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COMPARATIVE STUDY OF THE SEED EXTRACTS OF *Apiaceae* PLANTS IN ULTRASONIC CONDITIONS

BADANIA PORÓWNAWCZE EKSTRAKTÓW Z NASION ROŚLIN Z RODZINY *Apiaceae* W WARUNKACH ULTRADŹWIĘKOWYCH

Abstract: There are many bioactive compounds with known effects on microorganisms (fungi, bacteria, viruses) in plants of the *Apiaceae* family. The properties of selected extracts obtained with classic method, and their antifungal activities were described in previous works. In this work, the results of a study concerning the properties of the following seed extracts are described: parsley (*Petroselinum crispum*), common hogweed (*Heracleum sphondylium*), hogweed Sosnowsky (*Heracleum Sosnowsky*), ground-elder (*Aegopodium podagraria*), dill (*Anethum graveolens*), lowage (*Levisticum officinale*) and cow parsley (*Anthriscus sylvestris*). These extracts were obtained with the use of ethyl acetate as an extractant, at room temperature in ultrasonic bath. The analysis of the extracts was carried out on GC-MS an Agilent 6890N gas chromatograph. Antioxidant content in the extracts was measured with two different methods: with the use of ascorbic acid or linoleic acid in the first method and *N,N*-dimethyl-*p*-phenylenediamine (DMPD) in the second method. The obtained results showed that the extracts obtained with the proposed method contain more native agents so their biological activity should be higher.

Keywords: *Apiaceae*, seeds extracts, antioxidant activity, DMPD

There are many bioactive compounds with known effects on microorganisms (fungi, bacteria, viruses) in plants of the *Apiaceae* family. The properties of selected extracts obtained with classic method, and their antifungal activity (to *Fusarium culmorum, Botrytis cinerea* and *Alternaria alternata*) were described in previous works [1]. In this work, the results of a study concerning the properties of the following seed extracts are described: parsley (*Petroselinum crispum*), common hogweed (*Heracleum sphon-*

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dylium), hogweed Sosnowsky (*Heracleum Sosnowsky*), ground-elder (*Aegopodium podagraria*), dill (*Anethum graveolens*), lowage (*Levisticum officinale*) and cow parsley (*Anthriscus sylvestris*).

Materials and methods

All the chemicals used were of the analytical grade. The *N,N*-dimethyl-*p*-phenylenediamine dihydrochloride (DMPD), ascorbic acid, linoleic acid, pyrogallol and all analytical grade solvents, were purchased from Sigma Aldrich. Iron(II) chloride was purchased from Fluka Chemical Co.

The dry and ripe seeds were collected in summer 2008. Plant material was ground to powder in order to obtain extracts.

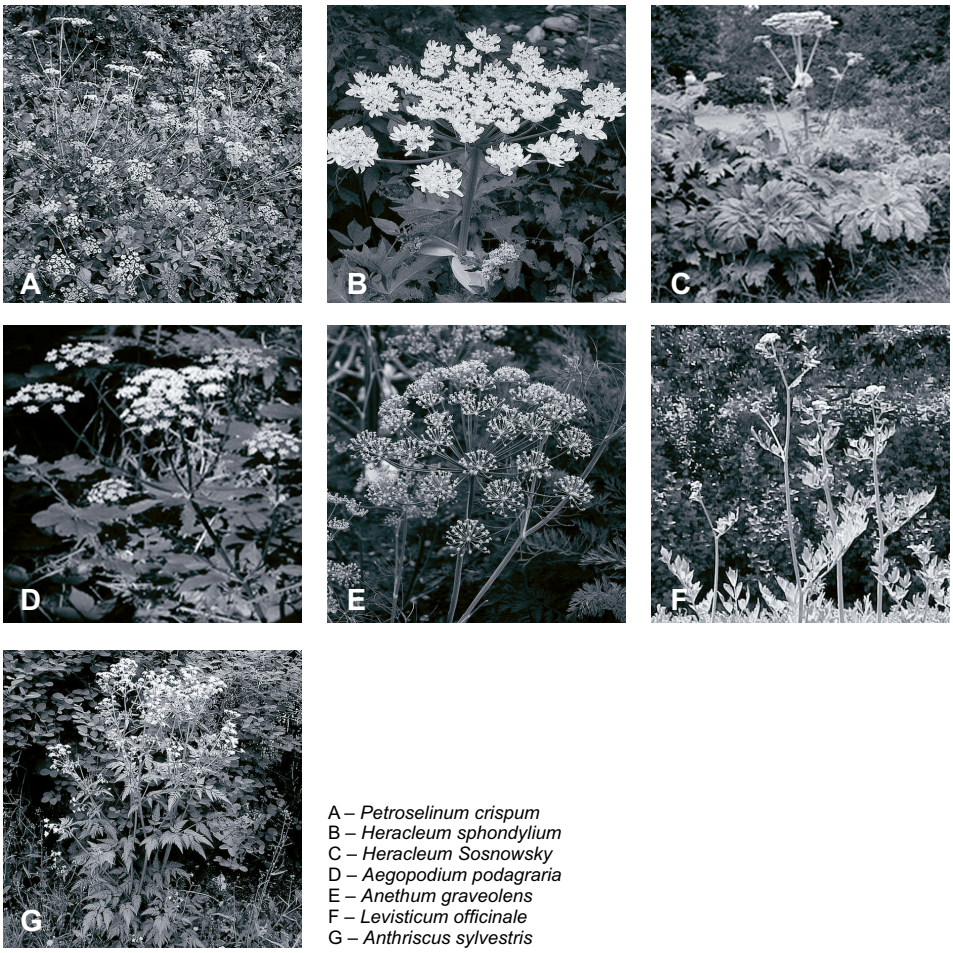

Fig. 1. The photos of plants used during researches

Ethyl acetate extracts were obtained by extracting the plant material: 24 hours at room temperature (maceration); 30 hours in Soxhlet apparatus; half an hour at room temperature in ultrasonic bath (35 kHz). The solvent was removed in a vacuum evaporation. The yield of extractions is showed in Table 1.

GC-MS analyses of extracts were performed with the use an Agilent 6890N series gas chromatograph equipped with an Agilent 5975C inert XL MSD (Agilent Technologies). A vaporization injector in the split mode (1:50) at 270 ^{o}C, with a HPMS5 capillary column was used. The oven temperature was programmed from 50 ^{o}C to 140 ^{o}C and the speed of temperature increase was 10 ^{o}C/min. At 140 ^{o}C the analysis was performed isothermally after 2 min; then the temperature was increased to 250 ^{o}C with the same speed of temperature increase as previously. At 250 ^{o}C the analysis was performed isothermally after 15 min.

High purity helium was used as the carrier gas. The identity of each compound was determined by means of a comparison of its spectral data with the data from the Wiley library spectral bank (G1035B; Rev D.02.00; Agilent Technologies).

Antioxidants content in the extracts was measured with two different methods with the use of ascorbic acid/linoleic acid in the first method [2] and *N,N*-dimethyl-*p*-phenyl-enediamine (DMPD) in the second method [3]. The total phenolate compounds content in the extracts was determined by a colorimetric assay, using the Folin-Ciocalteu reagent and pyrogallol as a standard [4].

Results and discussion

The lowest efficiency of the extraction was obtained when the maceration at room temperature was used, according to the expected results. This group of extracts was not used in our following consecutive investigations.

The efficiencies of extractions performed in ultrasonic bath (USBE) were higher in all the cases, than the efficiencies of extractions performed for a long time, at high temperature, in Soxhlet apparatus. The efficiency of the extraction was from 6.3 % for ground elder to 35.6 % for hogweed Sosnowsky (Table 1).

Table 1

The yield of ethyl acetate extraction [%]

Plants	Hot extract (Soxhlet apparatus)	Cold extract (maceration)	Ultrasonic bath
Common hogweed (*Heracleum sphondylium*)	9.6	4.1	26.2
Hogweed Sosnowsky (*Heracleum Sosnowsky*)	10.8	6.9	45.6
Dill (*Anethum graveolens*)	5.0	2.0	15.1
Lowage (*Levisticum officinale*)	9.6	5.4	13.6
Parsley (*Petroselinum crispum*)	8.2	3.2	24.9
Ground-elder (*Aegopodium podagraria*)	1.4	0.4	6.3
Cow parsley (*Antriscus sylvestris*)	30.1	7.6	23.8

In high temperature extraction, some of compounds were decomposed (unsaturated compounds and glycosides, with discharge aglycone with considerably smaller

Table 2

Contents of group of chemical components identified in extracts (GC-MS)

Compounds	Common hogweed		Hogweed S.		Dill		Lowage		Parsley		Ground-elder		Cow parsley	
	SA	UB	SA	UB	SA	UB	SA	UB	SA	UB	SA	UB	SA	UB
n-octyl alcohol	—	3.7	1.4	8.7	—	—	—	—	—	—	—	—	—	—
Other alcohols	—	—	7.7	—	—	—	0.3	—	—	—	—	8.9	2.0	—
Octyl esters	44.9	54.2	14.0	32.6	—	—	—	—	—	—	—	—	—	—
Terpenes	1.1	0.5	—	—	72.0	97.0	46.6	74.1	3.8	14.3	1.0	1.2	56.2	65.1
Furanecumarin and coumarin	9.0	4.3	52.2	20.8	2.1	1.2	20.8	2.2	3.7	—	—	—	16.7	10.2
Phenols	—	—	1.1	0.2	0.9	0.3	—	—	—	—	—	—	2.4	1.8
Unsaturated ketones	0.6	—	—	—	—	—	0.5	—	—	—	—	—	—	—
Unsaturated esters	1.1	4.1	—	—	7.2	1.5	—	—	—	—	—	—	3.3	5.0
Unsaturated fat acids	11.1	21	4.6	4.8	3.6	—	7.8	6.8	—	—	30.8	39.5	—	—
Saturated fat acids	—	—	—	—	—	—	0.5	—	—	—	—	—	—	—
Saturated esters	—	7.7	5.6	25.7	—	—	—	—	—	—	—	—	3.3	5.0
Sitosterol	0.4	—	—	—	—	—	—	—	—	—	—	—	—	—
Hydrocarbons	0.6	—	2.5	—	2.9	—	—	—	—	—	—	—	—	—
Unsaturated aldehydes	—	—	—	—	—	—	1.2	—	—	—	32.6	26.1	3.3	6.7
Sesquiterpenes	—	—	—	—	—	—	0.3	—	4.4	—	—	—	0.5	—
Phthalide	—	—	—	—	—	—	6.0	8.1	—	—	—	—	—	—
Phenylpropene derivative	—	—	—	—	—	—	—	—	77.1	84.7	—	—	—	—
Others	—	—	—	—	—	—	—	—	0.2	—	2.2	—	—	—
Others, not identifying	32.1	4.5	10.9	7.2	11.3	0.0	6.0	8.8	10.8	1.0	33.4	14.3	11.6	2.4

SA – Soxhlet apparatus, UB – Ultrasonic bath.

molecular mass). In comparison with the extracts made in Soxhlet apparatus, the extracts obtained in ultrasonic baths (USBE) did not include at all, or included only a very small quantity of not-cumarin phenols. The corresponding values for cow parsley amount to 2.4 and 1.8 %, for hogweed Sosnowsky – 1.1 and 0.2 %, also less cumarins and furanocumarins (for hogweed Sosnowsky 52.2 and 20.6 %, and for lowage – 20.8 and 2.2 %). At the same time, more terpenes and unsaturated compounds with different chemical nature were found. In the case of extracts from common hogweed and hogweed Sosnowsky – the presence of *n*-octyl alcohol and octyl esters was detected.

Table 3

Antioxidant activity

Plants	Soxhlet apparatus			Ultrasonic bath		
	AECD	DMPD	PyE	AECD	DMPD	PyE
Common hogweed (*Heracleum sphondylium*)	53	2.3	49	87	1.9	31
Hogweed Sosnowsky (*Heracleum Sosnowsky*)	143	3.1	182	198	2.3	123
Dill (*Anethum graveolens*)	116	1.7	35	136	1.6	26
Lowage (*Levisticum officinale*)	44	2.4	103	42	2.2	78
Parsley (*Petroselinum crispum*)	132	2.1	44	157	1.8	23
Ground-elder (*Aegopodium podagraria*)	45	0.6	10	162	0.4	0
Cow parsley (*Antriscus sylvestris*)	46	0.9	92	60	0.4	0

AECD – antioxidant activity measured by the ascorbic acid/linoleic acid assay, in mg/g dry mass of extract; DMPD – antioxidant activity measured by the *N,N*-dimethyl-*p*-phenylenediamine dihydrochloride/iron(II) chloride assay, in milligram Trolox equivalent/g dry mass of extract; PyE – total phenolic compounds, determined as pyrogallol equivalents, in mg per g of dry mass, by Folin-Ciocalteu method.

The received results showed that extracts, obtained with the proposed method, are characterized by more primeval properties; in consequence, they should have a higher biological activity. This effect was especially observed in the extracts of hogweed Sosnowsky and common hogweed's seeds, including more *n*-octyl alcohol and octyl acetate than the plant extracts obtained in Soxhlet apparatus. The research works conducted by the Department of Plant Protection of Agricultural University in Krakow show that the fungistatic activity of this substances is high. This effect is confirmed by the retardation of growth of fungi *Fusarium culmorum*, *Botrytis cinerea*, *Alternaria alternate*, with oil from hogweed Sosnowsky seeds, which includes a lot of *n*-octyl alcohol and octyl ester. A strong inhibition of the tested fungi was also observed in nutrient solution with additives of this substances [5].

The total content of phenols in the obtained extracts and their antioxidative activity were determined, in order to observe the relation between these quantities and the biological activity of plant extracts, which were described in numerous publications [6]. Antioxidant content in the extracts was measured with two methods, based on different mechanisms of chemical process – according to the well-established information of extracts' composition. In DMPD method a transfer of hydrogen atom occurs, characteristic of phenolic compounds. In the case of the use of linoleic acid and ascorbic acid, there occurs a transfer of electron in non-enzymatic formation of peroxides [7, 8].

It was found that the antioxidative capacity, which is determined by the complexion DMPD with Fe^{3+} is decreased. It is connected with the presence of phenols and conform with the results of total content determination. The antioxidative properties of extracts obtained with both methods, studied by means of inhibition of not enzymatic oxidation of linoleic acid give similar results, or even higher, in the case when the USBE method was used. It is connected with a bigger content of terpenes and other unsaturated compounds. According to the experimental works of Jeng-Leun Mau [9], all the compounds of isoprene structure reveal inhibitive effect in relation to peroxidation of linoleic acid. Moreover, terpenes are in synergetic relation to other antioxidants which are present in extracts.

Conclusions

To recapitulate, it should be supposed that the extracts received with the described method, have to be characterized by the same or a higher fungistatic activity. This conclusion will be subject to further investigations.

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Abstrakt: W roślinach z rodziny *Apiaceae* występuje wiele substancji znanych ze swej aktywności biologicznej w stosunku do mikroorganizmów (grzybów, bakterii i wirusów). We wcześniejszych badaniach opisano właściwości wybranych ekstraktów otrzymanych metodą klasyczną, ich skład i aktywność w stosunku do grzybów patogennych. W obecnej pracy przedstawiono właściwości ekstraktów otrzymanych z nasion: pietruszki (*Petroselinum crispum*), barszczu zwyczajnego (*Heracleum sphondylium*), barszczu Sosnowskiego (*Heracleum Sosnowsky*), podagrycznika (*Aegopodium podagraria*), kopru ogrodowego

(*Anethum graveolens*), lubczyku (*Levisticum officinale*) i trybuli (*Anthriscus sylvestris*), przez ekstrakcję octanem etylu, w temperaturze pokojowej w łaźni ultradźwiękowej. Analizę otrzymanych ekstraktów prowadzono metodą GC-MS (Agilent 6890N). Właściwości antyutleniające oznaczano dwiema metodami: z udziałem kwasu askorbinowego lub linolowego oraz w obecności *N,N*-dimetyl-*p*-fenylenodiaminy (DMPD). Uzyskane rezultaty pokazały, że ekstrakty otrzymane proponowaną metodą mają bardziej pierwotne właściwości, a co za tym idzie, powinny wykazywać większą aktywność biologiczną.

Słowa kluczowe: *Apiaceae*, ekstrakty z nasion, aktywność antyoksydacyjna, DMPD