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Fitoterapia 75 (2004) 162–167

FITOTERAPIA

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Monitoring the antioxidant activity of extracts originated from various *Serratula* species and isolation of flavonoids from *Serratula coronata*

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Received 15 July 2003; accepted 9 December 2003

Abstract

The antioxidant effect of aqueous methanolic herb extracts of *Serratula coronata*, *S. wolffii* and *S. tinctoria* was investigated using both enzyme-dependent and enzyme-independent systems. The extracts displayed concentration-dependent inhibition of lipid peroxidation. Flavonoids and ecdysteroids present in the extracts were evaluated as antioxidant components. The flavonoid-containing fraction of the herb extract of *S. coronata* was more effective in lipid peroxidation than the ecdysteroid-containing fraction. This paper also reports the isolation of quercetin 3-*O*-methyl ether, apigenin, luteolin, quercetin, luteolin 4′β-D-glucoside and quercetin 4′β-D-glucoside from *S. coronata*.

Keywords: *Serratula coronata*; *Serratula* spp.; Antioxidant activity; Flavonoid

1. Introduction

A great number of ecdysteroid containing whole plant preparations have been commercially available as dietary supplements, biostimulators, and OTC preparations

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doi:10.1016/j.fitote.2003.12.009

[1,2]. These products are used for their anabolic, adaptogenic, hypocholesterolemic and neuroprotective effects, attributed to their ecdysteroid content.

The *Serratula* species synthesize a wide spectrum and high level of ecdysteroids and large amounts of flavonoids and are also sources of herbal remedies or food supplements [3]. The ecdysteroid composition and the 20-hydroxyecdysone content of certain *Serratula* species are described in our previous works [4,5]. However, nothing was published on the flavonoidal constituents of *Serratula* species.

Both the flavonoids and ecdysteroids have antioxidant activity and may be defined as active ingredients of *Serratula* preparation. The protective effect of flavonoids is attributed to their oxygen radical scavenging and enzyme inhibitory capabilities [6,7]. The kinetics of their chemiluminescence parameters revealed that ecdysteroids also terminate the free radical-mediated oxidation of lipids in vitro. Especially, 20-hydroxyecdysone (the main ecdysteroid) was found to be an effective lipid peroxidation (LPO) inhibitor in D-hypovitaminosis [8]. 20-Hydroxyecdysone acts by virtue of its iron-chelating or ion-reducing properties; therefore it interferes with the active iron(III) form of lipoxygenases [9].

The aim of the present study is to evaluate the antioxidant capacity of the herb extracts of *Serratula coronata*, *S. wolffii* and *S. tinctoria*, and the antioxidant effects of the flavonoid- and ecdysteroid-containing fractions of *S. coronata*. We also report here the isolation of the main flavonoids from *S. coronata*.

2. Experimental

2.1. Plant materials

The aerial parts of *S. coronata* L., *S. wolffii* Andrae and *S. tinctoria* L. (Caryophyllaceae) were collected in May 1996 in the vicinity of Budapest, Hungary. Voucher specimens (*S. coronata* S91, *S. wolffii* S94 and *S. tinctoria* S95) have been deposited in the Herbarium of the University of Horticulture, Budapest, Hungary.

2.2. Extraction for measurement of the antioxidant effect

The air-dried, crushed and powdered plant materials (5 g) were extracted with 50% MeOH in an ultrasonic bath. The extracts yielded residues of approximately 30% for *S. coronata*, 28% for *S. wolffii*, 33.6% for *S. tinctoria*.

2.3. Measurements of antioxidant activities

The enzyme-independent LPO was assayed on a standard ox-brain homogenate, and the enzyme-dependent LPO on rat liver microsomes [10,11]. In vitro experiments were conducted in duplicate and means were calculated. No error was computed; the differences between the two samples were within approximately 1%. Saturation curves were fitted to the measurement data and IC_{50} values (the correlation at which 50% of the maximum LPO inhibition is exerted) were calculated by means of the computer program GraphPad Prism 2.01.

2.4. Determination of flavonoid content

The total flavonoid content in the EtOAc-soluble fraction of each 50% MeOH extract was determined by the aluminium chloride method described in DAB 10. Each sample was analyzed three times, and a calibration graph with three points for quercetin was used. The amounts of flavonoids were expressed as quercetin (g) per 100 g of dry extract. For accuracy in the determination of the flavonoid content the *Serratula* extracts were spiked with 1 and 2 mg/ml of quercetin solution and the recovery was calculated.

2.5. Isolation of flavonoids

Dry aerial parts (118 g) of *S. coronata* were collected and extracted with 4 l of 50% MeOH using an ultrasonic bath. The extract was concentrated in vacuum to give a residue (28.18 g), which was dissolved with the aid of 140 ml of MeOH and 70 ml of acetone were added. The precipitate was filtered and washed with 3×50 ml of MeOH:acetone (1:1 v/v) solution. The filtrate and the washing solution were combined and evaporated to dryness. The residue (19.4 g) was re-dissolved in MeOH and the precipitation procedure was repeated two times [5]. The prepurified dry extract (13.9 g) was dissolved in 50 ml 50% MeOH and further purified by solvent–solvent distribution with benzene. The layer was concentrated in vacuum to give a dry residue (11.4 g) that was dissolved in MeOH and evaporated into 20 g polyamide. The polyamide with the adsorbed material was suspended in H_2O, and topped on a polyamide column (230 g, 55×8 cm). The column was eluted with H_2O (100 ml), H_2O–MeOH (8:2, 200 ml), and with MeOH (300 ml). Elution with MeOH resulted in a solution containing the crude flavonoids (4.89 g). 0.5 g of the 4.89 g flavonoid-containing fraction was dissolved in MeOH, adsorbed into 1 g polyamide and further separated on the 2nd polyamide column (10 g polyamide, 33×2 cm). EtOAc (60 ml), EtOAc:MeOH 95:5 (470 ml), 92:8 (360 ml), 85:15 (410 ml), 7:3 (70 ml), 1:1 (190 ml) were used for elution. Fractions were collected, 10 ml each.

Fractions 30–68 (0.17 g) were combined, concentrated in vacuum, dissolved in MeOH and further purified on Sephadex LH-20 column (1×53 cm). The elution was made with MeOH (1 ml fractions). Fractions 30–41 of Sephadex LH-20 column (0.06 g) were separated by PTLC on polyamide using CH_2Cl_2:MeOH 8:2. Bands with R_f 0.32 and R_f 0.16 were scraped and the flavonoids eluted with MeOH to give compound **1** (12 mg) and compound **2** (13 mg), respectively. Compound **3** (17 mg) and **4** (31 mg) were directly obtained from the 45–62 (24 mg) and 67–85 (41 mg) fractions of the Sephadex LH-20 column by crystallization.

Fractions 114–131 from the polyamide column (0.12 g) were combined, purified on Sephadex LH-20 (2×39 cm, eluted with MeOH, collected in 1 ml fractions). Chromatography on Sephadex LH-20 column resulted in compounds **5** (2 mg) and **6** (3 mg) from fractions 23–25 and 31–37, respectively.

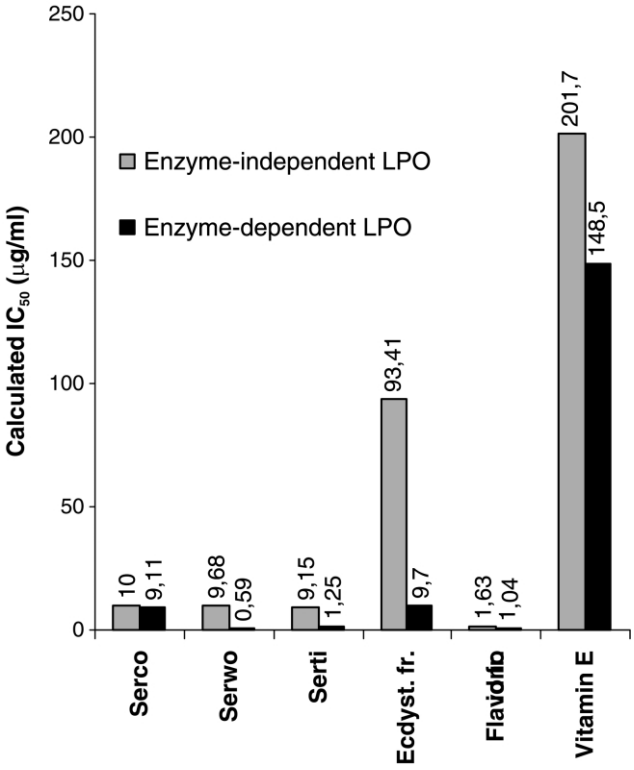

Fig. 1. Antioxidant activities of plant extracts[a], ecdysteroid and flavonoid-containing fractions of *S. coronata*, Vitamine E[b]. ([a]Serco: *S. coronata*; Serwo: *S. wolffii*; Serti: *S. tinctoria*, [b]Vitamine E = α-Tocopheryl succinate).

The structure elucidation of compounds **1**–**6** was performed by ^{1}H-NMR, ^{13}C-NMR, MS, IR and UV spectroscopy. The spectroscopic data of the isolated flavonoids agreed with the literature [12,13].

3. Results and discussion

The 50% aqueous methanolic extracts of the three *Serratula* species demonstrated the dose-dependent inhibition of LPO (Fig. 1). They were tested against the auto-oxidation of a standard ox-brain homogenate and against NADPH-dependent LPO in rat liver microsomes. Each of the extracts was found to be more effective in both tests than α-tocopherol acid succinate, applied as a positive control. All three extracts exhibited somewhat more potent effects in the enzyme-dependent test than in the enzyme-independent test, indicating the moderate direct enzyme-inhibitory activity of the extract, as a component of the total antioxidant effect.

The total flavonoid content of the extract of *Serratula* species was determined by means of spectrophotometry (Table 1). The quantification was controlled by the use

Table 1
Determination of the total flavonoid-content of *S. coronata*, *S. wolffii* and *S. tinctoria* by spectrophotometry

Quercetin (mg/ml)*	Total flavonoids (mg/ml)		
	S. coronata	*S. tinctoria*	*S. wolffii*
0	0.718	0.579	0.771
1	1.761	1.561	1.821
2	2.754	2.548	2.819
Average recovery (%)	101.95	98.82	102.19
Total flavonoid content (%)	0.90	0.73	0.95
R.S.D. (%)	1.99	1.56	1.48

$n = 3$. R.S.D. = Relative standard deviation.
* Added as standard.

of peak addition methods. Determining recoveries from pre-analyzed solution, spiked with two different amounts of quercetin, validate the accuracy of the method. Comparison of the results of the samples and spiked samples yielded an accuracy of 98–102%.

To differentiate the LPO-inhibitory activities originating from either the ecdysteroids or the flavonoids of *S. coronata*, the extract was separated. The separation was based on the difference in the absorption/elution characteristics of ecdysteroids and flavonoids on polyamide [5]. Separation takes place after prepurification of the crude extract. The prepurification includes fractionate precipitation and solvent–solvent distribution. The prepurified extract was adsorbed on polyamide and the ecdysteroids were eluted through the successive applications of water and 20% aqueous methanol. The flavonoids remained on the polyamide, and were next eluted with 100% methanol.

The LPO-inhibitory activities of the flavonoid- and ecdysteroid-containing fractions are depicted in Fig. 1. The ecdysteroid-containing fraction displayed an approximately 10-fold higher LPO-inhibitory activity in the enzyme-dependent than in the enzyme-independent system. The calculated IC_{50} values of the flavonoid-containing fraction were approximately 60 and 10-fold lower than those of the ecdysteroids in the enzyme-independent and enzyme-dependent systems, respectively.

To establish which flavonoids are responsible for the powerful LPO-inhibitory activity, six flavonoids were isolated: quercetin3-methyl ether (**1**), apigenin (**2**), luteolin (**3**), quercetin (**4**), luteolin4′β-D-glucoside (**5**) and quercetin4′β-D-glucoside (**6**). The antioxidant effects of these flavonoids are direct consequences of their chemical structures [7]. Three of them (**1**, **3**, **4**) contain vicinal hydroxy groups, and in all of them a 2,3-double bond is conjugated to a 4-oxo group. An additional hydroxy group on heterocycle (**4** and **6**) enhances the antioxidant capacity. Among the isolated flavonoids, **4** has earlier been found to exert inhibitory effects on non-enzymatic hydroxyl radical formation, iron-ion-dependent LPO, and ascorbate-

induced non-enzymatic LPO. Compound **3** inhibits NADH-oxidase and the oxidation of succinic acid [1]. Antioxidant effects of **2** and **5** have also been described [7].

The LPO-inhibitory activities of the ecdysteroids are moderate, and more expressed in the enzyme-dependent than in enzyme-independent system. The activity may be connected to the direct enzyme-inhibitory effect rather than to the free radical-scavenging activity of any specific part of the chemical structure of the ecdysteroids. The common presence of the flavonoids and ecdysteroids in the extract may lead to a potentiating effect in the enzyme-independent LPO system, or other types of compounds may affect the activities of the extract.

The ecdysteroid content of *S. coronata* is about twice that of the flavonoid content. The majority of the LPO activity of this species is, therefore attributed to its flavonoids. The powerful antioxidant effects of the *S. coronata* extract might also make an important contribution to the uses of the products made of this species. The results of further investigations will demonstrate the contributions of the individual flavonoids to the overall effect.

Acknowledgments

This project was supported by Hungarian National Research Fund (OTKA T032618, T035054).

References

[1] Opletal L, Sovová M, Dittrich M, Solich P, Dvorák J, Krátky F, Cerovsky J, Hofbauer J. Ceska Slovenská Farmacie 1997;46:247.
[2] Lafont R, Dinan L. J Insect Sci 2003;3.7:1.
[3] Kholodova YuD. Ukrainskii Biokhimicheskii Zhurnal 2001;73:21.
[4] Báthori M, Máthé I, Csikkel-Szolnoki A. ACE 1998;1:13.
[5] Báthori M, Máthé I, Praszna L, Rischák K, Kalász H. J Planar Chromatogr 1996;9:264.
[6] Harborne JB, Williams CA. Phytochemistry 2000;55:481.
[7] Pietta PG. J Nat Prod 2000;63:1035.
[8] Kuzmenko A, Morozova RP, Nikolenko IA, Koniets GV, Kolodova YuD. Biochemistry (Moscow) 1997;62(6):609.
[9] Osynskaia LP, Saad LM, Kholodova YD. Ukr Biokhim Zh 1992;64(1):114.
[10] Stocks J, Gutteridge JMC, Sharp RJ, Dormandy TL. Clin Sci Mol Med 1974;47:215.
[11] Engineer FN, Sridhar R. Biochem Biophys Res Commun 1991;179:1101.
[12] Mabry TJ, Markham KR, Thomas MB. Systematic identification of flavonoids. Berlin, Heidelberg, New York: Springer, 1970. p. 28.
[13] Harborne JB. The flavonoids advances in research since 1986. London, Glasgow, New York: Chapman and Hall, 1994. p. 448, 463.