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Antibacterial and antioxidant activities of three Turkish species of the genus *Centaurea*

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SUMMARY

A number of species of the genus *Centaurea* (Family: Asteraceae), distributed in various parts of Asia, Europe and North America, have been used in traditional plant-based medicine and reported to possess various medicinal properties. As part of our continuing evaluation of plants from the genus *Centaurea* for their phytochemistry and biological activities, the dichloromethane (DCM) and methanol (MeOH) extracts of the seeds of Turkish *Centaurea* species, *C. bornmuelleri*, *C. huber-morathii* and *C. schiskinii*, were screened for antioxidant and antibacterial activities. Among the three species, *C. huber-morathii* displayed the most prominent antibacterial activity. Both the MeOH and DCM extracts of this plant showed activity against *Citrobacter freundii*, *Enterococcus faecalis* and *Salmonella goldcoast* with the MIC values within the range of 1×10^{-2} to 1×10^{-3} mg/ml. The MeOH extract of *C schiskinii* showed activity (MIC = 1×10^{-1} mg/ml) against *Citrobacter freundii* and *Staphylococcus aureus*. While the DCM extract of *C. bornmuelleri* was only active against *Staphylococcus aureus* (MIC = 1×10^{-2} mg/ml), the MeOH extract did not show any inhibitory activity at test concentrations. The DCM and MeOH extracts of all three species demonstrated good degree of antioxidant property in the DPPH assay with the RC_{50} values ranging from 72×10^{-2} to 31×10^{-3} mg/ml. Among these extracts, the MeOH extract of *C. huber-morathii* was the most active antioxidant extract (MIC = 31×10^{-3} mg/ml).

Key words: *Centaurea bornmuelleri*; *Centaurea huber-morathii*; *Centaurea schiskinii*; DPPH assay; Natural antioxidant; Antibacterial

INTRODUCTION

The genus *Centaurea* L. of the family Asteraceae (*alt.* Compositae) is a large genus composed of *ca.* 500 species of hardy, herbaceous, perennial and annual plants, distributed in many parts of Asia, Europe and North America (Clapham *et al.*, 1952; GRIN database, 2005). A number of species of this genus have been used in traditional plant-based medicines and reported to possess various types of medicinal properties. For example, *Centaurea acaulis*, *C. centaurium*, *C. cyaneus*, *C. monantha*, *C. nigra*, *C. salonitana* and *C. scabiosa* possess anti-tumour and anticancer

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properties, *C. aspera* produces hypoglycaemic effect, *C. behen* is well known as an aphrodisiac and effective for the treatment of jaundice, *C. calcitrapa* possesses diuretic, depurative and tonic properties, and is used to treat common fever and jaundice, *C. cyanus* is used as an astringent, diuretic and emmenagogue, and a remedy for fever, etc. (Grieve, 2002; Phytochemical and Ethnobotanical Database, 2005).

As part of our continuing evaluation of plants from the genus *Centaurea* for their phytochemistry and biological activities (Sarker *et al.*, 1997a, b; Sarker *et al.*, 1998a-c; Sarker *et al.* 2001; Cooper *et al.*, 2002; Kumarasamy *et al.*, 2002; Ribeiro *et al.*, 2002; Ferguson *et al.*, 2003; Kumarasamy *et al.*, 2003a, b; Middleton *et al.*, 2003; Shoeb *et al.*, 2004a, b) we now report on the antibacterial and antioxidant properties of the dichloromethane and methanol extracts of the seeds of Turkish *Centaurea* species, *C. bornmuelleri, C. huber-morathii* and *C. schiskinii.*

MATERIALS AND METHODS

Plant materials

Seeds of *Centaurea bornmuelleri* Hausskn. Ex. Bornm, *C. huber-morathii* Wagenitz and *C. schiskinii* Tzelev were collected from West and East Anatolia, Turkey, during September-October 2002. Voucher specimens (PH800004, PH800001 and PH800003, respectively) have been deposited in the herbarium of Plant and Soil Science Department, University of Aberdeen, Scotland (ABD).

Extraction

Ground seeds (~100 g) of *Centaurea bornmuelleri, C. huber-morathii* and *C. schiskinii* Tzelev were Soxhlet-extracted sequentially using solvents (1.1 liter each) of increasing polarity, *n*-hexane, dichloromethane (DCM) and methanol (MeOH). Solvent was evaporated from the extracts using a rotary evaporator at a temperature not exceeding 50°C.

Preparation of the extract solutions for bioassays

The DCM and MeOH extracts (0.025 g) were dissolved in 5mL dimethyl sulfoxide (DMSO) to obtain stock solutions of 5 mg/ml concentrations.

Antibacterial assay

Antibacterial activity of the extracts was tested against 11 species of Gram-positive and Gram-negative pathogenic bacteria (Table 1). The bacterial cultures used were from the properly identified and appropriately maintained stock cultures from the Microbiological Research

Table 1. Antibacterial activity of the seeds of *Centaurea bornmuelleri, C. huber-morathii* and *C. schiskinii*

Bacterial species	MIC (mg/ml)							
		C. bornmuelleri		*C. huber-morathii*		*C. schiskinii*		Ciprofloxacin
		MeOH	DCM	MeOH	DCM	MeOH	DCM	
Bacillus cereus	9689[a]	-	-	-	-	-	-	2.5×10^{-8}
Citrobacter freundii	9750[a]	-	-	1×10^{-3}	1×10^{-2}	1×10^{-1}	-	2.5×10^{-7}
Enterococcus faecalis	8156[b]	-	-	1×10^{-3}	1×10^{-2}			2.5×10^{-7}
Escherichia coli	8110[a]	-	-	-	-	-	-	2.5×10^{-7}
Escherichia coli	4174[a]	-	-	-	-	-	-	2.5×10^{-6}
Klebsiella aerogenes	9528[a]	-	-	-	-	-	-	2.5×10^{-6}
Pseudomonas aeruginosa	6750[a]	-	-	-		-		2.5×10^{-8}
Salmonella goldcoast	13175[a]	-	-	1×10^{-3}	1×10^{-2}	-	1×10^{-2}	2.5×10^{-5}
Serratia marcescens	1377[a]	-	-	-	-	-	-	2.5×10^{-6}
Staphylococcus aureus	10788[a]	-	1×10^{-2}	-	-	1×10^{-1}	-	2.5×10^{-8}
Staphylococcus aureus (MRSA)	11940[a]	-	-	-	-	-		2.5×10^{-5}

[a] = NCTC; [b] = NCIB. - = No inhibitory activity at test concentrations. No inhibition with negative control (1% DMSO).

Laboratory, School of Pharmacy, The Robert Gordon University. The antibacterial test was performed using the 96 well microplate-based broth dilution method using resazurin solution (Sarker *et al.*, 2003; Drummond and Waigh, 2000; Lorian, 1996) as an indicator of bacterial growth. All tests were performed in triplicate.

Preparation of bacterial species

The bacterial cultures were prepared following the method outlined in a recent publication by Sarker *et al.* (2003). The concentration of bacterial solution used was 5×10^5 cfu/ml.

Preparation of resazurin solution

One tablet of resazurin was dissolved in 40 ml sterile distilled water to obtain standard resazurin solution.

Preparation of 96 well plates and assay

The top of the 96 well plates was labelled appropriately. Ciprofloxacin, a well known antibiotic, was used as positive control. Normal saline, resazurin solution and DMSO were used as negative controls. A 100 μl of the DCM and MeOH extracts in DMSO, ciprofloxacin, normal saline and resazurin solution were pipetted into the first row. The two extracts were added to two columns each while the controls were added to one column each. Normal saline (50 μl) was added to the rows 2 to 11. Using fresh sterile pipette tips, 50 μl of the contents of the first row was transferred to the second row. Serial dilutions were carried out until all the wells contained 50 μl of either extracts or controls in descending concentrations. Resazurin solution (10 μl) was added which was followed by the addition of 30 μl of triple strength broth (or triple strength glucose in the case of *Enterococcus faecalis*) to each of the wells. Finally, 10ml of bacterial solution of concentration 5×10^5 cfu/ml was added to all the wells starting with row 12. The plates were then wrapped with clingfilm to prevent bacterial dehydration and then incubated overnight for 18 hours at 37°C. The presence of bacterial growth was indicated by colour changes from purple to pink.

Antioxidant assay (DPPH assay)

2,2-Diphenyl-1-picrylhydrazyl (DPPH), molecular formula $C_{18}H_{12}N_5O_6$, was obtained from Fluka Chemie AG, Bucks. Quercetin was obtained from Avocado Research Chemicals Ltd, Shore road, Heysham, Lancs. The method used by Takao *et al.* (1994) was adopted with suitable modifications (Kumarasamy *et al.*, 2002; Sarker *et al.*, 2003). DPPH (4 mg) was dissolved in MeOH (50 ml) to obtain a concentration of 80 μg/ml.

Qualitative

Test samples were applied on a TLC plate and sprayed with DPPH solution using an atomiser. It was allowed to develop for 30 min. The colour changes (purple on white) were noted.

Quantitative

Stock solutions (10 mg/ml) of the plant extracts were prepared in MeOH. Serial dilutions were carried out to obtain concentrations of 5×10^{-1}, 5×10^{-2}, 5×10^{-3}, 5×10^{-4}, 5×10^{-5}, 5×10^{-6} 5×10^{-7}, 5×10^{-8}, 5×10^{-9}, 5×10^{-10} mg/ml. Diluted solutions (1 ml each) were mixed with DPPH (1 ml) and allowed to stand for 30 min for any reaction to occur. The UV absorbance was recorded at 517 nm. The experiment was performed in duplicate and the average absorption was noted for each concentration. The same procedure was followed for the positive standards quercetin and trolox.

RESULTS AND DISCUSSION

The antibacterial activity of the extracts was determined by a modified micro-plate-based broth dilution assay (also known as Checkerboard assay) using resazurin as an indicator of bacterial growth (Lorian, 1996; Drummond and Waigh, 2000; Sarker *et al.*, 2003). Use of resazurin indicator rendered

more accurate determination of MIC values than any other antibacterial assays, as inhibition of bacterial growth could be determined by the presence of blue colour of resazurin as opposed to pink colour indicating the presence of bacterial growth. The reduction of the indicator (resazurin) by microbial growth occurs in two stages, firstly, resazurin is irreversibly reduced to resorufin (pink), and this is taken as the indication of growth, and secondly, the resorufin can then be reversibly reduced to colourless dihydroresorufin (Drummond and Waigh, 2000). Among the three species, *C. huber-morathii* displayed the most prominent antibacterial activity (Table 1). Both the MeOH and DCM extracts of this plant showed activity against *Citrobacter freundii*, *Enterococcus faecalis* and *Salmonella goldcoast* with the MIC values within the range of 1×10^{-2} to 1×10^{-3} mg/ml. The fact that the activity was more prominent in the MeOH extract (MIC = 1×10^{-3}) than in DCM extract (MIC = 1×10^{-2}) indicated that the antibacterial compounds present in *C. huber-morathii* were of polar nature. The MeOH extract of *C schiskinii* showed activity (MIC = 1×10^{-1} mg/ml) against *Citrobacter freundii* and *Staphylococcus aureus*. While the DCM extract of *C. bornmuelleri* was only active against *Staphylococcus aureus* (MIC = 1×10^{-2} mg/ml), the MeOH extract did not show any inhibitory activity at test concentrations. The MIC values obtained for these extracts are certainly quite high compared to that of the positive control ciprofloxacin, but considering the fact that plant extracts contain hundreds of compounds, this result could be used as a valuable qualitative indication of the antibacterial potential of these extracts and the presence of antibacterial compound (s) in these extracts.

Table 2. Antioxidant activity of the seeds of *Centaurea bornmuelleri, C. huber-morathii* and *C. schiskinii*

Centaurea species	RC_{50} (mg/ml)	
	MeOH	DCM
C. bornmuelleri,	63×10^{-2}	10×10^{-2}
C. huber-morathii	31×10^{-3}	53×10^{-2}
C. schiskinii	15×10^{-2}	72×10^{-2}

RC_{50} value (mg/ml) of the positive control, trolox = 3.59×10^{-3} and quercetin = 2.88×10^{-5}

The DPPH antioxidant assay is based on the ability of 2,2-diphenyl-1-picryl-hydrazyl (DPPH), a stable free radical, to decolourise in the presence of antioxidants. The odd electron in DPPH radical is responsible for the absorbance at 517 nm, and also for visible deep purple colour. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolourised which can be quantitatively measured from the changes in absorbance. In the TLC-based qualitative antioxidant assay using DPPH spray, all extracts showed antioxidant properties indicated by the presence of a yellow/white spot on a purple background on the TLC plates. In the quantitative assay, all extracts displayed prominent antioxidant activity in the DPPH assay (RC_{50} values within the range 72×10^{-2} to 31×10^{-3} mg/ml). Among these extracts, the MeOH extract of *C. huber-morathii* exhibited the most potent activity (MIC = 31×10^{-3} mg/ml). The RC_{50} values for the positive standards, trolox and quercetin, were found to be 3.59×10^{-3} and 2.88×10^{-5} mg/ml, respectively. It is remarkable to note that, even in its crude form, the MeOH extract was only about 10 times less active than the well known antioxidant compound, trolox.

This is the first report on the assessment of antibacterial and antioxidant potentials of the DCM and MeOH extracts of three Turkish species of the genus *Centaurea*. As the most prominent antibacterial and antioxidant activities, in most cases, were observed with the MeOH extracts, it can be assumed that the compound (s) responsible for these activities are of polar nature.

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