1992).

The aim of the present study was primarily to investigate the antinociceptive and antipyretic effects of *Centaurea solstitialis* ssp. *solstitialis* aerial parts in order to evaluate its folkloric utilization against headache and to reduce fever and isolation of active constituent(s) through bioassay-guided fractionation techniques and chemical characterization of the active ingredient(s). On the other hand, a comparative study was run parallel using the same chemical and pharmacological procedures on another species – *Centaurea depressa* – which is reported to be utilized only as food in Turkish folk medicine. This second part was done to reveal that other *Centaurea* species may possess relevant activities even though not recorded in folk medicines.

2. Materials and methods

2.1. Plant material

Roots and aerial parts of *Centaurea solstitialis* L. subsp. *solstitialis* and *Centaurea depressa* Bieb. were collected from Ankara, Esenboga-Balikhisar in June 2000. Voucher specimens are deposited in the Herbarium of Faculty of Pharmacy, Gazi University (GUE-2246, GUE-2249).

2.2. Extraction and fractionation

2.2.1. Preparation of extracts for preliminary activity testing

For the preliminary activity screening, roots [*Centaurea solstitalis* subsp. *solstitialis* (CSR) and *Centaurea depressa* (CDR)] and aerial parts [*Centaurea solstitalis* subsp. *solstitialis* (CSAP) and *Centaurea depressa* (CDAP)] of each plant material was extracted separately with either ethanol (EtOH) or distilled water (H_2O).

Preparation of aqueous extracts: An aliquot of (50 g) powdered root or aerial part was extracted twice with cold distilled H_2O (250 ml) by stirring for 2 h at room temperature. Combined H_2O extract was then lyophilized to yield "H_2O extract" (*Centaurea solsititialis*-R-H_2O: 3.91 g, *Centaurea solsititialis*-AP-H_2O: 4.12 g, *Centaurea depressa*-R-H_2O: 1.75 g, *Centaurea depressa*-AP-H_2O: 6.32 g).

Preparation of ethanol extracts: An aliquot of powdered roots or aerial parts (50 g) was extracted twice with 80° EtOH in a 40 °C water bath for 2 h and combined extract was evaporated to dryness under reduced pressure to yield 'EtOH extract' (*Centaurea solsititialis*-R-EtOH: 1.82 g, *Centaurea solsititialis*-AP-EtOH: 2.78 g, *Centaurea depressa*-R-EtOH: 1.33 g, *Centaurea depressa*-AP-EtOH: 4.06 g).

2.2.2. Fractionation of the extracts

Two thousand grams of powdered aerial parts of each plant species were extracted with EtOH (20 L) by frequent stirring in a 40 °C water bath for 10 days, this process was repeated several times to remove the extractable components (extraction was followed with TLC control). Combined extract was evaporated to dryness under reduced pressure to yield 'EtOH extract' (yields; *Centaurea depressa*-AP-EtOH: 135.47 g, *Centaurea solsititialis*-AP-EtOH: 87.58 g). The ethanol extracts were then dissolved in 800 ml of EtOH:H_2O (9:1) mixture and extracted with *n*-hexane (14 × 300 ml) in a separatory funnel. Combined hexane extract was evaporated under reduced pressure to yield 'Hexane fraction' (*Centaurea solsititialis*-AP-Hexane Fr.: 17.72 g, *Centaurea depressa*-AP-Hexane Fr.: 17.96 g). EtOH was removed from the remaining extract and diluted with distilled H_2O to 250 ml and further extracted by successive solvent extractions with chloroform (6 × 300 ml) and *n*-butanol saturated with H_2O (6 × 250 ml). Each extract was then evaporated to dryness under reduced pressure to yield "*Centaurea solsititialis*-AP-$CHCl_3$ Fr." (15.89 g), "*Centaurea depressa*-AP-$CHCl_3$ Fr." (16.26 g) "*Centaurea solsititialis*-AP-BuOH Fr." (21.2 g), "*Centaurea depressa*-AP-BuOH Fr." (17.82 g) and the remaining aqueous extract was also evaporated under reduced pressure to dryness to yield "*Centaurea solsititialis*-AP-R H_2O Fr." (22.8 g) and "*Centaurea depressa*-AP-R H_2O Fr." (47.38 g).

2.2.3. Chromatographic separation and isolation of active constituents

2.2.3.1. Fractionation of Centaurea solstitialis-AP-$CHCl_3$ fraction. Four grams of *Centaurea solstitialis*-AP-$CHCl_3$ fraction was subjected to chromatographic separation on a silicagel column (Kieselgel 60 Merck, 0.040–0.63 mm, 230–400 mesh, ASTM) using $CHCl_3$/MeOH (99:1), (96:4), (90:10), (85:15) and $CHCl_3$/MeOH/H_2O (80:20:1), (70:30:2), (60:40:5) as solvent systems and eluted fractions were combined after thin layer chromatographical (TLC) analysis as follows: SG Fr.1–51 (320 mg), SG Fr.52–60 (274.3 mg/kg), SG Fr.61–68 (348.6 mg), SG Fr.69–74 (498.8 mg), SG Fr.75–92 (354.6 mg), SG Fr.93–121 (1031.8 mg) and SG Fr 122–153 (868.3 mg). SG Fr.69–74 (498.8 mg) was further subjected to chromatographic separation with gel permeation applying on a Sephadex LH-20 column (25–100 μm, Lot 124H0053, Sigma Chem. Co.) using MeOH as solvent system. Eluted fractions were checked by TLC for the spots using $CHCl_3$/MeOH (8:2) mobile system and combined as follows: LH Fr.1–4 (135 mg), LH Fr.5–6 (122 mg), LH Fr.7–8 (54.7 mg), LH Fr.9–14 (121.7 mg). LH Fr.5–6 was then subjected to preparative TLC separation using $CHCl_3$/MeOH (8:2) and two compounds were obtained.

The chemical structures of these compounds were elucidated as solstitialin A and acetyl solstitialin (sesquiterpene lactones) by comparison of the spectral data with those of the previously reported data (Yesilada et al., 2004).

2.2.3.2. Fractionation of Centaurea depressa-AP-$CHCl_3$ fraction. Three grams of *Centaurea depressa*-AP-$CHCl_3$ fraction was subjected to chromatographic separation on a silicagel column (Kieselgel 60 Merck, 0.040–0.63 mm, 230–400 mesh, ASTM) using the same solvent systems described above. Eluted fractions were then combined using TLC control as follows: SG Fr.1–13 (123.6 mg), SG Fr.14–24 (239.8 mg), SG Fr.25–56 (329.2 mg/kg), SG Fr.57–96 (460 mg), SG Fr.97–110 (260 mg), SG Fr.111–141 (867.5 mg). SG Fr.57–96 (460 mg) was further subjected to chromatographic separation with gel permeation applying on a Sephadex LH-20 column using MeOH as solvent system. Eluents were checked by TLC using $CHCl_3$/MeOH (8:2) mobile system and combined as follows: LH Fr.1–2 (83.7 mg), LH Fr.3–4 (86.3 mg), LH Fr.5–6 (55.2 mg), LH Fr.7 (11.4 mg), and LH Fr.8–20 (97.5 mg/kg). LH Fr.8–20 was further separated into two components on preparative TLC plate using $CHCl_3$/MeOH (8:2). The structures of these components were again determined as solstitialin A and acetyl solstitialin by comparison of the spectral data with those of the previously reported data (Yesilada et al., 2004).