Available online at www.sciencedirect.com
ScienceDirect
Phytochemistry 67 (2006) 2370–2375
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Americanin, a bioactive dibenzylbutyrolactone lignan, from the seeds of *Centaurea americana*

Mohammad Shoeb [a], Stephen M. MacManus [b], Yashodharan Kumarasamy [b], Marcel Jaspars [c], Lutfun Nahar [d], Paul Kong Thoo-Lin [d], Hossein Nazemiyeh [e], Satyajit D. Sarker [f,*]

[a] *Department of Chemistry, University of Dhaka, Dhaka 1000, Bangladesh*
[b] *School of Pharmacy, The Robert Gordon University, Schoolhill, Aberdeen AB10 1FR, Scotland, UK*
[c] *Department of Chemistry, University of Aberdeen, Aberdeen, Scotland, UK*
[d] *School of Life Sciences, The Robert Gordon University, Aberdeen AB25 1HG, UK*
[e] *Faculty of Pharmacy and Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*
[f] *School of Biomedical Sciences, University of Ulster at Coleraine, Cromore Road, Coleraine BT52 1SA, Co. Londonderry, Northern Ireland, UK*

Received 5 June 2006; received in revised form 7 August 2006

Abstract

The reversed-phase preparative HPLC analysis of the methanol (MeOH) extract of the seeds of *Centaurea americana* afforded a dibenzylbutyrolactone lignan, 3″-*O*-caffeoyl arctiin (named americanin), together with five known lignans, arctiin, arctigenin, matairesinol, matairesinoside and lappaol A, and two known phytoecdysteroids, 20-hydroxyecdysone and makisterone A. While the structures of the known compounds were determined by direct comparison of the spectral data with published data, the structure of americanin was elucidated by UV, MS and a combination of 1D and 2D NMR spectral analyses. The antioxidant properties and toxicity of the extracts and the isolated compounds were determined by the DPPH and the brine shrimp lethality assays, respectively.
© 2006 Elsevier Ltd. All rights reserved.

Keywords: *Centaurea americana*; Asteraceae; Compositae; Lignan; Phytoecdysteroid; DPPH; Brine shrimp lethality assay; Makisterone A; Antioxidant; Chemotaxonomy

1. Introduction

Centaurea americana Nutt. (Family: Asteraceae *alt.* Compositae), commonly known as "Jolly Joker" or "Basket flower", is an annual, indigenous to Northern Americana-Coahuila and Nuevo Leon in Mexico and Arizona, Arkansas, Kansas, Louisiana, Missouri, New Mexico, Oklahoma and Texas in the USA, and also cultivated in several other countries (USDA-ARS-GRIN Database, 2006). A sesquiterpene lactone, cynaropicrin (Rojatkar et al., 1997; Ohno et al., 1973), and two lignans, arctiin and matairesinoside (Cooper et al., 2002), have previously been isolated from this plant. The presence of phytoecdysteroids in the seeds of *C. americana* was also reported on the basis of the B_{II} bioassay and the ecdysteroid-specific RIA results (Sarker et al., 1997a,b). As a part of our ongoing studies on the genus *Centaurea* (Kumarasamy et al., 2003a,b, 2002a,b; Sarker et al., 2001, 2005; Shoeb et al., 2004, 2005), we now report on the assessment of the extracts of the seeds of *C. americana* for antioxidant activity and general toxicity using, respectively, the DPPH and the brine shrimp lethality assays, and on the isolation, identification and bioactivity of eight secondary metabolites including a new dibenzylbutyrolactone lignan, americanin (**1**), five other known lignans, arctiin (**2**), matairesinoside (**3**), matairesinol (**4**), arctigenin (**5**) and lappaol A (**6**), and two known phytoecdysteroids, 20-hydroxyecdysone (**7**) and

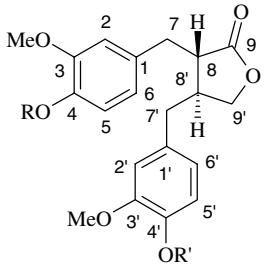
* Corresponding author. Tel.: +44 28 7032 4302; fax: +44 28 7032 4965.
E-mail address: s.sarker@ulster.ac.uk (S.D. Sarker).

0031-9422/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2006.08.012

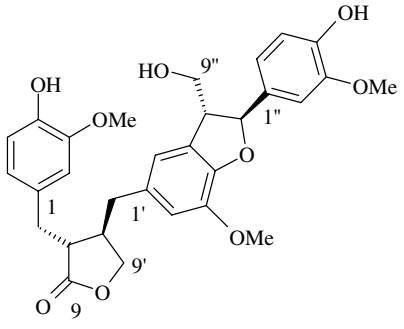
makisterone A (**8**), from the methanol extract of the seeds of *C. americana*.

2. Results and discussion

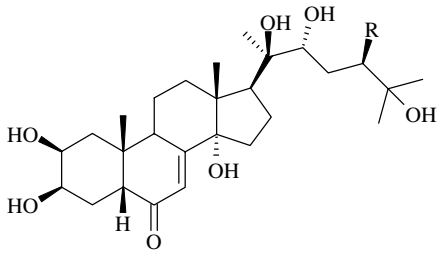
The reversed-phase preparative HPLC analysis of the methanol (MeOH) extract of the seeds of *C. americana*, in addition to previously reported lignan glucosides, arctiin (**2**) and matairesinoside (**3**) (Cooper et al., 2002), afforded a new dibenzylbutyrolactone lignan, 3″-*O*-caffeoyl arctiin (named americanin, **1**), three other known lignans, arctigenin (**5**), matairesinol (**4**), and lappaol A (**6**), and two known phytoecdysteroids, 20-hydroxyecdysone (**7**) and makisterone A (**8**). While the structures of the known compounds (**2**–**8**) were determined by direct comparison of their spectral data with published data (Shoeb et al., 2004; Rahman et al., 1990; Nishibe et al., 1984; Sarker et al., 1997a; Lafont and Wilson, 1996), the structure of the new lignan, americanin (**1**), was elucidated by UV, MS and a combination of 1D and 2D NMR spectral analyses.



Compounds	R	R’
1	β-D-(3-*O*-caffeoyl)-glucopyranosyl	Me
2	β-D-glucopyranosyl	Me
3	β-D-glucopyranosyl	H
4	**H**	H
5	**H**	Me



6



Compounds	R
7	H
8	Me

An ESIMS spectrum of compound **1** displayed the *pseudo*molecular ion peak at *m/z* 719 $[M+Na]^+$, suggesting $Mr = 696$ and the molecular formula $C_{36}H_{40}O_{14}$. The HRCIMS spectrum showed the *pseudo*molecular ion peak *m/z* 714.276133 $[M+NH_4]^+$, calculated 714.276153 for $C_{36}H_{44}NO_{14}$. In the ^1H and ^{13}C NMR spectra of **1** (Table 1), in addition to the signals attributable to an arctiin (**2**) skeleton (Shoeb et al., 2004), displayed signals associated with a caffeoyl moiety [δ_H 7.03 (*d*, $J = 2.0$ Hz), 6.93 (*dd*, $J = 8.0$, 2.0 Hz) and 6.74 (*d*, $J = 8.0$ Hz), 7.57 (*d*, $J = 16.0$ Hz) and 6.32 (*d*, $J = 16.0$ Hz); δ_C 167.8, 149.3, 148.3, 145.6, 126.7, 121.8, 115.3, 114.2 and 114.0]. The attachment of this caffeoyl moiety at C-3″ of the glucose unit of arctiin (**2**) was confirmed from a ^3J ^1H–^{13}C long-range correlation from δ_H 5.12 (H-3″) to the carbonyl carbon of the caffeoyl moiety [δ_C 167.8 (C-9‴)] observed in the HMBC spectrum of **1** (Table 1). While a ^1H–^1H COSY spectrum revealed all scalar ^1H–^1H couplings, an HSQC spectrum confirmed the assignment of all methine, methylene and methyl signals. A ^1H–^1H NOESY helped the confirmation of the relative stereochemistry at the chiral centres in the molecule. Thus, the identity of compound **1** was confirmed as 3″-*O*-caffeoyl-arctiin (named americanin). To the best of our knowledge this is a new natural product.

Except arctiin (**2**) and matairesinoside (**3**), all other compounds isolated in this study are reported from *C. americana* for the first time. While 20-hydroxyecdysone (**7**), an insect-moulting hormone, has previously been reported from a few other species of the genus *Centaurea*, this is the first report on the occurrence of makisterone A (**8**) within this genus. However, phytoecdysteroids **7**, **8** and their analogues have been isolated from a number of other genera within the family Asteraceae (Sarker et al., 1997a). The distribution of lignans **1**–**6** within the genus *Centaurea* is summarised in Table 2.

In the DPPH assay (Takao et al., 1994), the *n*-hexane and the DCM extracts exhibited low levels of free radical scavenging (antioxidant) activities, but the MeOH extract showed significant levels of activity ($RC_{50} = 5.2 \times 10^{-2}$ mg/mL) (Table 3). This indicated that the antioxidant principles present in the seeds of *C. americana* were polar in

Table 1
^{1}H NMR (400 MHz, coupling constant J in Hz in parentheses), ^{13}C NMR (100 MHz) data and long-range HMBC correlations for **1**

Carbon number	Chemical shift δ in ppm		HMBC correlations (^{1}H → ^{13}C)	
	^{1}H	^{13}C	^2J	^3J
1	–	131.5	–	–
2	6.57 *d* (2.0)	112.4	C-1, C-3	C-4, C-6, C-7
3	–	149.6	–	–
4	–	148.0	–	–
5	6.79 *d* (8.8)	111.9	C-4, C-6	C-1, C-3
6	6.56 *dd* (2.0, 8.8)	121.0	C-1, C-5	C-2, C-7
7	2.81 *dd* (14.1, 6.1) 2.87 *dd* (14.1, 6.8)	34.2	C-1, C-8	C-2, C-6, C-9, C-8′
8	2.65 *m*	46.4	C-7, C-9, C-8′	C-1, C-7′, C-9′
9	–	180.2	–	–
1′	–	133.5	–	–
2′	6.88 *d* (2.0)	109.2	C-1′, C-3′	C-4′, C-6′, C-7′
3′	–	149.3	–	–
4′	–	145.8	–	–
5′	7.04 *d* (8.4)	116.8	C-4′, C-6′	C-1′, C-3′
6′	6.71 *dd* (2.0, 8.4)	121.7	C-1′, C-5′	C-2′, C-7′
7′	2.50 *m*	37.7	C-1′, C-8′	C-2′, C-6′, C-9′, C-8
8′	2.41 *m*	41.3	C-7′, C-9′, C-8	C-1′, C-7, C-9
9′	4.16 *dd* (7.6, 9.2) 3.91 *dd* (7.6, 9.2)	71.7	C-8′	C-8, C-9, C-7′
1″	4.96 *d* (7.2)	101.1	C-2″	C-3″, C-5″
2″	3.65[a]	68.4	C-1″, C-3″	–
3″	5.12 *bd*	77.4	C-4″	C-1″, C-5″, C-9‴
4″	3.62[a]	72.1	C-3″	C-2″, C-6″
5″	3.48[a]	76.8	C-6″	C-1″, C-3″
6″	3.84[a] 3.71[a]	61.0	C-5″	C-4″
1‴	–	126.7	–	–
2‴	7.03 *d* (2.0)	114.2	C-1‴, C-3‴	C-4‴, C-6‴, C-7‴
3‴	–	149.3	–	–
4‴	–	148.3	–	–
5‴	6.74 *d* (8.0)	115.3	C-4‴, C-6‴	C-1‴, C-3‴
6‴	6.93 *dd* (2.0, 8.0)	121.8	C-1‴, C-5‴	C-2‴, C-7‴
7‴	7.57 *d* (16.0)	145.6	C-1‴, C-8‴	C-2‴, C-6‴, C-9‴
8‴	6.32 *d* (16.0)	114.0	C-7‴, C-9‴	C-1‴
9‴	–	167.8	–	–
3-OMe	3.72 *s*	55.4	–	C-3
3′-OMe	3.71 *s*	55.5	–	C-3′
4′-OMe	3.70 *s*	55.6	–	C-4′

[a] Overlapped peaks, identified from ^{1}H–^{1}H COSY and ^{1}H–^{13}C HSQC; spectra in CD_3OD.

nature. Except for the phytoecdysteroids **7** and **8**, which did not show any activity at test concentrations, all test compounds (**1**–**6**), isolated from the MeOH extract, displayed significant levels of free radical scavenging activity, and the RC_{50} values of **2**–**6** were identical to those published in the literature (Shoeb et al., 2004, 2005) (Table 3). The new compound **1**, which is a caffeoyl derivative of arctiin (**2**), had a RC_{50} value of 3.2×10^{-3} mg/mL, significantly higher than that of arctiin ($RC_{50} = 16.0 \times 10^{-2}$ mg/mL). This higher level of activity in **1** could be attributed to two extra phenolic hydroxyl groups present in its caffeoyl moiety. The antioxidant activity of **1**–**6**, like any other natural phenolic antioxidants, is a consequence of the presence of the phenolic moieties in the structures. 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.

The brine shrimp lethality assay, which has been proven to be an effective and rapid assay method to screen compounds for potential general toxicity and cytotoxic activity (Meyer et al., 1982), was used to determine the general toxicity of the extracts as well as the compounds **1**–**8**. However, owing to high degree of lipophilicity, *n*-hexane extract could not be tested in this assay. While none of the extracts demonstrated any significant toxicity towards the brine shrimp ($LD_{50} = >800$ mg/mL), the isolated lignans (**1**–**6**) showed considerable toxicity. It has previously been observed that it is not at all surprising to have active 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 (Kumarasamy et al., 2003a; Kumarasamy et al., 2006). The LD_{50} values of lignans **2**–**6** were identical to those published previously (Shoeb et al., 2004, 2005) (Table 3). Arctigenin (**5**) exhibited the highest degree of toxicity with a LD_{50} value of 2 μg/mL, which is surprisingly lower than that of the positive control, podophyllotoxin, a known cytotoxic lignan. The glucosidation of **5**, yielding arctiin (**2**), considerably reduced the toxicity (~50 times). It is interesting to note that the addition of the caffeoyl moiety on to the arctiin (**2**) skeleton forming **1**, slightly increased the levels of toxicity ($RC_{50} = 76.0$ and 98.0 μg/mL for **1** and **2**, respectively). None of the phytoecdysteroids (**7** and **8**) was particularly toxic in this assay (Table 3).

3. Experimental

3.1. General experimental procedures

UV spectra were obtained using a Hewlett-Packard 8453 UV–Vis spectrometer. NMR spectra were recorded in CD_3OD on a Varian Unity INOVA 400 MHz NMR Spectrometer 400 (400 MHz for ^{1}H and 100 MHz for ^{13}C) using the residual solvent peaks as internal standard. MS experiments on a Quattro II triple quadrupole instrument. HPLC separation was performed in a Dionex prep-HPLC System coupled with Gynkotek GINA50 autosampler and Dionex UVD340S Photo-Diode-Array detector. A Luna C18 preparative HPLC column (10 m × 250 mm × 21.2 mm) was used. Sep-Pak DSC-18 Supelco 10 g cartridge was used for pre-HPLC fractionation. HMBC spectra were optimized for a long-range J_{H-C} of 9 Hz and NOESY experiment was carried out with a mixing time of 0.8 s.

3.2. Plant material

The seeds of *C. americana* Nutt. were purchased from the B&T, World Seeds Sarl, Paguignan, 34210 Olonzac,

Table 2
Distribution of compounds **1**–**8** within the genus *Centaurea*

Species	Compounds								References
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	
C. affinis	−	−	−	+	+	−	−	−	Janackovic et al. (2004)
C. alexandria	−	+	−	−	−	−	−	−	Erdemgil et al. (2006)
C. americana	+	+	+	+	+	+	+	+	Cooper et al. (2002), present work
C. aspera	−	−	−	+	−	−	−	−	Marco et al. (2005)
C. calcitrapa	−	−	−	+	+	−	−	−	Dawidar et al. (1989)
C. cuneifolia	−	−	−	−	+	−	−	−	Aslan and Oksuz (1999)
C. dealbata	−	+	+	−	+	−	+	−	Shoeb (2005), Sarker et al. (1997b)
C. isaurica	−	+	−	−	−	−	−	−	Flamini et al. (2004)
C. macrocephala	−	+	+	+	+	+	−	−	Shoeb et al. (2004), Ribeiro et al. (2002)
C. melitensis	−	+	−	−	−	−	−	−	Erdemgil et al. (2006)
C. Montana	−	−	−	−	+	−	−	−	Christensen and Lam (1991b)
C. moschata	−	−	−	−	−	−	+	−	Sarker et al. (1997b)
C. nicaensis	−	−	−	−	−	+	−	−	Bruno et al. (1996)
C. napifolia	−	−	−	−	−	+	−	−	Bruno et al. (1995)
C. nervosa	−	−	−	−	+	−	−	−	Christensen and Lam (1991a)
C. nicolai	−	−	−	+	−	−	−	−	Vajs et al. (1999)
C. nigra	−	+	+	+	−	−	−	−	Middleton et al. (2003)
C. persica	−	−	−	+	−	−	−	−	Sanz et al. (1990)
C. Phrygia	−	−	−	−	+	−	−	−	Christensen and Lam (1991a)
C. ptosimopappa	−	−	−	+	+	−	−	−	Celik et al. (2006)
C. raphanina	−	−	−	+	−	−	−	−	Panagouleas et al. (2003)
C. rothrockii	−	−	−	−	−	−	+	−	Sarker et al. (1997b)
C. rupestris	−	−	−	−	−	−	+	−	Sarker et al. (1997b)
C. scabiosa	−	−	+	+	−	−	−	−	Ferguson et al. (2002)
C. sclerolepis	−	+	+	−	−	−	−	−	Erdemgil et al. (2006)
C. scoparia	−	−	−	+	+	−	−	−	Youssef and Frahm (1995)
C. solstitialis	−	−	−	+	+	−	−	−	Bruno et al. (1991)
C. sphaerocephala	−	+	−	+	−	+	−	−	Bastos et al. (1990)
C. schischkinii	−	+	+	+	+	−	−	−	Shoeb et al. (2005)
C. tweediei	−	−	−	+	+	−	−	−	Fortuna et al. (2001)

Table 3
Antioxidant activity and brine shrimp toxicity of the extracts of *C. americana* and isolated compounds (**1**–**8**)

Compounds/extracts	DPPH assay (RC_{50} in mg/mL)	Brine shrimp lethality assay (LD_{50} in μg/mL)
n-Hexane extract	6.9×10^{-1}	ND
DCM extracts	2.8×10^{-1}	1100.0
MeOH extracts	5.2×10^{-2}	893.0
1	3.2×10^{-3}	76.0
2	16.0×10^{-2}	98.0
3	2.2×10^{-3}	16.0
4	2.0×10^{-3}	5.5
5	1.9×10^{-2}	2.0
6	3.6×10^{-2}	9.2
7	–	640
8	–	460
Podophyllotoxin	NA	2.79
Quercetin (in MeOH)	2.88×10^{-5}	NA
Trolox (in DCM)	2.58×10^{-3}	NA

– = no activity detected at test concentrations.
NA = not applicable.
ND = not done.

France. A voucher specimen (PHSH0004) representing this sample has been retained in the herbarium of the Plant and Soil Science Department, University of Aberdeen, Scotland (ABD).

3.3. Extraction

The dried and ground seeds of *C. americana* (100 g) were Soxhlet-extracted, successively, with *n*-hexane, dichloromethane (DCM) and methanol (MeOH) (1.1 L each). All these extracts were separately concentrated using a rotary evaporator at a maximum temperature of 45 °C.

3.4. Isolation of compounds

The MeOH extract (2.0 g) 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). Reversed-phase preparative HPLC purification (eluted with a linear gradient-water:MeOH = 40:60 to 20:80 over 50 min followed by 80% MeOH for 10 min, 20 mL/min) of the Sep-Pak fraction, which was eluted with 60% MeOH, afforded matairesinoside (**3**, 8.8 mg, $t_R = 16.5$ min), 20-hydroxyecdysone (**7**, 13.9 mg, $t_R = 21.0$ min), arctiin (**2**, 203.4 mg, $t_R = 25.0$ min), makisterone A (**8**, 8.2 mg, $t_R = 27.1$ min), matairesinol (**4**, 8.0 mg, $t_R = 29.0$ min), arctigenin (**5**, 15.5 mg, $t_R = 31.8$ min), americanin (**1**, 12.1 mg, $t_R = 33.0$ min) and lappaol A (**6**, 9.2 mg, $t_R = 34.2$ min).

*3.4.1. Americanin (**1**)*

Gum. $[\alpha]_D^{23}$ −34.5° (c 0.001, MeOH); UV: λ_{max} (MeOH) nm: 310, 282, 225; ESIMS (positive ion mode) *m/z* 719 $[M+Na]^+$; HRCIMS *m/z* 714.276133, $C_{36}H_{44}NO_{14}$ requires *m/z* 714.276153; ^1H and ^{13}C NMR (Table 1).

*3.4.2. Arctiin (**2**)*

Gum. $[\alpha]_D^{23}$ −55.3° (*c* 0.0033, MeOH); UV: λ_{max} (MeOH) nm: 279, 225; CIMS *m/z* 552 $[M+NH_4]^+$; ^1H and ^{13}C NMR (Shoeb et al., 2004).

*3.4.3. Matairesinoside (**3**)*

Gum. $[\alpha]_D^{23}$ −48.8° (*c* 0.002, MeOH); UV λ_{max} (MeOH) nm: 279, 222; ESIMS *m/z* 543 $[M+Na]^+$; ^1H and ^{13}C NMR (Shoeb et al., 2004).

*3.4.4. Matairesinol (**4**)*

Gum., $[\alpha]_D^{23}$ −47.2° (*c* 0.0022, MeOH); UV λ_{max} (MeOH) nm: 282, 228; ESIMS *m/z* 381 $[M+Na]^+$; ^1H and ^{13}C NMR (Shoeb et al., 2004).

*3.4.5. Arctigenin (**5**)*

Gum. $[\alpha]_D^{23}$ −42.6° (*c* 0.0015, MeOH); UV λ_{max} (MeOH) nm: 281, 220; ESIMS *m/z* 395 $[M+Na]^+$; ^1H and ^{13}C NMR (Rahman et al., 1990; Nishibe et al., 1984).

*3.4.6. Lappaol A (**6**)*

Amorphous solid. $[\alpha]_D^{23}$ −17.6° (*c* 0.0021, MeOH); UV λ_{max} (MeOH) nm: 282, 225; ESIMS *m/z* 559 $[M+Na]^+$; ^1H and ^{13}C NMR (Shoeb et al., 2004).

*3.4.7. 20-Hydroxyecdysone (**7**)*

Amorphous solid. UV: λ_{max} (MeOH) nm: 242; ESIMS (positive ion mode) *m/z* 481 $[M+H]^+$; ^1H and ^{13}C NMR (Sarker et al., 1997a; Lafont and Wilson, 1996).

*3.4.8. Makisterone A (**8**)*

Amorphous solid. UV: λ_{max} (MeOH) nm: 242; ESIMS (positive ion mode) *m/z* 495 $[M+H]^+$; ^1H and ^{13}C NMR (Lafont and Wilson, 1996).

3.5. Brine shrimp lethality assay

Brine shrimp eggs were purchased from Water Life, Middlesex, UK. The bioassay was conducted following the procedure published previously (Meyer et al., 1982). The LD_{50} values were determined from the 24 h counts using the Probit analysis method (Finney, 1971). The percentage mortalities were adjusted relative to the natural mortality rate of the control, following Abbots formula $P = (Pi - C)/(1 - C)$, where *P* denotes the observed nonzero mortality rate and *C* represents the mortality rate of the control.

3.6. 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., 2002a,b). DPPH (4 mg) was dissolved in MeOH (50 mL) to obtain a concentration of 80 μg/mL.

Qualitative assay: Test extracts and compounds (**1**–**8**) 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 *n*-hexane and DCM extracts were dissolved in DCM and the MeOH extract in MeOH to obtain a stock concentration of 10 mg/mL. Compounds **1**–**8** were dissolved in MeOH to obtain a concentration of 0.5 mg/mL. Dilutions were made to obtain concentrations of 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 half an hour 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 controls, quercetin (in MeOH) and trolox (in DCM).

Acknowledgement

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

References

Aslan, U., Oksuz, S., 1999. Chemical constituents of *Centaurea cuneifolia*. Turk. J. Chem. 23, 15–20.

Bastos, M., Kijjoa, A., Cardoso, J.M., Gutierrez, A.B., Herz, W., 1990. Lignans and other constituents of *Centaurea sphaerocephala* ssp. *polyacantha*. Planta Med. 56, 403–405.

Bruno, M., Diaz, J.G., Herz, W., 1991. Guaianolides and lignans from *Centaurea solstitialis* subsp. *Schouwii*. Phytochemistry 30, 4165–4166.

Bruno, M., Fazio, C., paternostro, P., Diaz, J.G., Herz, W., 1995. Sesquiterpene lactones and other constituents of *Centaurea napifolia*. Planta Med. 61, 374–375.

Bruno, M., Paternostro, M.P., Gedris, T.E., Herz, W., 1996. Sesquiterpene lactones and other constituents of *Centaurea nicaensis*. Phytochemistry 41, 335–336.

Celik, S., Rosselli, S., Maggio, A.M., Raccuglia, R.A., Uysal, I., Kisiel, W., Michalska, K., Bruno, M., 2006. Guaianolode and lignans from the aerial parts of *Centaurea ptosimopappa*. Biochem. Syst. Ecol. 34, 349–352.

Christensen, L.P., Lam, J., 1991a. Flavones and other constituents from *Centaurea* species. Phytochemistry 30, 2663–2665.

Christensen, L.P., Lam, J., 1991b. Acetylenes and other constituents from *Centaurea* species. Phytochemistry 30, 3289–3292.

Cooper, G., Laird, A., Nahar, L., Sarker, S.D., 2002. Lignan glucosides from the seeds of *Centaurea americana* (Compositae). Biochem. Syst. Ecol. 30, 65–67.

Dawidar, A.M., Metwally, M.A., Abou-Elzahab, M., Abdel-Mogib, M., 1989. Chemical constituents of two *Centaurea* species. Pharmazie 44, 735–736.

Erdemgil, Z., Rosselli, S., Maggio, A.M., Raccuglia, R.A., Celik, S., Michalska, K., Kisiel, W., Bruno, M., 2006. An unusual pregnane derivative and dibenzylbutyrolactone lignans from *Centaurea sclerolepis*. Pol. J. Chem. 80, 647–650.

Ferguson, C.A., Nahar, L., Finnie, D., Kumarasamy, Y., Reid, R., Mir-Babayev, N.F., Sarker, S.D., 2002. *Centaurea scabiosa*: a source of dibenzyl butyrolactone lignans. Biochem. Syst. Ecol. 31, 303–305.

Flamini, G., Stoppelli, G., Morelli, I., Ertugral, K., Dural, H., Tugay, O., Demirelma, H., 2004. Secondary metabolites from *Centaurea isaurica* from Turkey and their chemotaxonomical significance. Biochem. Syst. Ecol. 32, 553–557.

Fortuna, A.M., de Riscala, E.C., Catalan, C.A.N., Gedris, T.E., Herz, W., 2001. Sesquiterpene lactones from *Centaurea tweediei*. Biochem. Syst. Ecol. 29, 967–971.

Finney, D.J., 1971. Probit Analysis, third ed. Cambridge University Press, Cambridge.

Janackovic, P., Tesevic, V., Milosavljevic, S., Vajs, V., Marin, P.D., 2004. Sesquiterpene lactones, lignans and flavones of *Centaurea affinis*. Biochem. Syst. Ecol. 32, 355–357.

Kumarasamy, Y., Nahar, L., Cox, P.J., Jaspars, M., Sarker, S.D., 2002a. Screening seeds of Scottish plants for antibacterial activity. J. Ethnopharmacol. 83, 73–77.

Kumarasamy, Y., Fergusson, M., Nahar, L., Sarker, S.D., 2002b. Bioactivity of moschamindole from *Centaurea moschata*. Pharm. Biol. 40, 307–310.

Kumarasamy, Y., Cox, P.J., Jaspars, M., Nahar, L., Sarker, S.D., 2003a. Isolation, structure elucidation and biological activity of two unique alkaloids, hederacine A and B, from *Glechoma hederaceae*. Tetrahedron 59, 6403–6407.

Kumarasamy, Y., Nahar, L., Cox, P.J., Dinan, L.N., Ferguson, C.A., Finnie, D., Jaspars, M., Sarker, S.D., 2003b. Biological activities of lignans from *Centaurea scabiosa*. Pharm. Biol. 41, 203–206.

Kumarasamy, Y., Nahar, L., Lin, P.K.T., Sarker, S.D., 2006. Hederacine A and hederacine B from *Glechoma hederacea* inhibit the growth of colorectal cancer cells *in vitro*. Nat. Product Commun. 1, 33–35.

Lafont, R., Wilson, I., 1996. The Ecdysone Handbook, second ed. The Chromatographic Society, UK.

Marco, J.A., Sanz-Cervera, J.F., Yuste, A., Sancenon, F., Carda, M., 2005. Sesquiterpene from *Centaurea aspera*. Phytochemistry 66, 1644–1650.

Meyer, B.N., Ferrigni, R.N., Putnam, J.E., Jacobsen, L.B., Nichols, D.E., Mclaughlin, J.L., 1982. Brine shrimp: a convenient bioassay for active plant constituents. Planta Med. 45, 31–34.

Middleton, M., Cox, P.J., Jaspars, M., Kumarsamy, Y., Nahar, L., Reid, R., Sarker, S.D., 2003. Dibenzylbutyrolactone lignans and indole alkaloids from the seeds of *Centaurea nigra* (Asteraceae). Biochem. Syst. Ecol. 31, 653–656.

Nishibe, S., Tsukamoto, H., Hisada, S., 1984. Effects of *O*-methylation and *O*-glucosylation on carbon-13 nuclear magnetic resonance chemical shifts of matairesinol, (+)-pinoresinol and (+)-epipinoresinol. Chem. Pharm. Bull. 32, 4653–4657.

Ohno, N., Hirai, H., Yoshioka, H., Domingue, X.A., Mabry, T.J., 1973. Cynaropicrin – sesquiterpene lactone from *Centaurea americana*. Phytochemistry 12, 221–222.

Panagouleas, C., Skaltsa, H., Lazari, D., Skaltsounis, A., Sokovic, A., 2003. Antifungal activity of secondary metabolites of *Centaurea raphanina* ssp. Mixta, growing Wild in Greece. Pharm. Biol. 41, 266–270.

Rahman, M.M.A., Dewic, P.M., Jackson, D.E., Lucas, J., 1990. Lignans of *Forsythia intermedia*. Phytochemistry 29, 1971–1980.

Ribeiro, N.L., Nahar, L., Kumarasamy, Y., Mir-Babayev, N., Sarker, S.D., 2002. Flavonoids *C*-glucosides and a lignan from *Centaurea macrocephala* (Compositae). Biochem. Syst. Ecol. 30, 1097–1100.

Rojatkar, S.R., Pujar, P.P., Patewad, B.R., Nagasampagi, B.A., 1997. Guainolide from *Amberboa divericata*. Indian J. Heterocyclic Chem. 7, 67–68.

Sanz, J.F., Marco, J.A., Rustaiyan, A., 1990. Chemical constituents from *Centaurea persica* and *Senecio coronopifolius*. Pharmazie 45, 381.

Sarker, S.D., Girault, J.P., Lafont, R., Dinan, L., 1997a. Ecdysteroid xylosides from *Limnanthes douglasii*. Phytochemistry 44, 513–521.

Sarker, S.D., Savchenko, T., Whiting, P., Sik, V., Lafont, R., Dinan, L., 1997b. Occurrence of Ecdysteroids in the Genus *Centaurea* (Compositae): 20-Hydroxyecdysone from *Centaurea moschata*. Biochem. Syst. Ecol. 25, 367–368.

Sarker, S.D., Laird, A., Nahar, L., Kumarasamy, Y., Jaspars, M., 2001. Indole alkaloids from the seeds of *Centaurea cyanus* (Asteraceae). Phytochemistry 57, 1273–1276.

Sarker, S.D., Kumarasamy, Y., Shoeb, M., Celik, S., Yucel, E., Middleton, M., Nahar, L., 2005. Antibacterial and antioxidant activities of three Turkish species of the genus *Centaurea*. Orient. Pharm. Exp. Med. 5, 246–250.

Shoeb, M., 2005. Cytotoxic compounds from the genus *Centaurea*. Ph.D. Thesis, The Robert Gordon University, Aberdeen, UK.

Shoeb, M., Rahman, M.M., Nahar, L., Jaspars, M., MacManus, S.M., Delazar, A., Sarker, S.D., 2004. Bioactive lignans from the seeds of *Centaurea macrocephala*. DARU 12, 87–93.

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

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

USDA-ARS-GRIN Database, 2006. USDA, ARS, National Genetic Resources Program, Germplasm Resources Information Network (GRIN). National Germplasm Resources Laboratory, Beltsville, MD, USA. Available from: <http://www.ars-grin.gov/cgi-bin/npgs/html/taxgenform.pl>.

Vajs, V., Todorovic, N., Ristic, M., Tesevic, V., Todorovic, B., Janackovic, P., Marin, P., Milosavljevic, S., 1999. Guaianolides from *Centaurea nicolai*: antifungal activity. Phytochemistry 52, 383–384.

Youssef, D., Frahm, A.W., 1995. Constituents of the Egyptian *Centaurea scoparia*. 3. Phenolic constituents of the aerial parts. Planta Med. 61, 570–573.