ORIGINAL ARTICLE

CHEMICAL, ANTIOXIDANT AND ANTIBACTERIAL STUDIES OF ROMANIAN *HERACLEUM SPHONDYLIUM*

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Abstract

In order to obtain a chemical and biological characterization of *Heracleum sphondylium* subsp. *sphondylium*, we proposed the qualitative and quantitative analysis of polyphenolic compounds from roots, stems, leaves, flowers and fruits, as well as the antioxidant and antibacterial potential evaluation. For the identification and quantification of the phenolic compounds, chromatographic and spectrophotometric methods were employed. The antioxidant activity was evaluated using DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging, FRAP (ferric reducing antioxidant power) and EPR (electron paramagnetic resonance) methods. The antibacterial test was performed by means of agar diffusimetric method. The HPLC (high performance liquid chromatography) phenolic profile has revealed high amounts of rutin in flowers (983.88 mg/100 g) and in leaves (477.08 mg/100 g), other flavonoids: quercitrin (15.60 mg/100 g leaves), quercetin (13.38 mg/100 g flowers) and phenol acids: ferulic acid (13.04 mg/100 g) and chlorogenic acid (4.32 mg/100 g) in roots. The flowers and leaves extracts showed the highest antioxidant capacity, according to the phenolic content. The antimicrobial tests revealed a good inhibitory activity against *S. aureus* and *L. monocytogenes* for all samples. The results of the present investigation showed both qualitative and quantitative differences between the parts of *H. sphondylium,* so it is recommended to use in practice the extract obtained from a specific organ that furnishes the higher yield of active constituents.

Rezumat

În vederea caracterizării chimice şi biologice a speciei *Heracleum sphondylium* subsp. *sphondylium*, ne-am propus analiza calitativă şi cantitativă a compuşilor polifenolici din rădăcini, tulpini, frunze, flori şi fructe, precum şi evaluarea potenţialului lor antioxidant şi antibacterian. Identificarea şi dozarea compuşilor fenolici s-a realizat prin metode cromatografice şi spectrofotometrice. Activitatea antioxidantă a fost evaluată prin metodele DPPH (2,2-difenil-1-picrilhidrazil), FRAP (activitatea antioxidantă totală prin reducerea fierului) şi EPR (rezonanţa electronică paramagnetică), iar cea antibacteriană prin metoda difuzimetrică. Profilul fenolic a pus în evidenţă cantităţi mari de rutozidă în flori (983,88 mg/100 g) şi în frunze (477,08 mg/100 g), dar şi alte flavonoide: quercitrină în frunze (15,60 mg/100 g), quercetol în flori (13,38 mg /100 g) şi acizi fenolici: acid ferulic (13.04 mg/100 g) şi acid clorogenic (4.32 mg/100 g) în rădăcini. Extractele obţinute din flori şi frunze au avut cea mai mare capacitate antioxidantă, în concordanţă cu conţinutul în compuşi fenolici. Testele antimicrobiene au evidenţiat o activitate antibacteriană bună pe *S. aureus* şi *L. monocytogenes,* pentru toate probele. Rezultatele acestui studiu au arătat diferenţe calitative şi cantitative semnificative între cele cinci produse naturale medicinale, care stau la baza unei utilizări corecte în practică a extractelor obţinute din organele care conţin cele mai multe principii active.

Keywords: *Heracleum sphondylium*, plant parts, polyphenols, antioxidant, antibacterial

Introduction

The genus *Heracleum* L. from the *Apiaceae* family contains 3 species, 3 subspecies and a hybrid, spread in Romania. Among them, *Heracleum sphondylium* L. (hogweed) is an herbaceous plant with stem hollow, leaves pinnate, often with 5 broad, lobed and toothed segments, upper leaves with large inflated bases, white flowers, 5 to 10 mm in large umbels up to 15 cm across with 12 to 25 rays and petals of outer flowers very unequal [1, 2, 13]. The roots and aerial parts are widely used in traditional food and medicine for its tonic, aphrodisiac, vaso-dilator, antihypertensive, sedative, digestive properties, in the treatment of digestive disorders (dyspepsia, diarrhoea), hypertension, healing wounds, menstrual problems [1, 2]. In Romania, the extract of aerial parts is reputed to be aphrodisiac and antihypertensive

and to treat the male and female sterility, impotence, frigidity, hypertension etc. [14]. Modern biological studies have shown that *H. sphondylium* has a vaso-relexant action, *H. sphondylium* subsp. *artvinense* is antimicrobial, *H. sphondylium* subsp. *ternatum* is antioxidant, anti-bacterial, cytotoxic and the extract of *H. sphondylium* subsp. *sphondylium* upper aerial part revealed anti-oxidant, antifungal and germination inhibitory activity [3-5, 9-11, 14]. Phytochemical investigations on this species showed the predominance of furocoumarins (bergapten, isopimpinellin, heraclenin,) in fruits, seeds and roots and the presence of essential oil (with octyl acetate, octyl butanoate, *n*-octanol, apiol, *ar*-curcumene) [8-12, 14].

The present studies carried out on roots, stems, leaves, flowers and fruits harvested from Romanian (north-west area) *H. sphondylium* subsp. *sphondylium* are the first report in terms of the phenolic chemical composition and the evaluation of the biological potential for possible therapeutic applications in modern medicine.

Materials and Methods

Plant materials consisted of roots, stems, leaves, flowers and fruits of *H. sphondylium* subsp. *sphondylium* (Voucher No. 37), identified and harvested in 2015 from the spontaneous flora from Ciucea (Bihor, Romania) by Prof. Dr. biologist Mircea Tămaş. The samples preparation: 5 g of plant material powder were extracted with 50 mL of 70% ethanol (Merck), for 30 min on water bath, at 60ºC. The samples were then cooled down and centrifuged at 4500 rpm for 15 min. and the supernatants were recovered [6, 7, 16].

Chemicals: All necessary substances were purchased from Sigma-Aldrich (Steinheim, Germany), Merck (Darmstadt, Germany) and Alfa-Aesar (Karlsruhe, Germany).

HPLC analysis. HPLC-MS analysis was performed using the chromatographic conditions previously described [6, 7, 16]. Quantitative determinations were performed using an external standard method. The polyphenolic compounds of the extracts were identified based on their retention time, UV and MS spectra as compared to the standards. Calibration curves in the 0.5 - 50 mg/mL range with good linearity ($R^2 > 0.999$) for a five points plot were used to determine the concentration of polyphenols.

Determination of polyphenolic compounds. Quantitative determinations of total polyphenols (TPC), flavonoids and caffeic acid derivatives were carried out using spectrophotometric methods. Gallic acid, rutin and caffeic acid reagents were used as standards [6, 7, 16].

Evaluation of the antioxidant activity. The extracts were screened for antioxidant activity using three *in vitro* assay models, the DPPH (2,2-diphenyl-1-picrylhydrazyl), FRAP (ferric reducing/antioxidants power) and EPR (electron paramagnetic resonance) assays [6, 7, 15, 16]. The DPPH· scavenging activity assay is based on the spectrophotometric measurement of the DPPH concentration change, resulting from the reaction with an antioxidant. The FRAP method is based on the change in the colour of a complex with iron (III) ion of the 2,4,6-tri(2-pyridyl)-1,3,5-triazine (TPTZ) radical, due to the reduction of the ferric ion to the ferrous iron in this complex. As antioxidant standard Trolox was used. An absorbance curve was built in function of Trolox mass, the correlation coefficient (R^2) for this curve being 0.992. The final results were converted to µM Trolox equivalents/100 mL extract. EPR measurements for the DPPH test have been made on a Bruker EPR spectrometer which is equipped with X-band (9.54 GHz) Microwave Bridge; EPR spectra have been registered at various time points. The relative concentration changes of the para-magnetic species have been achieved with double integration of the spectra (Integral intensity) using X EPR software [6, 7].

Determination of the antibacterial activity

The extracts were investigated concerning the activity on the most common human pathogenic microbial strains: *S. aureus* (ATCC 49444), *L. monocytogenes* (ATCC 13076), *S. typhimurium* (ATCC 14028), *E.coli* (ATCC 25922), using a disc-diffusion assay previously described [16].

Results and Discussion

The concentrations of the identified polyphenolic compounds by HPLC method in all the five analysed samples are presented in Table I and the HPLC chromatograms are shown in Figures 1 and 2. Gentisic acid was identified only in *H. sphondylium* flowers while the caffeic acid was also found in leaves and fruits. Chlorogenic acid was identified in all the parts of the plant, but it was quantified only in the roots and stems extracts (about 4.5 mg/100 g). The *p*-coumaric acid was found in roots, leaves, flowers and fruits extracts, but in small quantities. Ferulic acid was present in flowers and leaves and in higher quantity in roots (13.04 mg/100 g). Three flavonoid glycosides (isoquercitrin, rutin, quercitrin) were found in high concentrations in leaves and flowers (eg. Iso-quercitrin: 14.37 mg/100 g flowers and 15.60 mg/ 100 g leaves; quercitrin 15.60 mg/100 g leaves). The levels of rutin in the five samples have dropped in the order: flowers > leaves > stems > fruits > roots, the flowers extracts being the richest in rutin (983.88 mg/100 g). Four flavonoid aglycones (luteolin, quercetin, kaempferol and apigenin) were identified, but only quercetin was present in all samples, the flowers containing the greatest amount (13.38 mg/ 100 g). Our results showed that the

extracts of leaves, flowers and fruits are rich in flavonoids, especially rutin, whereas the extract of roots contains phenol acids, especially ferulic acid.

Table I

Phenolic compounds in different organs of *H. sphondylium* (mg polyphenols/100 g plant material)

Phenolic compounds	RT ± SD (min)	H. roots	H. stems	H. leaves	H. flowers	H. fruits
Caffeic acid	5.60 ± 0.04			< 0.2	< 0.2	< 0.2
Chlorogenic acid	5.62 ± 0.05	4.32 ± 1.12	4.70 ± 0.20	< 0.2	< 0.2	< 0.2
Gentisic acid	6.52 ± 0.04				< 0.2	
p-Coumaric acid	9.48 ± 0.08	1.22 ± 0.04		< 0.2	2.06 ± 0.23	0.38 ± 0.04
Ferulic acid	12.8 ± 0.10	13.04 ± 0.95		< 0.2	1.06 ± 0.15	
Isoquercitrin	19.60 ± 0.10		0.81 ± 0.13	15.60 ± 0.59	14.37 ± 0.62	
Rutin	20.20 ± 0.15	0.11 ± 0.02	40.79 ± 3.20	477.08 ± 7.93	983.88 ± 15.11	11.99 ± 0.65
Quercitrin	23.64 ± 0.13			15.60 ± 2.39	0.92 ± 0.07	
Quercetin	26.80 ± 0.15	< 0.2	< 0.2	0.61 ± 0.08	13.38 ± 1.11	0.11 ± 0.03
Luteolin	29.10 ± 0.19					0.88 ± 0.11
Kaempferol	32.48 ± 0.17				1.27 ± 0.06	
Apigenin	33.10 ± 0.15				< 0.2	

Notes: H, *Heracleum*; SD, standard deviation. Values are the mean ± SD (n = 3).

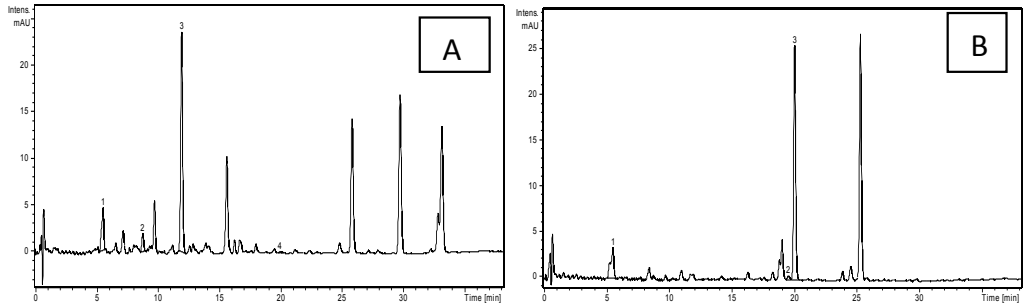

Figure 1.

HPLC chromatograms of *H. sphondylium* roots (A) and stems (B)

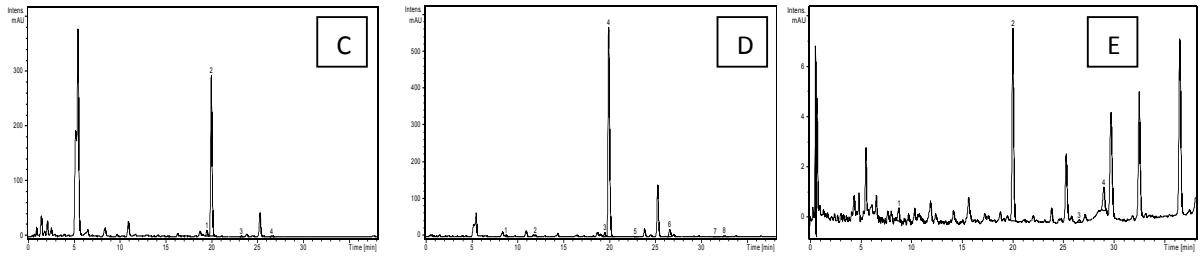

Figure 2.

HPLC chromatograms of *H. sphondylium* leaves (C), flowers (D) and fruits (E)

Table II

Polyphenolic contents and antioxidant activity of *H. sphondylium* extracts

***Heracleum* samples**	**TPC (g GAE/100 g)**	**Flavonoids (g RE/100 g)**	**Caffeic acid derivatives (g CAE/100 g)**	**DPPH IC_{50} µg/mL)**	**FRAP µM Trolox/100 mL**	**EPR Integral intensity**
roots (H_1)	0.56 ± 0.08	0.02 ± 0.005	-	> 200	153 ± 7.00	115 ± 5
stems (H_2)	0.18 ± 0.01	0.10 ± 0.01	-	> 200	63 ± 6.00	113 ± 7
leaves (H_3)	3.48 ± 0.51	1.03 ± 0.06	2.26 ± 0.23	116.22 ± 2.78	1128 ± 12.00	101 ± 9
flowers (H_4)	3.32 ± 0.27	2.84 ± 0.35	0.43 ± 0.05	168.94 ± 6.05	710 ± 9.00	59 ± 4
fruits (H_5)	0.69 ± 0.10	-	0.26 ± 0.03	> 200	48 ± 2.00	104 ± 6
Trolox	-	-	-	11.20 ± 0.20	2073.91 ± 14.09	
DPPH						543 ± 37

Notes: Each value is the mean ± SD of three independent measurements. GAE, RE, CAE: gallic acid, rutin, caffeic acid equivalents; IC_{50}: half maximal inhibitory concentration.

The levels of TPC in the five samples decreased in the order: leaves > flowers > fruits > roots > stems. The flowers contain a large amount of flavonoids (2.84%). The highest level of caffeic acid derivatives

was found in leaves (2.26%), while in roots and stems were not found. Similar results to ours were obtained for the aerial parts of *H. sphondylium* from Serbia [11].

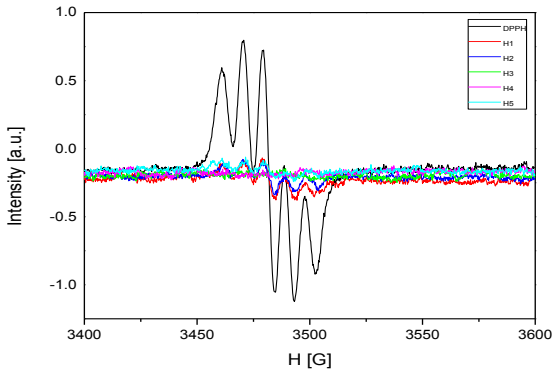

Figure 3.
EPR - the rate of interaction between the extracts and DPPH radical

The antioxidant potential was determined by three methods. The antioxidant properties values obtained by DPPH and FRAP revealed a great antioxidant activity of the tested leaves and flowers extracts, while the roots, stems and fruits extracts revealed a weak antioxidant capacity (IC_{50} > 200 µg/mL). Other authors reported a good antioxidant activity of *H. sphondylium* aerial parts extract (Piteşti, Romania) [5]. Concerning the EPR method, as expected, the integral intensity of DPPH is notably reduced by the antioxidant extracts with the integral intensity values of the five extracts (Table II, Figure 3). Thus the flowers extract exhibited an antioxidant effect by two times higher than the other samples. The EPR measurement are in line with the DPPH and FRAP assays results.

The results of the *in vitro* activity of *H. sphondylium* extracts against bacteria are summarized in Table III. The results showed a variation in the antimicrobial properties of the five extracts. Gram-negative bacteria tested did not show any sensitivity to all extracts (inhibition diameter - 6 mm), while all samples demonstrated some activity against gram-positive strains. The most pronounced activity on *S. aureus* (inhibition diameter - 18 mm) was shown by the leaves and flowers extracts, quite similar with the gentamicin. The roots and fruits extracts showed a moderate antibacterial activity against *L. monocytogenes* (roots) and *S. aureus* (fruit) and low activity against the other tested bacterial pathogens. The results of the present investigation suggested that the extracts obtained from different parts of *H. sphondylium,* the leaves and flowers extracts especially, exhibited activity against Gram-positive bacteria.

Table III
Antibacterial activity of *H. sphondylium* extracts

***Heracleum* samples**	**Zone of inhibition (mm)**			
	Staphylococcus aureus	*Listeria monocytogenes*	*Escherichia coli*	*Salmonella typhimurium*
roots	10 ± 1.50	16 ± 1.00	6 ± 0.00	6 ± 0.00
stems	8 ± 0.50	12 ± 0.50	6 ± 0.00	6 ± 0.00
leaves	18 ± 2.00	12 ± 0.30	6 ± 0.00	6 ± 0.00
flowers	18 ± 0.00	14 ± 0.10	6 ± 0.00	6 ± 0.00
fruits	16 ± 0.02	10 ± 0.00	6 ± 0.00	6 ± 0.00
Gentamicin	19 ± 0.60	18 ± 1.00	22 ± 0.50	18 ± 0.00

Each value is the mean ± SD of four independent measurements. Gentamicin (10 µg/well) was used as positive control.

Conclusions

In the present investigation, it was determined the phenolic composition, the antioxidant and anti-bacterial activities for Romanian *Heracleum sphondylium* subsp. *Sphondylium,* with the aim of better phyto-chemical and biological assessments. The comparative phytochemical study on roots, stems, leaves, flowers and fruits, showed large qualitative and quantitative differences between the organs of the plant. The leaves and flowers extracts were rich in active principles (TPC, flavonoids and caffeic acid derivatives). The antioxidant activity evaluated by the DPPH, FRAP and EPR methods indicated that the leaves and flowers extracts were more anti-oxidant, related with the polyphenolic total content. Concerning the antibacterial effect, *H. sphondylium* aerial parts extracts (leaves, flowers) could be use as agents especially against *S. aureus* strains. Therefore, our results highlight that the leaves and flowers of *H. sphondylium* subsp. *sphondylium* may be used as a source of antioxidant flavonoids, as well as antibacterial agents in pharmaceuticals and food chains.

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