SHOEB, M., MACMANUS, S.M., JASPARS, M., KONG THOO LIN, P., NAHAR, L., CELIK, S. and SARKER, S.D. 2007. Bioactivity of two Turkish endemic centaurea species, and their major constituents. *Revista Brasileira de Farmacognosia/Brazilian journal of pharmacognosy* [online], 17(2), pages 155-159. Available from: https://doi.org/10.1590/S0102-695X2007000200003

Bioactivity of two Turkish endemic centaurea species, and their major constituents.

SHOEB, M., MACMANUS, S.M., JASPARS, M., KONG THOO LIN, P., NAHAR, L., CELIK, S. and SARKER, S.D.

2007

OpenAIR @RGU

This document was downloaded from https://openair.rgu.ac.uk

CC BY NC ND

Received 01/19/07. Accepted 04/20/07

Revista Brasileira de Farmacognosia
Brazilian Journal of Pharmacognosy
17(2): 155-159, Abr./Jun. 2007

Artigo

Bioactivity of two Turkish endemic *Centaurea* species, and their major constituents

Mohammad Shoeb[1], Stephen M. MacManus[2], Marcel Jaspars[3], Paul Kong-Thoo-Lin[4], Lutfun Nahar[4], Sezgin Celik[5], Satyajit D. Sarker[6]*

[1]Department of Chemistry, University of Dhaka, Dhaka-1000, Bangladesh,
[2]School of Pharmacy, The Robert Gordon University, Schoolhill, Aberdeen AB10 1FR, UK,
[3]Marine Natural Products Laboratory, Department of Chemistry, University of Aberdeen, Aberdeen AB24 3UE, UK,
[4]School of Life Sciences, The Robert Gordon University, St Andrew Street, Aberdeen AB25 1HG, UK,
[5]Department of Biology, Faculty of Science and Literature, Canakkale Onsekiz Mart University, 17020 Canakkale, Turkey,
[6]School of Biomedical Sciences, University of Ulster at Coleraine, Cromore Road, Coleraine BT52 1SA, Co. Londonderry, Northern Ireland, UK

RESUMO: "Bioatividade e os principais constituintes químicos de duas espécies de *Centaurea,* endêmicas da Turquia". A atividade antioxidante, a toxicidade geral e a citotoxidade dos extratos metanólicos de *Centaurea urvillei* subs. *armata* e *C. mucronifera* foram analisados, respectivamente, pelo ensaio DPPH e pelos ensaios de letalidade de *Artemia salina* e de citotoxidade MTT. A análise dos extratos metanólicos em CLAE de fase reversa apresentou duas lignanas biotivas do tipo dibenzilbutirolactona, matairesinosídeo (**1**) e arctiina (**2**). As estruturas destas lignanas foram elucidadas através de análises espectroscópicas completas bem como por comparação direta dos dados experimentais com os respectivos dados da literatura.

Unitermos: *Centaurea urvillei* subs. *armata*, *Centaurea mucronifera*, Asteraceae, Compositae, lignana, arctiina, matairesinosídeo, DPPH, MTT, *Artemia salina*, toxicidade.

ABSTRACT: The antioxidant activity, general toxicity and cytotoxicity of the methanol extracts of *Centaurea urvillei* subs. *armata* and *C. mucronifera* have been assessed, respectively, by the DPPH assay, the brine shrimp lethality and the MTT cytotoxicity assays. The reversed-phase HPLC analysis of the methanol extracts afforded two bioactive dibenzylbutyrolactone-type lignans, matairesinoside (**1**) and arctiin (**2**). The structures of these lignans were elucidated by comprehensive spectroscopic analyses as well as by the direct comparison of experimental data with respective literature data.

Keywords: *Centaurea urvillei* subs. *armata, Centaurea mucronifera,* Asteraceae, Compositae, lignan, arctiin, matairesinoside, DPPH, MTT, brine shrimp lethality assay.

INTRODUCTION

Centaurea urvillei subs. *armata* Wagenitz and *C. mucronifera* DC. (Family: Asteraceae *alt.* Compositae), are two Turkish endemic perennials that are distributed in the Mediterranean, the Middle and the Eastern Anatolian regions of Turkey (Wagenitz, 1975). To our knowledge, no reports on the isolation of any plant secondary metabolites or any pharmacological properties of these plants are available to date. However, many species of the genus *Centaurea* have long been used in traditional medicine to cure various ailments, *e.g.* diabetes, diarrhoea, rheumatism, malaria, hypertension, treatment of drug dependence etc., and a variety of secondary metabolites have been reported from different species of this genus (Sarker et al., 1997; Carlini et al., 2006). As a part of our continuing phytochemical and bioactivity studies on the species of the genus *Centaurea* (Shoeb et al., 2006a,b; 2005; 2004a,b; Sarker et al., 2005; 2001; 1998; 1997), we now report on the assessment of the extracts of *C. urvillei* subs. *armata* and C. *mucronifera* for antioxidant activity, general toxicity and cytotoxicity, respectively, by the DPPH assay, the brine shrimp lethality and the MTT cytotoxicity assays, and the isolation and identification of two major bioactive lignans, matairesinoside (**1**) and arctiin (**2**), from these plants.

MATERIAL AND METHODS

General

* *E-mail: s.sarker@ulster.ac.uk, Tel. +44-28-70324302*

UV spectra were obtained in MeOH using a Hewlett-Packard 8453 UV-vis spectrometer. MS analyses were performed on a Quattro II triple quadrupole instrument. NMR spectra were recorded in CD_3OD on a Varian Unity INOVA 400 MHz NMR Spectrometer 400 (400 MHz for ^1H and 100 MHz for ^{13}C) using the residual solvent peaks as internal standard. HPLC separation was performed using a Dionex prep-HPLC system coupled with Gynkotek GINA50 autosampler and Dionex UVD340S Photo-Diode-Array detector and/or A JASCO PU-1580 Intelligent HPLC Pump, coupled with JASCO DG-1580-53 Degasser and JASCO LG-1580-02 Ternary Gradient Unit. A Luna C_{18} preparative (10 μM, 250 mm × 21.2 mm) and/or a Luna C_{18} semi-preparative HPLC column (5 μM, 250 mm x 10 mm) were used. Sep-Pak Vac 35 cc (10 g) C_{18} cartridge (Waters) was used for pre-HPLC fractions. HMBC spectra were optimised for a long range $J_{H\text{-}C}$ of 9Hz and the NOESY experiment was carried out with a mixing time of 0.8s.

Plant material

The aerial parts of *Centaurea urvillei* subs. *armata* and C. *mucronifera* were collected from Anatolia, Turkey. Voucher specimens PHSH80012 and PHSH0013, respectively, for *C. urvillei* subs. *armata* and C. *mucronifera* have been retained in the herbarium of the Plant and Soil Science Department, University of Aberdeen, Scotland (ABD).

Extraction, isolation and structure elucidation

Dried ground aerial parts of *C. urvillei* subs. *armata* and *C. mucronifera* (100 g) were separately, Soxhlet-extracted, successively, with *n*-hexane, dichloromethane and methanol (MeOH) (1 L each). The MeOH extract (2.0 g) of *C. urvillei* was fractionated by solid-phase extraction method using a Sep-Pak C_{18} (10 g) cartridge eluting with a step gradient: 40, 60, 80 and 100% MeOH in water (200 mL each). Prep-HPLC (eluted with a linear gradient- water:MeOH = 75:25 to 30:70 over 50 min followed by 70% MeOH for 10 min, 20 mL/min) of the Sep-Pak fraction, which was eluted with 40% MeOH, yielded compounds **1** (12.4 mg, t_R = 20.6 min) and **2** (56.0 mg, t_R = 24.5 min). The 'dereplication' of the Sep-Pak fractions of the MeOH extract (100 mg) of *C. mucronifera* using the HPLC-PDA analyses revealed the presence of exactly the same lignans (**1** and **2**) in the Sep-Pak fraction, which was eluted with 40% MeOH.

Matairesinoside (**1**): Gum, $[\alpha]^{23}_D$ -48.8° (c 0.002, MeOH); UV λ_{max} (MeOH): 279, 222; IR ν_{max} (neat): 3373, 1760, 1600, 1514, 1452 and 1270 cm^{-1}; ESIMS *m/z* 543 $[M+ Na]^+$; ^1H (400 MHz, CD_3OD) and ^{13}C NMR (100 MHz, CD_3OD): as published data (Shoeb et al., 2004a).

Arctiin (**2**): Gum, $[\alpha]^{23}_D$ -55.3° (c 0.0033, MeOH); UV λ_{max} (MeOH): 279, 225; IR ν_{max} (neat): 3459, 1765, 1591, 1460 and 1266 cm^{-1}; CIMS *m/z* 552 $[M+ NH_4]^+$; ^1H (400 MHz, CD_3OD) and ^{13}C NMR (100 MHz, CD_3OD): as published data (Shoeb et al., 2004a).

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). DPPH (4 mg) was dissolved in MeOH (50 mL) to obtain a concentration of 80 μg/mL.

Qualitative assay: Test samples (MeOH extract, **1** and **2**) 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 assay: The MeOH extract, and test compounds **1** and **2** were dissolved in MeOH to obtain a concentration of 1.0 mg/mL. Dilutions were made to obtain concentrations of 5 x 10^{-1}, 5 x 10^{-2}, 5 x 10^{-3}, 5 x 10^{-4}, 5 x 10^{-5}, 5 x 10^{-6} 5 x 10^{-7}, 5 x 10^{-8}, 5 x 10^{-9}, 5 x 10^{-10} mg/mL. Diluted solutions (1.00 mL each) were mixed with DPPH (1.00 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 triplicate and the average absorption was noted for each concentration. The same procedure was followed for the positive standards (quercetin).

Brine shrimp lethality assay

Shrimp eggs were purchased from The Pet Shop, Kittybrewster Shopping Complex, Aberdeen, UK. The bioassay was conducted following the procedure described by Meyer et al. (1982). The eggs were hatched in a conical flask containing 300 mL artificial seawater. The flasks were well aerated with the aid of an air pump, and kept in a water bath at 29-30 °C. A bright light source was left on and the nauplii hatched within 48 h. The methanol extracts of two species and the test compounds (**1** and **2**) were dissolved in 20% aq. DMSO to obtain a concentration of 1 mg/mL. These were serially diluted two-times, and seven different concentrations were obtained. A solution of each concentration (1 mL) was transferred into clean sterile universal vials with pipette, and aerated sea-water (9 mL) was added. About 10 nauplii were transferred into each vial with pipette. A check count was performed and the number alive after 24 h was noted. LD_{50}s were determined using the Probit analysis method (Finney, 1971).

MTT cytotoxicity assay

CaCo-2 cells were maintained in Earle's minimum essential medium (Sigma), supplemented

with 10% (v/v) foetal calf serum (Labtech Int.), 2 mM L-glutamine (Sigma), 1% (v/v) non-essential amino acids (Sigma), 100 IU/mL penicillin and 100 µg/mL streptomycine (Sigma). Exponentially growing cells were plated at 2×10^4 cells cm^{-2} into 96-well plates and incubated for 72 h before the addition of drugs. Stock solution of compounds was initially in DMSO or H_2O and further diluted with fresh complete medium.

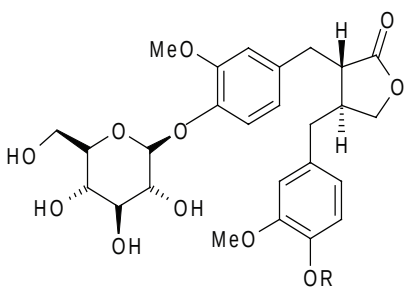
The growth-inhibitory effects of the methanol extracts of two *Centaurea* species and the test compounds (**1-2**) were assessed using the standard tetrazolium MTT assay (Mosmann, 1983). After 72 h of incubation at 37 °C, the medium was removed, and 100 µL of MTT reagent (1 mg/mL) in serum free medium was added to each well. The plates were incubated at 37 °C for 4 h. At the end of the incubation period, the medium was removed and pure DMSO (200 µL) was added to each well. The metabolised MTT product dissolved in DMSO was quantified by reading the absorbance at 560 nm on a micro plate reader (Dynex Technologies, USA). The IC_{50} values were calculated from the equation of the logarithmic line determined by fitting the best line (Microsoft Excel) to the curve formed from the data. The IC_{50} value was obtained from the equation y = 50 (50% value).

RESULTS AND DISCUSSION

The MeOH extracts of *C. urvillei* subs. *armata* and *C. mucronifera* were tested for antioxidant activity, general toxicity and cytotoxicity, respectively, by the DPPH assay, the brine shrimp lethality and the MTT cytotoxicity assays. The crude MeOH extracts of *C. urvillei* subs. *armata* and *C. mucronifera* showed significant levels of free radical scavenging activity (antioxidant activity) (IC_{50} = 51.6 x 10^{-2} and 53.6 x 10^{-2} mg/mL) in the DPPH assay (Table 1) which was due to the presence of two lignans, matairesinoside (**1**) and arctiin (**2**) with significant antioxidant properties (IC_{50} = 2.2 x 10^{-3} and 16.0 x 10^{-2} and mg/mL, respectively).

The brine shrimp lethality assay has been demonstrated to be an effective, robust and rapid assay method for primary screening of compounds for potential cytotoxic activities (Meyer et al., 1982). The MeOH extracts of *C. urvillei* subs. *armata* and *C. mucronifera* were found to possess similar levels of general toxicities with the LD_{50} values of 115.5 x 10^{-2} and 120.0 x 10^{-2} mg/mL, respectively (Table 1). Again, the lignans **1** and **2,** as the major components present in the extracts, contributed to this activity (LD_{50} values of 1.6 x 10^{-2} and 9.8 x 10^{-2} mg/mL, respectively).

While none of the extracts demonstrated any significant cytotoxicity against the CaCo-2 colon cancer cell line in the MTT assay (IC_{50} = >1000 µg/mL), the isolated lignans (**1** and **2**) showed considerable cytotoxicity (Table 1). The IC_{50} values for matairesinoside (**1**) and arctiin (**2**) were 220 and 288 µM. It has previously been observed that it is not at all surprising to have active



1 R = H
2 R = Me

Table 1. Antioxidant (DPPH assay) and brine shrimp toxicity (Brine Shrimp Lethality assay), and cytotoxic (MTT assay) activities, of the MeOH extracts of *Centaurea urville* subs. *armata* and *C. mucronifera*, and the lignans (**1** and **2**).

MeOH extracts/ Compounds	Antioxidant activity IC_{50} (mg/mL)	Brine shrimp toxicity LD_{50} (mg/mL)	Cytotoxicity IC_{50} (µM)[a], µg/mL[b]
C. urvillei	51.6×10^{-2}	115.5×10^{-2}	>100[b]
C. mucronifera	53.6×10^{-2}	120.0×10^{-2}	>100[b]
Matairesinoside (**1**)	2.2×10^{-2}	1.6×10^{-2}	220.0[a]
Arctiin (**2**)	16.0×10^{-3}	9.8×10^{-2}	288.0[a]
Quercetin (positive control)	2.88×10^{-5}	-	-
Podophyllotoxin (positive control)	-	2.79×10^{-3}	0.06

compounds from inactive extracts or fractions as the amounts of active compounds present in the amounts of extracts or fractions tested can be too small to show any activity (Shoeb et al., 2006a).

Reversed phase HPLC analyses of the 40% Sep-Pak fraction of the methanol extracts of *C. urvillei* subs. *armata* and *C. mucronifera* afforded two known bioactive dibenzylbutyrolactone-type lignans, matairesinoside (**1**) and arctiin (**2**). The structures of these compounds were elucidated by comprehensive spectroscopic analyses, and also by direct comparison with the respective published data (Shoeb et al., 2004a). Compounds **1** and **2** displayed characteristic UV absorption maxima of dibenzylbutyrolactone-type lignans (Shoeb et al., 2004a). The ^{1}H and ^{13}C NMR spectra of these compounds also supported this fact. A CIMS spectrum of **2** revealed the $[M+NH_4]^+$ ion peak at *m/z* 552, suggesting *M*r = 534, and the molecular formula $C_{27}H_{34}O_{11}$. The ^{1}H and ^{13}C NMR spectral data of **2** were identical to those published for arctiin (**2**) (Shoeb et al., 2004a). A combination of HMQC, HMBC, COSY and NOESY 2D NMR spectral analyses led to the unambiguous assignment of all ^{1}H and ^{13}C NMR signals of **2** and confirmed unequivocally its identity as arctiin (**2**).

The ^{1}H and ^{13}C NMR data of **1** were similar to those of arctiin (**2)** with the exception that one methoxyl signal was missing. The CIMS spectrum of **1** revealed $[M+NH_4]^+$ ion at *m/z* 538, suggesting *Mr* = 520, and the molecular formula $C_{26}H_{32}O_{11}$ which also confirmed the findings from the NMR data that it contained 14 mass units less than arctiin (**2**), i.e. instead of a methoxyl group, it had a hydroxyl group present. The ^{1}H and ^{13}C NMR data of **1** were in good agreement with the published data of matairesinoside (Shoeb et al., 2004a). Using the HPLC-PDA-based 'dereplication' protocol, the lignans **1** and **2** were also unambiguously identified in the 40% Sep-Pak fraction of the MeOH extract of *C. mucronifera*, and so was not processed for further purification.

These lignans were previously reported from a few other species of the genus *Centaurea* (ISI database, 2006; Shoeb et al., 2004a). However, this is the first report on the occurrence of matairesinoside (**1**) and arctiin (**2**) in *C. urvillei* subs. *armata* and *C. mucronifera*. The co-occurrence of these dibenzylbutyrolactone lignans (**1** and **2**) within the genus *Centaurea* might be chemotaxonomically significant.

The overall bioactivity profiles of the MeOH extracts of *C. urvillei* subs. *armata* and *C. mucronifera* were almost identical (Table 1). It is interesting, but not unexpected, to note that both extracts have the same chemical profiles as well, and contain matairesinoside (**1**) and arctiin (**2**) as the major bioactive compounds.

ACKNOWLEDGEMENTS

We thank the EPSRC National Mass Spectrometry Service Centre (Department of Chemistry, University of Wales Swansea, Swansea, Wales, UK) for CIMS analyses.

REFERENCES

Carlini EA, Rodrigues E, Mendes FR, Tabach R, Gianfratti B 2006. Treatment of drug dependence with Brazilian herbal medicines. *Rev Bras Farmacogn 16*: 690-695.

Finney D 1971. *Probit analysis*, 3rd eD., Cambridge University Press, Cambridge.

ISI Database 2006. *Institute for Scientific Information, UK*. Available on-line through Web of Science at: http://wos.mimas.ac.uk.

Kumarasamy Y, Fergusson M, Nahar L, Sarker SD 2002. Biological activity of moschamindole from *Centaurea moschata*. *Pharm Biol 40*: 307-310.

Meyer BN, Ferrigni RN, Putnam JE, Jacobsen LB, Nichols DE, Mclaughlin JL 1982. Brine shrimp - A convenient general bioassay for active plants constituents. *Planta Med 45*: 31-34.

Mosmann T 1983. Rapid colorimetric assay for the cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods 65*: 55-63.

Sarker SD, Kumarasamy Y, Shoeb M, Celik S, Yucel E, Middleton M, Nahar L 2005. Antibacterial and antioxidant activities of three Turkish species of the gemus *Centaurea*. *Oriental Pharmacy and Experimental Medicine 5*: 246-250.

Sarker SD, Laird A, Nahar L, Kumarsamy Y, Jaspars M 2001. Indole alkaloids from the seeds of *Centaurea cyanus* (Asteraceae). *Phytochemistry 57*: 1273-1276.

Sarker SD, Dinan L, Sik V, Underwood E, Waterman PG 1998. Moschamide: an unusual alkaloid from the seeds of *Centaurea moschata. Tetrahedron Lett 39*: 1421-1424.

Sarker SD, Savchenko T, Whiting P, Sik V, Dinan LN 1997. Moschamine, *cis*-moschamine, moschamine, moschamindole and moschamindolol: four novel indole alkaloids from *Centaurea moschata*. *Nat Prod Lett 9*: 189-199.

Shoeb M, MacManus SM, Kumarasamy Y, Jaspars M, Nahar L, Kong-Thoo-Lin P, Nazemiyeh H, Sarker SD 2006a. Americanin, a bioactive dibenzylbutyrolactone lignan, from the seeds of *Centaurea americana*. *Phytochemistry 67*: 2370-2375.

Shoeb M, MacManus SM, Jaspars M, Trevidu J, Nahar L, Kong-Thoo-Lin P, Sarker SD 2006b. Montamine, a unique dimeric indole alkaloid, from the seeds of *Centaurea montana* (Asteraceae), and its *in vitro* cytotoxic activity against the CaCo-2 colon cancer cells. *Tetrahedron 62*: 11172-11177.

Shoeb M, Celik S, Jaspars M, Kumarasamy Y, MacManus SM, Nahar L, Kong-Thoo-Lin P, Sarker SD 2005. Isolation, structure elucidation and bioactivity of schischkiniin, a unique indole alkaloid from the seeds of *Centaurea schischkini Tetrahedron 61*: 9001-9006.

Shoeb M, Rahman MM, Nahar L, Jaspars M, MacManus S, Delazar A, Sarker SD 2004a. Bioactive lignans from the seeds of *Centaurea macrocephala. DARU - Journal of Tehran School of Pharmacy 12*: 87-93.

Shoeb M, Jaspars M, MacManus SM, Majinda RRT, Sarker SD 2004b. Epoxylignans from the seeds of *Centaurea cyanus*. *Biochem Syst Ecol 32*: 1201-1204.

Takao T, Watanabe N, Yagi I, Sakata K 1994. A simple screening method for antioxidants and isolation of several antioxidants produced by marine bacteria from fish and shellfish. *Biosci Biotech Biochem 58*: 1780-1783.

Wagentiz G 1975. *Flora of Turkey and the East Aegean Islands*, Davis, P.H, Eds., University Press, Edinburgh, 5.