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Antioxidant and antibacterial activities of the methanolic extract of *Centaurea zuvandica* Sosn

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Abstract: A methanol crude extract and a sesquiterpene lactone, cynaropicrin, isolated from *Centaurea zuvandica* aerial parts, were evaluated for antioxidant activity using FTC and TBA methods. The antimicrobial activity of the crude extract was also tested against six strains including gram-positive and gram-negative bacteria by disk diffusion method. The methanolic extract and cynaropicrin obtained of *Centaurea zuvandica* aerial parts possessed high antioxidant activity when tested with both methods. The MeOH extract was also inhibited the growth of Staphylococcus aureus, Bacillus cereus and Escherichia coli but had no inhibitory effects on Streptococcus faecalis, Pseudomonas aeruginosa and Klebsiella pneumoniae. These results indicated the possibility of using MeOH extract of *Centaurea zuvandica* for medicinal uses and food prevention.

Keywords: *Centaurea zuvandica*; Cynaropicrin; Antioxidant activity; Antibacterial activity; Methanolic extract

Introduction

The large genus *Centaurea* (family Compositae, tribe Cardueae, subtribe Centaureinae) comprises about 500 species distributed around the Mediterranean area and in west Asia. Seventy-four species of the genus *Centaurea* are found in Iran, among which 38 are endemic [1,2]. Previous chemical investigations on *Centaurea* species have shown the presence of flavonoids [3] sesquiterpene lactones, specially guaianolides [4-6] and germacranolide types sesquiterpene lactones [3]. These components are known for the main materials having various biological activities, including anti-tumor, anti-ulcer, anti-inflammatory, neuro-cytotoxic and cardiotonic activities [7-9]. The sesquiterpene lactones are usually characterized by a α-methylene-γ-lactone group, which may react with sulphydryl group of proteins by a Michael addition [10]. The reaction between these groups could be responsible for the toxic effect of sesquiterpene lactones [11].

In our chemical investigation of the methanolic extract of air-dried aerial parts of *Centaurea zuvandica*, cynaropicrin as a main compound was isolated and characterized by spectral techniques (FT-IR, ^{1}H NMR, ^{13}C NMR, HMQC and ^{1}H-^{1}H COSY). This compound showed 89% lysis against trypomastigote forms of *T. cruzi* in concentration of up to 100 mg/ml and inhibited the growth of gram-positive bacteria and yeast strains [12].

Also cynaropicrin showed non-specific significant cytotoxicity against five human tumor cell lines with ED_{50} values ranging from 0.29~1.37 μg/ml [13].

There is a strong need for effective antioxidants from natural sources as alternatives to synthetic food additives in order to prevent deterioration of foods, drugs and cosmetics. The extracts and essential oils of many plants have been investigated for their antioxidant activity [14-16]. To our knowledge, no information is available on the antioxidant and antibacterial properties of the extract of *C. zuvandica*. Therefore, this study evaluated the antioxidant activity of the methanol extract and cynaropicrin isolated of aerial parts of this plant.

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Materials and methods

General experimental procedure

^{1}H NMR (500 MHZ) and ^{13}C NMR (125.8 MHZ) spectra were recorded on a Bruker Avance-DRX 500 spectrometer in $CDCl_3$ with TMS as internal standard. The IR spectra were recorded on a Shimadzu FT-IR Bruker Vector 22 spectrophotometer. Column chromatography on silica gel 60 (Merk, 70-230 and 230-400 mesh) and TLC on silica gel PF-254 (Merk). The absorbance was measured using a GBA UV-Vis Spectrophotometer (model Cintra-10, Victoria, Australia).

Plant materials

The aerial parts of *Centaurea zuvandica* Sosn. (Compositae), endemic for Iran, were collected during the full flowering stage from plants growing wild at Gadook region, north of Iran, in May 2007. A voucher specimen has been deposited in the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Science, Tehran, Iran.

Chemicals

α-Tocopherol was purchased from Sigma (Sigma, Aldrich GmbH, Sternheim, GERMANY)**.** Butylated hydroxyl toluene (BHT), linoleic acid, thiobarbituric acid (TBA), ammonium thiocyanate, ethanol, methanol and the other chemicals and reagents were purchased from Merck (Darmstat, Germany).

Extraction and isolation

The dried and powdered aerial parts (700 g) of *C. zuvandica* were extracted with methanol (2000 ml), by maceration at room temperature for 72 h. The solvent was removed under the vacuum at temperature below 50°C and suspended in hot MeOH (10 ml) and then cooled to -15°C. After standing overnight at -15°C, the waxy precipitate was removed by filtration and the filtrate evaporated in vacuum to afford a syrupy residue (32 gr). The extract (5 gr) was chromatographed over silica gel under vacuum and eluted with n-hexane, gradually increasing the polarity with ethyl acetate and then methanol. Fifteen fractions were collected. Fraction 8 (0.72 gr) was submitted to preparative TLC (silica gel), eluting with ethyl acetate-n-hexane 7:3 (v/v), to afford 0.387 gr of cynaropicrin.

Antioxidant assays

In this study, antioxidant activity of the methanol extract and cynaropicrin (final concentration 0.02% w/v) of *Centaurea zuvandica* were tested by using the ferric thiocyanate (FTC) and thiobarbituric acid (TBA) methods (*17-19*). α-Tocopherol (vitamin E) and butylated hydroxy toluene (BHT) were used as standards in both methods. One sample without antioxidant activity was also used as negative control.

a) Ferric Thiocyanate (FTC) method

A mixture of 4 mg of each sample in 4 ml absolute ethanol, 4.1 ml of 2.52% linoleic acid in absolute alcohol, 8 ml of 0.05 M phosphate buffer (pH 7.0) and 3.9 ml of water was placed in a vial with a screw cap and then placed in an oven at 40 °C in the dark. To 0.1 ml of this solution was added 9.7 ml of 75% ethanol and 0.1 ml ammonium thiocyanate. Precisely 3 minutes after an addition of 0.1 ml of 0.02 M ferrous chloride in 3.5% hydrochloride acid to the reaction mixture, the absorbance was measured at 500 nm every 24 hours until the absorbance of the control reached maximum.

b) Thiobarbituric acid (TBA) method

An amount of 2 ml of 20% trichloroacetic acid (TCA) and 2 ml of 0.67% 2-thiobarbituric acid (TBA) aqueous solutions were added into 1 ml of sample solution, prepared with the FTC method. The mixture was placed in a boiling water bath for 10 minutes. After cooling, it was centrifuged at 3000 rpm for 20 minutes and the absorbance of the supernatant was measured at 532 nm.

To calculate the percentage of antioxidant activity, after reading the absorbance of samples at 500 nm for FTC method and at 532 nm for TBA method, the percentage of activity was calculated according to the following equation:

$$AI\ (\%) = \frac{(A_0 - A) \times 100}{A_0}$$

where A_0 is the absorbance of the control reaction (reaction, containing no test compound) and A is the absorbance of the test compound. The values obtained for the control samples were taken for 100% lipid peroxidation.

Determination of reducing power

The determination of reducing power was performed as described by Yen and Duh (1993) (*20*). Different amounts of methanolic extracts (0.48, 1.20, 2.40, 3.60, and 4.80 mg/ml) were mixed with phosphate buffer (5.0 ml, 0.2 M, pH 6.6) and 1% potassium ferricyanide (5 ml), and incubated at 50 °C for 20 min; 5 ml of 10% trichloroacetic acid were added, and the mixture was centrifuged at 2500 rpm for 10 min. The upper layer of the solution (5 ml) was mixed with distilled water (5 ml) and 0.1% ferric chloride (1 ml) and the absorbance was read at 700 nm. Increase in the absorbance of the

reaction mixture indicated increase in the reducing power.

Screening of antimicrobial activity

The methanolic extract of *C. zuvandica* was investigated for antibacterial activity by using the disc diffusion method against six species of bacteria. The following strains of gram- positive and gram-negative bacteria were provided from Persian Type Culture Collection (PTCC) and American Type Culture Collection (ATCC): *Bacillus cereus* (ATCC 11778), *Staphylococcus aureus* (PTCC 25923), *Streptococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 8739), *Klebsiella pneumoniae* (ATCC 10031) and *Pseudomonas aeruginosa* (ATCC 9027). Three to five identical colonies from each agar plate were lifted with a sterile wire loop and transferred into a tube containing 5 ml of saline buffer. Turbidity of each bacterial suspension was adjusted with saline buffer to reach an optical comparison to that of a 0.5 McFarland standard, resulting in a suspension containing approximately 1 to 2×10^8 CFU/ml. Within 15 minutes after adjusting the turbidity of the inoculum suspension, tryptic soy agar (TSA) plates were inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking two more times, rotating the plate approximately 60^o each time to ensure even distribution of the inoculum. As a final step, the rim of the agar was also swabbed. Paper discs (diameter 6.4 mm) were placed on in the inoculated agar surfaces and impregnated with 8 µl of extract at 100 mg/ml. All plates were incubated aerobically at 37 °C for 24 h. The antimicrobial activity was estimated by measuring the radius of the zone inhibition. Each test was performed in three replications and the results analyzed for statistical significance. Gentamicin and tetracycline with positive responses were used as controls for plates.

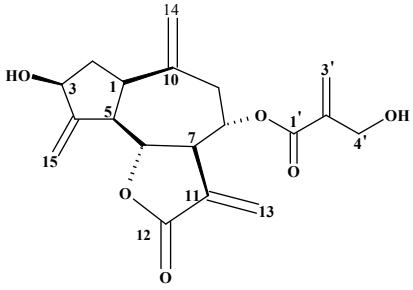
Statistical analysis

All experiments were repeated three times and the data were calculated as means ± SD. The antioxidant activity of the extract, cynaropicrin and positive controls were compared by ANOVA one-way test, ($P < 0.05$), using SPSS program.

Result and discussion

In this research a sesquiterpene lactone with guaianolide structure was obtained from air-dried aerial parts of *C. zuvandica*, as a colorless oil. Based on the spectral data obtained and the reported chemical structures of sesquiterpene lactones in the literature, the structure of this compound (Figure1) was determined to be 8-O- (2-hydroxymethyl-2-propenoyl)-3-hydroxy-(15),10(14),11(13)-guaiatriene-12,6-olide (cynaropicrin) (*4*). Then antioxidant activities of the crude extract and cynaropicrin isolated of *C. zuvandica* were measured by the FTC method and compared with the TBA method at a concentration of 0.02% in methanolic solutions. The inhibitions of activities against lipid peroxidation in linoleic acid were evaluated by measuring the concentration of the TBA-reactive substances and FTC. The FTC method measures the amount of peroxide produced during the initial stage of lipid oxidation. Subsequently, at a later stage of lipid oxidation, peroxide decomposes to form carbonyl compounds that are measured by using the TBA method.

Figure 1. The chemical structure of cynaropicrin isolated from *C. zuvandica*.



Results indicated that the methanol extract and cynaropicrin possessed strong antioxidant activity (low absorbance values) by both the FTC and TBA methods. The antioxidant activity was then compared with those of vitamin E (a natural antioxidant) and BHT (a synthetic antioxidant) (Figures 2-4).

Figure 2. Antioxidant activity of methanol extract and cynaropicrin isolated of *C. zuvandica* aerial parts determined with the FTC method.

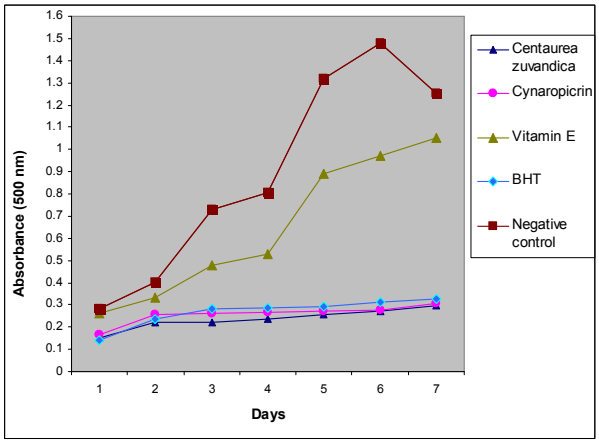


Figure 3. Antioxidant activity of methanol extract and cynaropicrin isolated of *C. zuvandica* aerial parts determined with the TBA method.

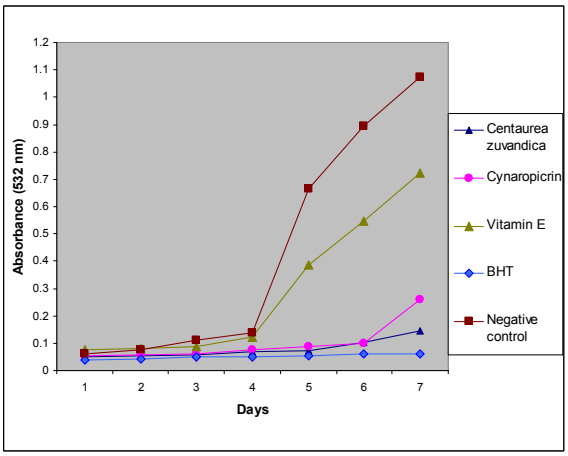
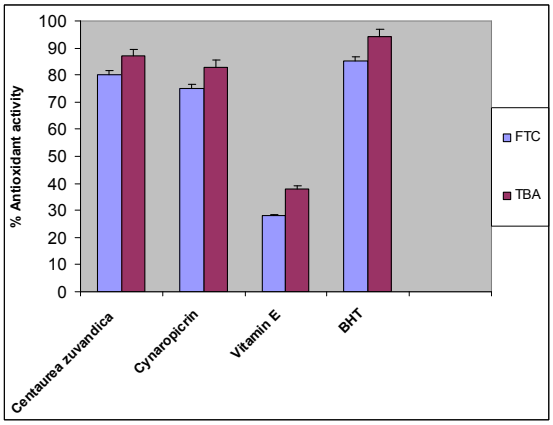


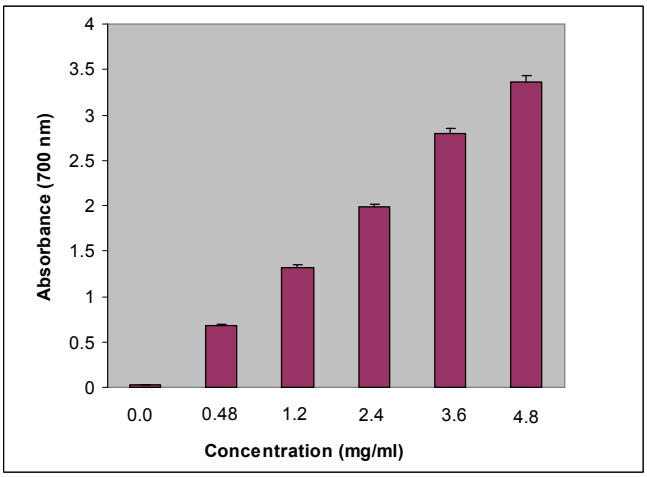
Figure 4. A comparison between antioxidant activity (% ± SD) using the FTC and TBA methods of methanol extract and cynaropicrin isolated of *C. zuvandica* aerial parts.



The antioxidant activity detected with the TBA method was higher than that detected with the FTC method. This might suggest that amount of peroxide in the initial stage of lipid peroxidation was less than the amount of peroxide in the secondary stage. Furthermore, the secondary product was much more stable for a period of time.

Figure 5 shows the reducing power of extract using the potassium ferricyanide method at various extract concentrations. It appears that antioxidative activity may have a mutual correlation with the reducing effect. The reducing properties are generally associated with the presence of reductones and the antioxidant activity of reductones is believed to break radical chains by donation of a hydrogen atom, indicating that the antioxidative properties are concomitant with the development of the reducing power (*21*). Therefore, the marked antioxidative activity in MeOH extract may be associated with its higher reducing power.

Figure 5. Reducing power of methanolic extract of *C. zuvandica.* Mean values ± standard deviations (n = 3) with the same letter are not significantly different (P < 0.05).



The effects of extract at 100 mg/ml on the test bacteria are presented in Table 1. Methanol (control) had no inhibitory effects on the six bacteria tested. The results of antibacterial activity test of the methanolic extract according to the disc diffusion method indicated that, the most sensitive bacterium was *Bacillus cereus* and the most resistant bacteria were *Streptococcus faecalis*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae.* The results obtained suggest that *C. zuvandica* can be used in treating diseases caused by the test organisms and might be a potential source of natural antioxidant.

Acknowledgments

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References

[1] Rechinger, K. H. *Centaurea*, Carduus. In: *Flora lranica Compositae*, No. 139; Rechinger, K.H.; Hedge, I. C. Eds.; Akademische Druck and Verlagsanstalt, Graz, Austria, **1980**, PP. 327, 226, respectively.

[2] Mozaffarian, V. *A Dictionary of Iranian Plant Names*. Farhang Moaser Publishers, Tehran, Iran, **1996**.

[3] Rustaiyan, A.; Niknejad, A.; Aynehchi, Y. Chemical constituents of *Centaurea brugueriana*. *Planta Med.* **1982,** *44*, 185-186.

[4] Rustaiyan, A.; Niknejad, A.; Zdero, C.; Bohlmann, F. Guaianolide from *Centaurea behen*. *Phytochemistry*, **1981**, *20*, 2427-2429.

[5] Rustaiyan, A.; Ardebili, S. New guaianolides from *Centaurea kandavanesis*. *Planta Med*. **1984,** *46*, 363-364.

[6] Rustaiyan, A.; Nazarians, L.; Bohlmann, F. Guaianolides from *Acroptilon repens* (*Centaurea picris*). *Phytochemistry*, **1981,** *20*, 1152-1153.

[7] Robles, M.; Aregullin, M.; West, J.; Rodriguez, E. Recent studies on the zoopharmacognosy, pharmacology, and neurotoxicology of

sesquiterpene lactone. *Planta Med.* **1995**, *61*, 199–203.

[8] Taniguchi, M.; Kataoka, T.; Suzuki, H.; Uramoto, M.; Ando, M.; Arao, K.; Magae, J.; Nishimura, T.; Otake, N.; Nagai, K. Costunolide and dehydrocostus lactone as inhibitors of killing function of cytotoxic T lymphocytes. *Biosci.*, *Biotechnol., Biochem.* **1995**, *59*, 2064–2067.

[9] Cho, J. Y.; Kim, A.R.; Jung, J. H.; Chun, T.; Rhee, M. H.; Yoo, E. S. Cytotoxic and pro-apoptotic activities of cynaropicrin, a sesquiterpene lactone, on the viability of leukocyte cancer cell lines, *Europ. J. Pharm.* **2004**, *492*, 85-94.

[10] Milbrodt, M.; Schröder, F.; König, W. A. 3,4-β-epoxy-8-deoxycumambrin B, a sesquiterpene lactone from *Tanacetum parthenium*. *Phytochemistry*, **1997**, *44*, 471-474.

[11] Noldin, V. F.; Cechinel Filho, V. Composicao quimicae atividades biologicas das folhas de *Cynara scolymus* I. (Alcachofra) cultivada no, Brasil. *Quim. Nova*, **2003,** *26*, 331-334.

[12] Schinor, E. C.; Salvador, M. J.; Ito, I. Y.; Dias, D.A. Trypanocidal and antimicrobial activities of *Moquinia Kingii*, *Phytomedicine*, **2004**, *11*, 224-229.

[13] Choi, S. Z.; Choi, S. U.; Lee, K. R. Cytotoxic sesquiterpene lactones from *Saussurea calciola, Arch. Pharm. Res.* **2005***, 28,* 1142-1146.

[14] Aruoma, O. I. Free radicals, oxidative stress, and antioxidants in human health and disease. *J. Am. Oil Chem. Soc.* **1998**, *75*, 199–212.

[15] Amakura, Y.; Umino, Y.; Tsuji, S.; Ito, H.; Hatano, T.; Yoshida, T. Constituents and their antioxidative effects in eucalyptus leaf extract used as a natural food additive. *Food Chem*. **2002**, *77*, 47–56.

[16] Da Silva, J. M. R.; Darmon, N.; Fernandez, Y.; Mitjavila, S. Oxygen free radical scavenger capacity in aqueous models of different procyanidins from grape seeds. *J. Agric. Food Chem.* **1991**, *39*, 1549–52.

[17] Osawa, T.; Namiki, M. A novel type of antioxidant isolated from leaf wax of *Eucalyptus* leaves. *Agric. Biol. Chem.* **1981**, *45*, 735.

[18] Kikuzaki, H.; Nakatani, N. Antioxidant effects of some ginger constituents. *J. Food Sci.* **1993**, *58*, 1407-1410.

[19] Ottolenghi, A. Interaction of ascorbic acid and mitochondria lipids. *Arch. Biochem. Biophys.* **1959**, *79*, 355

[20] Yen, G. C.; Duh, P. D. Antioxidantive properties of methanolic extracts from *peanut hulls*. *J. Am. Oil Chem. Soc*. **1993,** *70*, 383-386.

[21] Gordon, M. F. The mechanism of antioxidant action in vitro. In: *Food Antioxidants **1***, Hudson, B. J. F. Eds.; Elsevier Applied Science, London, **1990**, PP. 1-18.