Antioxidant activity and chemical constituents of *Warionia saharae* Benth. & Coss. (Compositae) from Algeria

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Abstract: Phytochemical investigations of chloroform and ethyl acetate extracts from the aerial parts of *Warionia saharae* Benth. & Coss. from Algeria resulted in the isolation of ten known compounds: β-sitosterol, stigmasterol, scopoletin, cirsimaritin, chrysoeriol, hispidulin, luteolin, esculetin, (2R,3R) taxifolin, quercetin. Their structures were elucidated on the basis of extensive spectroscopic analysis and comparison with the related known compounds. The EtOAc extract which was evaluated for DPPH radical scavenging activity and of lipid peroxide inhibition showed potent antioxidant ability compared to the quercetin and ascorbic acid used as positive control.

Keywords: Antioxidant activity; Asteraceae; Coumarins; Flavonoids; *Warionia saharae*.

Introduction

The genus *Warionia* which belongs to the Asteraceae family is represented by only one species: *W. saharae* Benth. & Coss. an endemic shrub, growing in the South of Algeria and Morocco (Ozenda 1958). In Algeria, the leaves of *W. saharae* are used in folk medicine for their gastrointestinal properties. In the traditional medicine of Morocco the leaves are used to treat inflammatory diseases, such as rheumatoid arthritis (Bellkhadar 1997).

An earlier phytochemical study on *W. saharae* focused on the main components of the essential oils and led to the isolation of eudesmol, linalool and nerolidol (Ramault et al. 1985). Other investigations of this species, from Morocco, reported the presence of sesquiterpene lactones with guaianolide skeleton-type and their cytotoxic and anti-inflammatory activities (Hilmi et al. 2002; Hilmi et al. 2003).

The present paper describes the phytochemical studies of the chloroform and the ethyl acetate soluble parts of the aqueous-EtOH extract of the aerial parts of an Algerian species.

Material and methods

Plant material

*Warionia saharae* was collected during the flowering phase in April 2005, in the southeast of Algeria, and was identified by Mr. Benabdellahkem (Director of the protection of nature agency, Bechar, Algeria). An authenticated voucher specimen (CWS04/05) was deposited at the Herbarium of the VAREN laboratory of Mentouri University of Constantine.
Antioxidant activity and chemical constituents of Warionia saharae

Reagents

All solvents used were of analytical grade, 1,1-Diphenyl-2-picrylhydrazyl (DPPH°), thiobarbituric acid (TBA), and all other chemicals were of highest purity available quercetin, ascorbic acid, and were prustasched from sigma Aldrich chemical company (St Louis. USA). Column chromatography was carried out using silica gel (230-400 mesh, Merck, Darmstadt, Germany) and TLC was performed using precoated silica gel F254 plates and detection was achieved at 254 and 366 nm, and by spraying with anisaldehyde.

Apparatus

The IR spectra were recorded on a Shimadzu FTIR 8201TC spectrophotometer with KBr discs. The UV spectra were recorded on an Evolution 300 spectrophotometer. 1H and 13C NMR spectra were recorded in CDCl3 and CD2OD at 250 and 62.9 MHz, respectively, with a Bruker DPX 250 spectrometer. Chemical shifts are given in δ (ppm). The coupling constants (J) are in Hertz.

Extraction and separation

Air-dried aerial parts (650 g) of Warionia saharae (Asteraceae) were macerated at room temperature with EtOH–H2O (70:30, v/v) for 24 h, three times. After filtration, the filtrate was concentrated and dissolved in H2O (300 mL). The resulting solution was successively extracted with petroleum ether, CHCl3, EtOAc and n-butanol. The organic phases were dried with Na2SO4, filtered and concentrated in vacuum at room temperature to obtain the following extracts: petroleum ether (5 g), chloroform (13.8 g), EtOAc (11.45 g) and n-butanol (23.75 g).

A part of the chloroform extract (12 g) was chromatographed on a 70-230 mesh silica gel column eluted with a gradient of n-hexane/EtOAc to give 38 fractions obtained by combining the eluates on the basis of TLC analysis. F17 (118 mg, n-hexane/EtOAc, 95:5) was purified with a mixture of n-hexane/Ethyl acetate to give β-sitosterol as a major component 1 (51 mg). F19 (90 mg, n-hexane/EtOAc, 95:5) was purified with a mixture of n-hexane/EtOAc to obtain a mixture (51%, 49%) of β-sitosterol and stigmasterol 2 respectively (58.7 mg). After purification on preparative plates of silica gel (CHCl3/EtOAc/Methanol 5:2:0.5), F30 (85 mg, n-hexane/EtOAc, 58:42) gave scopoletin 3 as white crystals (24 mg).

The ethyl acetate extract was subjected to column chromatography on silica gel (70-230 mesh), eluted with n-hexane/CHCl3/Methanol with increasing polarity, to give 30 fractions. F22 (375 mg, n-hexane/CHCl3/Methanol, 45:50:5) was washed with a mixture of CHCl3/EtOAc to give esculetin 4 as white crystals (28.6 mg). F23 (825 mg, n-hexane/CHCl3/Methanol, 40:50:10) was chromatographed on preparative silica gel 60 GF254 plates (CHCl3/Methanol (9.5:0.5) to give circsimaritin 5 (63.8 mg), chrysoeriol 6 (29 mg), hispidulin 7 (25 mg) and luteolin 8 (19 mg). F26 (634 mg, n-hexane/CHCl3/Methanol, 40:50:10) was subjected to preparative silica gel TLC (CHCl3/Methanol, 9.5:0.5) allowing the isolation of two pure flavonoids (2R,3R)-taxifolin (distylin) 9 (6 mg) and quercetin 10 (11 mg).

The identity of compounds 1-10 was confirmed on the basis of UV, IR and 1H and 13C NMR spectra in comparison with literature data (Lendl et al. 2005; Jung et al. 1990; Kosuke et al. 2006; Hiroki et al. 1984; Renée et al. 2010; Franz et al. 1998; Tsukasa et al. 1999; Ocampo et al. 1994; Agrawal et al. 1980; Sakushima et al. 1983).

Determination of DPPH radical scavenging activity

A solution of 0.2 mM DPPH in methanol was prepared and 1 ml of this solution was mixed with 1 ml of extract in methanol (10 to 150μg/ml). The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. A control sample containing the same volume of solvent in place of extract was used to measure the maximum DPPH absorbance. The absorbance of the mixture was measured spectrophotometrically at 517 nm (Magalhaes et al. 2006). Ascorbic acid and quercetin were used as references. Results were expressed as percentage of inhibition of the DPPH radical according to the following equation:

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% Inhibition of DPPH° = (1 - Absorbance of sample / Absorbance of control) x 100

Inhibition of lipid peroxide (LPO) formation induced by Fe²⁺/ascorbic acid system

The reaction mixture containing rat liver homogenate (0.1 ml, 25% w/v) in Tris-HCl (30 mM), ferrous ammonium sulfate (0.16 mM), ascorbic acid (0.06 mM) and different concentrations of the extract (from 50 to 600 µg/ml) in a final volume of 0.5 ml was incubated for 1h at 37°C and the resulting TBARS was measured (Halliwell and Gutteridge 1990). A 0.4 ml aliquot of the reaction mixture was treated with sodium dodecyl sulfate (0.2 ml, 8.1%), thiobarbituric acid (1.5 ml, 0.8%), and acetic acid (1.5 ml, 20%, pH 3.5), made to a total volume of 4 ml by adding distilled water, and kept in a water bath at 95°C for 1 h. After cooling, 4 ml of n-BuOH was added. After shaking and centrifuging, the organic layer was separated and the absorbance measured at 532 nm (Seeram and Nair 2002). Ascorbic acid and quercetin were used as references.

All assays were carried in triplicates and results expressed as means ± standard deviation.

IC50-value (µg extract/ml) is the effective concentration which proves 50% of activity, was calculated for each assay. Statistical comparisons were done with Student’s test. Differences were considered to be significant at P< 0.05.

Results and discussion

Compounds 1-10 (Figure 1) were isolated from CHCl₃ and EtOAc extracts respectively. The chloroform extract was chromatographed on silica gel column to give β-sitosterol as a major component (1) (Lendl et al. 2005), a mixture of β-sitosterol and stigmasterol (2) (Jung et al. 1990) and scopoletin as white crystals (3) (Kosuke et al. 2006). The ethyl acetate extract was also subjected to column chromatography on silica gel followed by purification on preparative Silica gel plates to give esculetin as white crystals (4) (Hiroki et al. 1984), cirsimaritin (5) (Renée et al. 2010), chrysoeriol (6) (Franz et al. 1998), hispidulin (7) (Tsukasa et al. 1999), luteolin (8) (Agrawal et al. 1980), (2R, 3R)-taxifolin or distylin (9) (Sakushima et al. 1983) and quercetin (10) (Okamura et al. 1994).

Figure 1: Chemical structures of compounds 1–10.

The structures of the compounds were elucidated by UV, IR, EIMS and extensive application of one and two dimensional NMR spectroscopy as well as by comparing their spectroscopic data with those reported in the literature. All these compounds were isolated for the first time from the genus Waronia except hispidulin (Hilmi 2002).

And there is no previous report regarding antioxidant effect of this species. One of the mechanisms to investigate total antioxidant activities is to study the radical scavenging effect based on the following observation: The purple
color of DPPH solution fades quickly when it reacts with radical scavengers (Magalhaes et al. 2006).

![Figure 2: DPPH radical scavenging activities of EtOAc extract of Warionia saharae and standards. Each value represents a mean ± SD (n=3), P<0.05](image)

As depicted in Figure 3, The EtOAc extract of Warionia saharae (92 %) exerted a good protection via reducing thiobarbituric reacting substance (TBARS) generated by Fe²⁺/ascorbic acid system at concentration (400 µg/mL) in a dose-dependent manner. The EtOAc extract (IC₅₀ = 27.9 ± 0.6) was found to be a better inhibitor of lipid peroxide compared with the reference standards of ascorbic acid (IC₅₀ = 75.4 ± 2.1) and of quercetin (IC₅₀ = 32.7 ± 1.3).

The phenolic compounds influence the lipid peroxidation (LPO) process through either a simple or complex mechanism including free radical scavenging divalent metal chelating and so on (Seeram and Nair 2002). The iron ion dependent LPO will be affected by phenolic compounds capacity of metal chelating. The inhibitory effect by some flavones and flavonols possessing either an ortho-dihydroxy (catechol moiety), 3- or 5-hydroxy groups was ascribed to this mechanism (Pietta 2000). Since the products 5, 6, 7, 8, 10 possess both a carbonyl chelated hydroxyl and for 8 and 10 an ortho-dihydroxy group, a part of their antiperoxidative action could be based on the same mechanism (Valko et al. 2006).

Moreover, more the phenolic compounds contain free hydroxyls, more free radical scavenging activity increases (Rice-Evans et al. 1996).

This fact was confirmed by other authors suggesting that the phenolic compounds concentration is not the only factor determining the antioxidant activity. The antioxidant capacity and the antiperoxidative action could be affected by the interaction and synergy of the compounds present in the extract (Heo et al. 2007).

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Antioxidant activity and chemical constituents of Warionia saharae


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