Food and Chemical Toxicology 49 (2011) 3328–3335

Contents lists available at SciVerse ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Antioxidant and antigenotoxic properties of compounds isolated from *Marrubium deserti* de Noé

Nabila Zaabat [a,b], Anne-Emmanuelle Hay [a], Serge Michalet [a], Nicole Darbour [a], Christine Bayet [a], Inès Skandrani [c], Leila Chekir-Ghedira [c], Salah Akkal [b], Marie-Geneviève Dijoux-Franca [a,*]

[a] *UMR 5557 CNRS-UCBL – Ecologie Microbienne, Equipes 6/1/5, CESN, ISPB, 8 Avenue Rockefeller, F69373 Lyon cedex, France*
[b] *Département de chimie, Laboratoire de recherche en phytochimie et analyse physicochimique, Université Mentouri, B.P. 325 Route Ain El Bey, Constantine 25017, Algeria*
[c] *Laboratoire de Biologie Moléculaire et Cellulaire, Faculté de Médecine Dentaire de Monastir, Rue Avicenne, 5000 Monastir, Tunisia*

ARTICLE INFO

Article history:
Received 28 April 2011
Accepted 31 August 2011
Available online 6 September 2011

Keywords:
Marrubium
Flavonoids
Phenylethanoids
Labdane diterpenoids
Genotoxicity
Antioxidant

ABSTRACT

In our continual course toward the valorization of traditionally used endemic flora through the analysis of its chemobiodiversity, the phytochemical analysis of aerial parts of *Marrubium deserti* de Noé was undertaken. Dichloromethane and methanol extracts led to the isolation of terpenoid derivatives among which two were new labdane diterpenes named marrulibacetal A and desertine, respectively. Six of them were known compounds (a mixture of the isomers cyllenin A and 15-epi-cyllenin A, marrubiin, marrulactone, marrulibacetal and β-stigmasterol) and seven known phenolic compounds were also isolated: apigenin and several 7-*O*-substituted derivatives (apigenin-7-*O*-β-neohesperidoside, apigenin-7-*O*-glucoside, terniflorin and apigenin-7-*O*-glucuronide) together with two phenylethanoid glucosides (acteoside and forsythoside B). The structures and relative configurations of the new compounds were elucidated by MS and a series of 1D and 2D NMR analyses. Some pure compounds have been evaluated for their antioxidant activities through different methods: DPPH and ABTS assays as well as CUPRAC assay. Genotoxic and antigenotoxic activities of extracts and pure compounds were also evaluated in vitro on *Escherichia coli* PQ37 cells by the SOS Chromotest. Some of the isolated compounds like phenylethanoid derivatives showed stronger antioxidant capacity than trolox and were also able to significantly inhibit β-galactosidase induction caused by the mutagen agent nitrofurantoin.

1. Introduction

The genus *Marrubium* (Lamiaceae) includes about 97 species which are widespread along the Mediterranean Sea and in the temperate regions of the Eurasian zone. Many *Marrubium* species are reported in the literature to be used in folk medicine. For example, aqueous and hydroalcoholic extracts of flowered aerial parts of different *Marrubium* sp. are reported for treating cough and as choleretic in digestive and biliary complaints (Newall et al., 1996; Wichtl and Anton, 1999). Moreover, they are traditionally used for their neurosedative and antiinflammatory activities (Girre, 2000). The best known and first isolated diterpenoid was from *M. vulgare* in 1842 and characterized as marrubiin. This furanic labdane diterpene is in part responsible for therapeutic properties observed for *Marrubium* sp. (Stulzer et al., 2006). The genus *Marrubium* is a rich source of diterpenoids, flavonoids and phenylethanoid glycosides (Takeda et al., 2000; Rigano et al., 2006; Piozzi et al., 2006; Dendougui et al., 2011). *Marrubium deserti* de Noé known as "Djaidi" or "Djaada" in Algeria's desert is endemic. Furthermore, it is frequently used in traditional medicine for treating digestive disorders as antispasmodic, and as local treatment against scorpion stings and allergy (Maiza et al., 1993; Ould El Hadj et al., 2003). The phytochemical investigations of *M. deserti* de Noé are reported here for the first time.

We also present the evaluation of antioxidant activities concerning phenolic compounds isolated from the MeOH extract (**8**–**13**). Oxidative stress has actually been described as a crucial etiological factor implicated in various human chronic diseases such as cancer, cardiovascular and neurodegenerative diseases, inflammation, diabetes mellitus and aging (Mariani et al., 2005; Uttara et al., 2009). This oxidative damage is achieved through the attack of free radicals on various biomolecules, particularly proteins, lipids and DNA, resulting ultimately in cell degradation and death. Plant phenolics have been widely studied for their antioxidant properties since they are able to (i) chelate metal ions involved in Reactive Oxygen Species (ROS) generation or (ii) scavenge free radicals and form stable intermediate structures, thus limiting free radical initiation or propagation (Moon and Shibamoto, 2009; Zheng and Wang, 2001). Recently, much interest has been focused on finding effective antioxidant from natural sources since they

* Corresponding author. Tel.: +33 (0) 478 777 000; fax: +33 (0) 478 777 565.
E-mail address: dijoux@univ-lyon1.fr (M.-G. Dijoux-Franca).

0278-6915/$ - see front matter © 2011 Published by Elsevier Ltd.
doi:10.1016/j.fct.2011.08.026

can be used as nutraceuticals and/or food additives in prevention of cardiovascular diseases for example. Moreover, some of the synthetic drugs largely used as antioxidants in the industry turned out to be cytotoxic (Labrador et al., 2007).

In another way, and in order to bring preliminary studies concerning the plant toxicity and its potential use in therapeutic, interest was given to the evaluation of genotoxic and antigenotoxic activities of phenolic compounds isolated. Actually, several compounds of this class have been demonstrated to have an antimutagenic effect on various mutagens or carcinogens (Nagabhushan and Bhide, 1988; Edenharder et al., 1993; Edenharder and Tang, 1997).

The aim of this study is to bring out some preliminary data concerning the security of use of this medicinal plant through the identification of the major chemical constituents and their biological activities as protective agents against oxidative stresses and antigenotoxic effect, or as potent toxic agents (genotoxicity).

2. Material and methods

2.1. Plant material

Aerial parts from *M. deserti* de Noé were collected in May 2007 in Algeria. They were identified by Prof. Hocine Laouer (Department of Biology, Faculty of sciences, University Ferhat Abbas, Setif, Algeria). A voucher specimen has been deposited at the Herbarium of the Museum d'Histoire Naturelle of Nice (France) (Number B 8991).

2.2. Chemicals

1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azino-*bis*-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and nitrofurantoin (NF) were purchased from Sigma (St. Louis, USA). *O*-nitrophenyl-β-D-galactopyranoside (ONPG), *p*-nitrophenylphosphate (PNPP) and all organic solvents were purchased from Merck (Darmstadt, Germany). Potassium persulfate ($K_2S_2O_8$), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), silica gel (40–63 and 63–200 μm), Sephadex LH20, Diol (40–63 μm) and reversed-phase silica gel C_{18} (40–63 μm) were purchased from Merck (Darmstadt, Germany). All other chemicals were reagent grade. The elucidation and purity of diterpenes, flavonoids and phenylethanoids were determined by TLC, ^{1}H and ^{13}C (1D and 2D) NMR spectroscopy and UV.

2.3. Extraction, fractionation and purification methods

The air dried powdered aerial parts of *M. deserti* de Noé (1.5 kg) were successively extracted with CH_2Cl_2 (DCM) (4 × 6 L) and MeOH (4 × 6 L) at room temperature for 48 h. After concentration under reduced pressure, DCM (30.0 g) and MeOH (150.0 g) extracts were obtained. 15.0 g of the first one was subjected to silica gel VLC with gradient steps using hexane–CH_2Cl_2 (50:50 and 0:100), CH_2Cl_2–EtOAc (90:10, 50:50 and 0:100) and EtOAc–MeOH (50:50 and 0:100) affording nine fractions A1–A9. The fraction eluted with CH_2Cl_2–EtOAc (90:10) A4 (10.0 g) was fractionated by consecutive liquid–liquid extraction. After solubilization in DCM (40 mL), addition of H_2O (100 mL) afforded B1 (44 mg), the second was obtained with MeOH–H_2O (50:50, 100 mL) to obtain B2 (42 mg) and finally B3 (DCM part). B3 (9.0 g) was applied to a Sephadex LH20 column using a gradient [cyclohexane–CH_2Cl_2 (50:50, 25:75 and 0:100), CH_2Cl_2–MeOH (100:0, 50:50 and 0:100)]. 13 fractions were collected (C1–C13). C2 (3.8 g) was subjected to a silica gel VLC using hexane–EtOAc (100:0 to 50:50 with 10% steps) and EtOAc–MeOH (0:100, 50:50 and 0–100) gradients and yielded 14 fractions (D1–D14). Fraction D4 (1.4 g) was applied to a Sephadex LH20 column using cyclohexane–CH_2Cl_2 (80:20, 50:50, 0:100), CH_2Cl_2–MeOH (100:0, 50:50, 0:100) and gave five fractions (E1–E5). E1 (736 mg) was purified on a silica gel column eluting with hexane–CH_2Cl_2 (100:0 to 0:100), CH_2Cl_2–EtOAc (90:10 to 0:100) and EtOAc–MeOH (50:50 to 0:100). Fourteen fractions were obtained (F1–F14). A SPE of F4 (33 mg) over silica gel with CH_2Cl_2 added of a drop of MeOH, permitted to isolate β-stigmasterol **1** (15 mg). F10 and F11 (33 mg) were purified on a silica gel column with CH_2Cl_2–EtOAc (96:4 to 0:100) to give a mixture of the two isomeric compounds **2** and **3**. C3 (1.0 g) was purified on a Sephadex LH20 with 100% CH_2Cl_2 to yield compound **4** (132 mg). Fraction C6 (670 mg) was applied to CC on silica gel with a gradient system [cyclohexane–EtOAc (70:30 to 0:100); EtOAc–MeOH (100:0 to 0:100)] to afford 13 fractions G1–G13. Compound **5** (8 mg) was obtained from G11 (82 mg), after successive RP-18 SPE with MeOH–H_2O (30:70 to 100:0) as eluent, a Sephadex LH20 using CH_2Cl_2–MeOH (100:0; 50:50; 0:100) and another one eluting with cyclohexane–CH_2Cl_2 (100:0; 50:50; 0:100). Fraction C11 (80 mg) was chromatographed by CC over silica gel using a gradient of increasing MeOH in CH_2Cl_2 from 0% to 50% and yielding nine fractions (H1–H9). H4 (23 mg) and H6 (18 mg) were separately investigated on preparative RP-18 TLC using MeOH–H_2O (60:40) to give respectively compounds **6** (6 mg) and **7** (5 mg) on one hand, and compound **8** (5 mg) on the other hand.

The MeOH extract (150.0 g) was submitted to a liquid–liquid extraction between DCM (3 × 1400 mL), EtOAc (3 × 600 mL), BuOH (3 × 500 mL) and H_2O (3 × 500 mL). The resulting phases were concentrated under reduced pressure to give three viscous residues. EtOAc one (6.0 g) was subjected to a Sephadex LH20 column eluting with MeOH–H_2O (9:1) to give 18 fractions (I1–I18). A precipitate occurred in I7 (1.0 g) and 143 mg was obtained after filtration, which was purified by solid phase extraction (SPE) on RP-18 using MeOH in H_2O (10–100%), to give **9** (22 mg). The filtrate (873 mg) was successively submitted to MPLC on RP-18 using a gradient MeOH–H_2O (20:80 to 100:0), a preparative polyamid TLC using (H_2O–MeOH–Methyl Ethyl Cetone–Acetyl acetone) (13-3-3-1), and a flash chromatography on RP-18 with MeOH–H_2O (4:6). It permitted to obtain pure compounds **10** (24 mg) and **11** (21 mg). I9 (30 mg) was chromatographed over silica gel using MeOH in CH_2Cl_2 (10–100%) and yielded compound **12** (15 mg). In I16 (88 mg) also appeared a precipitate which was separated from the supernatant by centrifugation. The precipitate was purified as compound **13** (15 mg). The supernatant (23 mg) purified by SPE on diol with increasing gradient of MeOH in DCM (100:0 to 0:90) afforded apigenin **14** (5 mg).

From the BuOH phase (12.0 g), fractionation on Sephadex LH-20 using MeOH–H_2O (9:1) as eluent conducted to 15 fractions (J1–J15). J15 was purified on a Sephadex LH20 column with MeOH–H_2O (50:50) to yield compound **15** (8 mg).

Marrulibacetal A (**6**): (16s)-(9α-16α), (15α-16α)-dipoxy-13β,14β-dihydroxy-15α-methoxylabdane-6β(19)-olide. Amorphous powder; ^{1}H-NMR and ^{13}C-NMR (see Table 1). $[\alpha]_D = -10.77$ (25 °C). HRESIMS *m/z*: 419.2053 $[M + Na]^+$ (calcd. for $C_{21}H_{32}O_7Na$ 419.2045).

Desertin (**8**): Amorphous powder; ^{1}H-NMR and ^{13}C-NMR (see Table 1). $[\alpha]_D = -56.6$ (25 °C). HRESIMS *m/z*: 451.2300 $[M+Na]^+$ (calcd for $C_{22}H_{36}O_8Na$ 451.230790).

2.4. General experimental procedures

NMR spectra were recorded on a Brucker DRX 500 Spectrometer (500 MHz for ^{1}H and 125 MHz for ^{13}C) in $CDCl_3$ as solvent (internal reference, TMS). ESI-MS were recorded with a Thermo LCQ advantage, ion-trap spectrometer while HR-ESI/MS were recorded with Thermo Finnigan Mat 95 XL. UV spectra were recorded on a Shimadzu UV-1240 spectrophotometer. Vacuum liquid chromatography (VLC): silica gel 60 (40–63 μm) (Merck). Column chromatography (CC): Sephadex LH20; silica gel (60–200 μm) (Merck); Analytical and preparative TLC were carried out using: Merck silica gel Si 60 F_{254} (20 × 20 cm) or RP18 F_{254} (20 × 20 cm) or Lichroprep Diol (40–63 μm) or polyamid 11F_{254} (20 × 20 cm) aluminum sheets. For SPE, plastic syringes were filled with reverse phase, Merck LiChroprep RP-18 60 (40–63 μm),

Table 1
^{1}H and ^{13}C-NMR spectral datas of compounds **6** and **8** (500 and 125 MHz, respectively in $CDCl_3$) (δ multiplicity (*J* in Hz)).

	δ_H		δ_C	
	6	8	6	8
1α	1.96 m	1.65 m	27.8	28.4
1β		1.84 m		
2α	1.72 m	1.74 m	17.9	18.2
2β	1.44 m	1.51 m		
3α	2.12 m	2.12 m	28.2	28.3
3β	2.06 m	1.45 m		
4	–	–	43.9	43.9
5	2.38 d (5.0 Hz)	2.23 d (7.5 Hz)	44.6	45
6	4.78 t (5.0 Hz)	4.73 m	76.6	76.3
7α	2.22 m	2.14 m	32.3	31.6
7β	2.18 m	1.66 m		
8	2.07 m	2.06 m	33.5	32.5
9	–	–	80.3	75.3
10	–	–	40.9	40.1
11	1.77 m	1.29 m	20.7	28.5
12α	2.08 m	1.75 m	30	27.4
12β	1.86 m			
13	–	–	75.8	81.2
14	3.88 s	3.95 bs	78.6	80.2
15	4.93 d (1.5 Hz)	4.89 d (3.5 Hz)	109.8	110.8
16	5.47 s	4.75 s	105.7	108.6
17	1.10 d (6.8 Hz)	0.91 d	19.4	16.8
18	1.28 s	1.29 s	23.2	23
19	–	–	184.1	183.9
20	1.03 s	1.04 s	22	22.2
15-OMe	3.41 s	3.47 s	55.5	56.4
16-OMe	–	3.40 s	–	55.1