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ORIGINAL PAPER

AN INVESTIGATION INTO THE RELATIONSHIPS BETWEEN ANTIOXIDANT ACTIVITY AND CHEMICAL ELEMENTS AS WELL AS POLYPHENOLICS IN FUNGAL FRUITING BODIES GROWING ON *BETULA* L.

Marcin Szymański[1], Joanna Smolibowska[2], Arkadiusz Szymański[3]

[1] Centre for Advanced Technologies, Adam Mickiewicz University, Poland
[2] Department of Pharmacognosy Poznan University of Medical Sciences, Poland
[3] Faculty of Chemistry, Adam Mickiewicz University, Poland

Abstract

The kingdom of fungi is a numerous group of organisms with differences in their structure, coloration, nutrition, proliferation, and concentrations of chemical compounds. Discussed in this paper are the results of studies on antioxidant properties, total polyphenolics, percentage of elements in water extracts of six fungal species found on birch (*Betula* sp.): *Inonotus obliquus*, *Fomes fomentarius*, *Piptoporus betulinus*, *Trametes versicolor*, *Pycnoporus cinnabarinus* and *Daedaleopsis confragosa*. The data are analyzed with respect to their mutual correlations and relationships. The paper contains the results of determinations of total polyphenolics and antioxidant capacity achieved with methods based on DPPH and ABTS, and of the content of elements in extracts of fruiting bodies of six fungal species which occur on *Betula* L. The total content of polyphenolics (in terms of caffeic acid) in the test samples was between 0.24% for *Daedaleopsis confragosa* to 4.04% for *Inonotus obliquus*. On the other hand, IC50 found by the methods based on DPPH was from 114 for *Daedaleopsis confragosa* to 1.80 mg ml^{-1} for *Inonotus obliquus*, whereas IC50 found by the methods based on ABTS was between 107 for *Daedaleopsis confragosa* and 3.37 mg ml^{-1} for *Inonotus obliquus*. The Spearman rank correlation test for the results showed a linear relationship between the total content of polyphenolics and the parameters IC50 and the presence of such elements as sulfur, copper and potassium. The value of IC50 was correlated with the content of potassium, rubidium, and sulfur. Data clustering showed that the total content of polyphenolics was the closest to rubidium and potassium, followed by manganese. The value of IC50 was close to those of sulfur and sulfur, and then to the aluminum and iron.

Keywords: polypore fungi fruiting bodies, antioxidant activity, chemical elements, Spearman rank correlation, data clustering.

dr Marcin Szymański, Centre for Advanced Technologies, Adam Mickiewicz University, Umultowska 89c, 61-614 Poznań, Poland, e-mail: marcin.szymanski@amu.edu.pl

INTRODUCTION

The constituents of the fruiting bodies of *Inonotus obliquus* include lanostane-type triterpenes (Nakata et al. 2009, Knox et al. 1991), abietane-type diterpene (Liu et al. 2014), steroids, polyphenolic acids (Kuriyama et al. 2013), xylogalactoglucan (Wasser 2002). Triterpenes exhibit anti-tumor properties (Youn et al. 2008) and they start the neoplastic cell apoptosis (Song et al. 2013). The components of the anti-tumor effect also include an ability to inhibit the inflammatory process. Water extract from *Inonotus obliquuis* is reported to have a virucidal effect towards hepatitis C virus and to reduce its infective properties very rapidly (Shibnev et al. 2011).

Fomes fomentarius (L.) J. J. Kickx is the only European representative of the genus *Fomes*. Water extract of *Fomes fomentarius* was prepared and its content of polysaccharides and their monosaccharides: glucose, galactose, mannose and fucose was determined (Kardošová et al. 1969). Exopolysaccharides (EPS) and intramolecular polysaccharides (IPS) showed an anti-cancerogenic effect on stomach cancer cells (Chen et al. 2011). *Fomes fomentarius* also generates ergostane-type sterols. The compounds had a cytotoxic effect on selected cancer cell lines (colon, lungs, breasts, stomach), confirming the anti-tumor effect of *Fomes fomentarius* and indicating a new direction in medical therapy (Zang et al. 2013).

An analysis of the fruiting bodies of *Piptoporus betulinus* (Bull.) P. Karst showed the following composition: fatty acids, ergosterol peroxide and dehydroergosterol peroxide, polyporenic acid C, D-mannitol, D-arabinitol. Ergosterol peroxide (described for *Inonotus obliquus*) shows anti-tumor and immunomodulating effects, fatty acids are able to control lipid management and mannitol can protect the colon against cancer (Hybelbauerová et al. 2008). Water-soluble polysaccharides with an α-(1→3)-D link, having anti-tumor properties, are also present (Wiater et al. 2011). Piptamine is an antibiotic found in *Piptoporus betulinus* (Schlegel et al. 2000).

Trametes versicolor (L.) Lloyd is a common fungus, frequently found in deciduous forests and hardly ever in coniferous one. Methanolic extract of fruiting bodies of *Trametes versicolor* comprises a number of fatty acids (Hybelbauerová et al. 2008). The extract of *Trametes versicolor* increases survival of gastric, colon, or colorectal cancer patients, improves immune parameters, including increased secretion of NK cells, leukocyte count, IgG, IgM (Ramberg et al. 2010). Combining β-1,3-glucane with PSP activates immunostimulating and anti-tumor effects (Yang, Wu 1998). An extract of *Trametes versicolor* was shown to be a safe immunostimulant, which can support chemotherapy and improve the patient's general condition (Torkelson et al. 2012). The anti-tumor effect in breast cancer is attributed to the PSP and PSK (Krestin) polysaccharopeptides, which act on the immune system and mediators of inflammation (Standish et al. 2008). The *Trametes versicolor* extract demonstrates a very strong antiviral activity against influenza virus

type A (H1N1) and herpes simplex (high therapeutic index of 324.67) (Krupodorova et al. 2014).

Pycnoporus cinnabarinus (Jacq.) Fr. is found on the wood of deciduous trees. It is able to convert *p*-coumaric acid into caffeic acid. Coumaric acid is a hydroxyacid, derived from cinnamic acid. Its strong antioxidant properties have been investigated with the focus on an antisclerotic effect; it appeared to have the strongest effect on LDL lipoproteins among all hydrocinnamic acids. *Pycnoporus cinnabarinus* converts *p*-coumaric acid using two paths: one leads to the product – *p*-hydroxybenzaldehyde (and its further derivatives), the other does to caffeic acid (Alvarado et al. 2001, 2003). The red-to--orange pigmentation of *Pycnoporus cinnabarinus* is attributed to phenoxazinone pigments, including cinnabarin, tramesanguin and cinnabarinic acid (Levasseur et al. 2014). The role of the system is defined either as the backbone for the further construction of phenoxazinone antibiotics or as an important molecule which protects mammalian tissues against the effect of free radicals (Le Roes-Hill et al. 2009).

Daedaleopsis confragosa (Bolton) J. Schröt. is found all year. It grows preferably on injured deciduous trees, mainly birch (*Betula* sp.). Earlier studies indicate the presence of "atypical fatty acids". In a more recent study, the following compounds have been isolated: 3α-carboxyacetoxyquercinic acid, ergosterol peroxide, 3*α*-carboxyacetoxy-24-methylene-23-oxolanost-8-ene-26-oic acid, and 5*α*,8*α*-epidioxyergosta-6,22-dien-3*β*-ol (Rösecke, König 2000).

Most polyphenolic compounds found in plants and fungi have a strong antioxidant effect with beneficial influence on human health. However, plants and fungi often accumulate various elements, including heavy metals, during their development.

The aim of the study was to determine the chemical elements, polyphenolic compounds and antioxidant activity in selected fungal species occurring on birch. The data was analyzed with respect to their mutual correlations and relationships.

MATERIAL AND METHODS

The test material was collected between August and November in 2012 and in 2014. The fruiting bodies of *Inonotus obliquus* (one fruiting body was collected in August 2012), *Fomes fomentarius* (two fruiting bodies of the fungus from one tree were collected in November 2012), *Piptoporus betulinus* (two fruiting bodies of the fungus from one tree were collected in October 2013), *Trametes versicolor* (four fruiting bodies of the fungus from one tree were collected in October 2013), *Pycnoporus cinnabarinus* (four fruiting bodies of the fungus from one tree were collected in October 2014) and *Daedaleopsis confragosa* (four fruiting bodies of the fungus from one tree

were collected in November 2014) were picked in Puszcza Notecka (near Sieraków and Chojno). The material was dried in a dark and airy place at a room temperature. The fruiting bodies of fungi were identified based on morphological and anatomical properties. Quantitative determinations were carried out up to two months from the date of harvest.

Determination of total polyphenolics using the Folin-Ciocalteu (FC) reagent

An aqueous stock solution of caffeic acid (200 µg ml^{-1}) was prepared and, using the serial dilution method, solutions containing 0.01; 0.03; 0.04; 0.06; 0.07; 0.08; 0.09; 0.10; 0.125 mg of caffeic acid in 1 ml were prepared. Each of the test tubes containing water (7.4 ml) and the standard solution or test solution (0.1 ml) was filled with 0.5 ml of the FC reagent and, after the lapse of 2 min – with 2.0 ml of a 20% sodium carbonate solution. The solutions were supplemented with distilled water to obtain a volume of 10 ml and mixed.

A reference solution was obtained by combining the above reagents at same time intervals, with the exception of the caffeic acid solution (7.5 ml of distilled water, 0.5 of ml FC reagent, after the lapse of 2 min – 2.0 ml of a 20% solution Na_2CO_3). The solution was mixed and, after 30 min, the absorbances of the resulting colored solutions were measured at the wave length λ = 760 nm. Five assays were performed for each solution or nine for test solution.

Determination of anti-oxidative activity using the radical DPPH

An aqueous solution of gallic acid (2 mg ml^{-1}) was prepared, and, using the serial dilution method, solutions containing the following concentrations were prepared: 0.005; 0.010; 0.015; 0.020; 0.030; 0.040; 0.050 mg ml^{-1}.

Six dilutions were made for each of respective extracts.

To perform the assay, 2-ml Eppendorf test tubes were filled with 0.1 ml of the respective solutions of gallic acid or test extract and 0.7 ml of DPPH solution. This procedure was performed five times for each dilution. After adding DPPH, the solutions were closed up in light-protective casssete and placed on a shaker. After 30 min, a spectrophotometric measurement was performed at a wave length λ = 515 nm. The reference sample was water and methanol (0.1 ml and 0.7 ml, respectively). Before proceeding to the absorbance measurements for the gallic acid solutions, absorbance of the DPPH solution (0.1 ml of deionized water and 0.7 ml of DPPH solution) was measured in five repetitions.

Determination of anti-oxidative activity using the ABTS radical

A sample of gallic acid of exactly 0.0050 g was measured and transferred to a 10-ml measuring flask, dissolved in 6 ml of methanol and supplemen-

ted with deionized water, to obtain a stock solution at a concentration of 0.5 mg ml^{-1}. Then, using the serial dilution method, solutions of the concentrations: 0.005, 0.010, 0.015, 0.025, 0.0275 and 0.030 mg ml^{-1} were prepared. Six dilutions each were made for the respective extracts.

To perform the measurement, 2-ml Eppendorf test tubes were filled, in the following order, with: 50 µl of extract at a given concentration or the test extract at the specified dilution and 1.0 ml of the ABTS solution. Five assays were performed for each solution.

After adding ABTS and mixing, the samples were closed up in light-protective cassettes. A spectrophotometric measurement was performed at a wavelength λ = 734 nm after the lapse of 10 minutes. Water was the reference sample. Before proceeding to absorbance measurements for the samples, absorbance was measured for the ABTS solution (50 µl of deionized water and 1.0 ml of ABTS solution).

Determination of chemical elements

Selected chemical elements were determined using ICP-OES.

The test samples of the fruiting bodies were mineralized in a Mars 5 digestion microwave system by CEM Corporation, the USA, using 10 ml of concentrated HNO_3 Ultranal; deionized water was added to obtain a volume of 15 ml. The mineralization parameters were as follows: maximum temp. – 210°C, maintenance time – 10 min, power – 600 W, pressure – 195 PSI, and temp. rise time – 20 minutes.

The test samples were analyzed to detect aluminum, boron, copper, iron, manganese, sodium, rubidium, strontium, zinc, magnesium, phosphorus, sulfur, potassium, titanium, lead and arsenic.

The concentrations stated for the respective elements are mean values from six determinations.

RESULTS AND DISCUSSION

The results of determinations of polyphenolics and antioxidant properties in aqueous extracts of fungal fruiting bodies are shown in Table 1.

The tests have shown that the highest content of polyphenolics was in the extract of *Inonotus obliquus*: 4.04%. Much lower values were found in the extracts of *Pycnoporus cinnabarinus* and *Piptoporus betulinus*: 0.38% and 0.32%, respectively. The lowest concentration of polyphenolics was present in the extract of *Daedaleopsis confragosa*: 0.24%. A slightly higher concentration of the compounds was found in the extracts of *Fomes fomentarius* and *Trametes versicolor*, 0.27% and 0.25%, respectively.

The strongest effect on free radicals was observed for the extract of

Table 1

Results of the determinations of total polyphenolics and antioxidant capacity of the extracts of fungal fruiting bodies

Specification	Total of polyphenols (%)	IC_{50} (DPPH) (mg ml^{-1})	IC_{50} (ABTS) (mg ml^{-1})
Gallic acid (standard)	-	0.03	0.02
Piptoporus betulinus	0.32	14.5	32.4
Pycnoporus cinnabarinus	0.38	51.4	71.9
Daedaleopsis confragosa	0.24	114	107
Trametes versicolor	0.25	96	85.9
Fomes fomentarius	0.27	20.4	45.8
Inonotus obliquus	4.04	1.80	3.37

Inonotus obliquus (IC_{50} = 1.80 mg ml^{-1}), followed by the extract of *Piptoporus betulinus* (IC_{50} = 14.5 mg ml^{-1}). A considerable effect was also seen for the extract of *Fomes fomentarius* (IC_{50} = 20.4 mg ml^{-1}). The least perceptible antioxidant activity was observed for the extracts of *Daedaleopsis confragosa* (IC_{50} = 114 mg ml^{-1}) and *Trametes versicolor* (IC_{50} = 96 mg ml^{-1}). None of the above had a stronger effect than the standard (gallic acid).

Studies on antioxidant activity, as measured by means of the ABTS reagent and expressed as IC_{50}, indicate that the strongest effect on free radicals was observed for the extract of *Inonotus obliquus* (IC_{50} = 3.37 mg ml^{-1}), followed by that of *Piptoporus betulinus* (IC_{50} = 32.4 mg ml^{-1}). The extract of *Fomes fomentarius* also had a significant effect (IC_{50} = 45.8 mg ml^{-1}). The weakest antioxidant activity was shown by the extract of *Daedaleopsis confragosa* (IC_{50} = 107 mg ml^{-1}) and that of *Trametes versicolor* (IC_{50} = 85.9 mg ml^{-1}).

The results of determinations of chemical elements in the test samples of fungal fruiting bodies are shown in Tables 2-4.

The following concentrations of heavy metals were found: the highest content of chromium was found in *Trametes versicolor* (86.2 ppm), copper – in *Fomes fomentarius* (118 ppm), iron – in *Trametes versicolor* (1220 ppm), manganese – in *Inonotus obliquus* (229 ppm), zinc – in *Piptoporus betulinus* (144 ppm). Titanium was present only in *Pycnoporus cinnabarinus* and *Trametes versicolor* and was detected below the limit of determination in the other samples. Lead was determined only in *Trametes versicolor* and *Fomes fomentarius*, and was detected below the limit of determination in the other samples. Among light metals, the highest content of aluminum was found in *Trametes versicolor* (793 ppm), calcium – in *Fomes fomentarius* (5314 ppm), magnesium in *Trametes versicolor* (908 ppm), sodium in *Pycnoporus cinnabarinus* (43.5 ppm), potassium in *Inonotus obliquus* (46330 ppm), rubidium in *Inonotus obliquus* (453 ppm), and the highest level of strontium was found in *Pycnoporus cinnabarinus* (7.8 ppm). Among metalloids, the highest level

Table 2

Average heavy metal content in fungal fruiting bodies

Item	Cr		Cu		Fe		Mn		Zn		Ti		Pb	
	ppm	SD	ppm	SD	ppm	SD	ppm	SD	ppm	SD	ppm	SD	ppm	SD
PB	1.71	0.04	8.9	0.3	32.2	2.4	107	17	144	7	b.l.q.		b.l.q.	
PC	1.51	0.03	1.77	0.07	103	3	50.2	1.0	62.4	1.0	5.1	0.1	b.l.q..	
DC	b.l.q.		19.4	4.1	53.2	5.8	23.0	2.7	69.8	8.8	b.l.q.		b.l.q..	
TV	86.2	3.8	29.0	15.4	1220	64	55.8	1.5	42.3	0.9	20.4	1.1	2.5	0.3
FF	36.9	4.5	118	12	259	31	20.5	1.6	85.3	1.6	b.l.q.		2.2	0.4
IO	b.l.q.		1.66	0.18	8.9	1.7	229	21	33.5	1.3	b.l.q.		b.l.q.	

SD – standard deviation, b.l.q. – below the limit of quantification,
PB – *Piptoporus betulinus*, PC – *Pycnoporus cinnabarinus*, DC – *Daedaleopsis confragosa*,
TV – *Trametes versicolor*, FF – *Fomes fomentarius*, IO – *Inonotus obliquus*

Table 3

Average light metal content in fungal fruiting bodies

Item	Al		Ca		Mg		Na		K		Rb		Sr	
	ppm	SD	ppm	SD	ppm	SD	ppm	SD	ppm	SD	ppm	SD	ppm	SD
PB	4.2	0.8	575	15	895	52	15.0	0.4	4000	230	25.6	1.3	4.3	0.2
PC	90.4	2.1	2175	80	384	4	43.5	0.4	2485	82	12.6	0.3	7.8	0.1
DC	28.6	4.2	748	14	810	20	20.1	0.6	1789	14	6.1	1.1	2.6	0.2
TV	793	25	1573	74	908	9	24.0	1.7	2032	34	13.7	0.2	5.3	0.1
FF	9.8	0.2	5314	256	634	7	18.3	2.1	4619	89	37.5	1.6	7.2	0.6
IO	9.7	1.8	843	178	716	56	28.9	0.7	46330	1362	453	24	2.4	0.6

Table 4

Average content of metalloids and non-metals in fungal fruiting bodies

Item	B		As		P		S	
	ppm	SD	ppm	SD	ppm	SD	ppm	SD
PB	b.l.q.		b.l.q.		1293	75	751	88
PC	10.3	0.6	5.6	2.3	877	13	746	44
DC	6.8	0.2	b.l.q.		1128	32	1046	68
TV	b.l.q.		b.l.q.		8.1	0.2	916	39
FF	b.l.q.		b.l.q.		2.6	0.9	881	145
IO	8.2	0.5	b.l.q.		79.5	4.7	176	9

of boron was found in *Pycnoporus cinnabarinus* (10.3 ppm). Boron was found also in *Daedaleopsis confragosa* and *Inonotus obliquus*, in the other samples it was present below the limit of determination. Arsenic was present only in *Pycnoporus cinnabarinus* (5.6 ppm), in the other samples it was below the limit of determination. Among non-metals, the highest level of phosphorus was found in *Piptoporus betulinus* (1293 ppm), and that of sulfur – in *Daedaleopsis confragosa* (1046 ppm).

As a result of the testing of fruiting bodies of *Inonotus obliquus* in other scientific centers, low molecular weight polyphenols were isolated: caffeic acid, 3,4-dihydroxybenzalacetone (DBL), protocatechuic acid, gallic acid, syringic acid, 3,4-dihydroxybenzaldehyde and 2,5-dihydroxy terephthalic acid (Kuriyama et al. 2013). The ability to scavenge free oxygen and non-oxygen radicals has been assigned to polysaccharides. Five polysaccharide fractions were isolated by column chromatography (DEAE-Sepharose fast flow and SepharoseCL-6B). The antioxidant properties were measured *in vitro* (iron reduction, hydroxyl and peroxide radical activity). It turned out that all fractions contain uronic acids and protein substances. The mechanism of action of polysaccharide fractions is not yet known (Huang et al. 2012).

The compounds responsible for antioxidant properties in *Piptoporus betulinus* are phenolic acids (*p*-hydroxybenzoic, protocatechuic, vanillic), flavonoids, tocopherols and vitamins (Reis et al. 2011; Grienke et al. 2014).

The Spearman rank correlation test for the results, using the Statistica 10 program, showed relationships between the results. It was assumed that the criterion of value for the data correlation coefficient was $| r_s | > 0.6$ at the significance level of $p < 0.05$. It was shown that the presence of polyphenolics was connected mainly with the presence of sulfur, copper and potassium in a test sample. Antioxidant properties, found as IC_{50} by means of DPPH and ABTS, are connected with the presence of aluminum, rubidium, sulfur and potassium in the test sample. After an analysis of the above findings, the existing relationships between the presence of polyphenolics and antioxidant capacity were also confirmed.

Data clustering, also performed with the use of Statistica 10, is an attempt to sort the experimental data so as to enable them to be shown in the form of specific hierarchy tree structures. The data were transformed into standardized values to obtain dimensionless values, enabling the formation of a network of data relationships and classifications. A measure of data divergence or convergence was, in this case, the Euclidean distance, i.e., the geometrical distance in a multi-dimensional space. This analysis showed that the presence of polyphenolics was connected with the presence of rubidium, potassium and manganese in the test samples. As regards antioxidant properties (IC_{50} with DPPH and ABTS), they are most visibly connected with the presence of sulfur and, to a lesser extent, with aluminum and iron. The cluster analysis indicates some similarities with the results obtained in the Spearman rank correlation test: the correlation between polyphenolics

Table 5

Values of coefficients in the Spearman rank correlation for the parameters measured

Specification	Total of polyphenols	IC50 (DPPH)	IC50 (ABTS)	Al	Cu	Fe	Mn	Na	Rb	Sr	Zn	Ca	Mg	P	S	K
Total of polyphenols	1.0000	-0.8286	-0.8286	-0.4286	-0.7714	-0.5429	0.6000	0.4286	0.6000	-0.0286	-0.3143	0.0857	-0.4857	0.0286	-1.0000	0.7714
IC50 (DPPH)	-0.8286	1.0000	1.0000	0.7714	0.4857	0.6000	-0.6000	0.0857	-0.8857	0.2571	0.0286	0.0857	0.2000	0.0286	0.8286	-0.9429
IC50 (ABTS)	-0.8286	1.0000	1.0000	0.7714	0.4857	0.6000	-0.6000	0.0857	-0.8857	0.2571	0.0286	0.0857	0.2000	0.0286	0.8286	-0.9429
Al	-0.4286	0.7714	0.7714	1.0000	0.3143	0.7714	-0.3714	0.5429	-0.6000	0.4857	-0.4286	0.4857	0.0286	-0.3714	0.4286	-0.6571
Cu	-0.7714	0.4857	0.4857	0.3143	1.0000	0.7714	-0.7143	-0.5429	-0.1429	0.3714	0.4286	0.3714	0.2571	-0.4857	0.7714	-0.3143
Fe	-0.5429	0.6000	0.6000	0.7714	0.7714	1.0000	-0.6000	0.0286	-0.3143	0.7143	0.0286	0.6571	0.0857	-0.6000	0.5429	-0.4286
Mn	0.6000	-0.6000	-0.6000	-0.3714	-0.7143	-0.6000	1.0000	0.2000	0.4286	-0.5429	-0.4286	-0.5429	0.3714	0.3143	-0.6000	0.3714
Na	0.4286	0.0857	0.0857	0.5429	-0.5429	0.0286	0.2000	1.0000	-0.1429	0.1429	-0.8286	0.3714	-0.4286	-0.2000	-0.4286	-0.0286
Rb	0.6000	-0.8857	-0.8857	-0.6000	-0.1429	-0.3143	0.4286	-0.1429	1.0000	-0.2571	-0.1429	0.1429	-0.0857	-0.4286	-0.6000	0.9429
Sr	-0.0286	0.2571	0.2571	0.4857	0.3714	0.7143	-0.5429	0.1429	-0.2571	1.0000	0.2571	0.7143	-0.4286	-0.3143	0.0286	-0.1429
Zn	-0.3143	0.0286	0.0286	-0.4286	0.4286	0.0286	-0.4286	-0.8286	-0.1429	0.2571	1.0000	-0.2000	0.0286	0.3714	0.3143	-0.0857
Ca	0.0857	0.0857	0.0857	0.4857	0.3714	0.6571	-0.5429	0.3714	0.1429	0.7143	-0.2000	1.0000	-0.6000	-0.8286	-0.0857	0.2000
Mg	-0.4857	0.2000	0.2000	0.0286	0.2571	0.0857	0.3714	-0.4286	-0.0857	-0.4286	0.0286	-0.6000	1.0000	0.2000	0.4857	-0.3714
P	0.0286	0.0286	0.0286	-0.3714	-0.4857	-0.6000	0.3143	-0.2000	-0.4286	-0.3143	0.3714	-0.8286	0.2000	1.0000	-0.0286	-0.3143
S	-1.0000	0.8286	0.8286	0.4286	0.7714	0.5429	-0.6000	-0.4286	-0.6000	0.0286	0.3143	-0.0857	0.4857	-0.0286	1.0000	-0.7714
K	0.7714	-0.9429	-0.9429	-0.6571	-0.3143	-0.4286	0.3714	-0.0286	0.9429	-0.1429	-0.0857	0.2000	-0.3714	-0.3143	-0.7714	1.0000

and the presence of potassium, connections between antioxidant properties and the presence of sulfur and aluminum, although no predictable relationships between polyphenolics and antioxidant properties were found. The values of the coefficients found in the Spearman rank correlation analysis of the test results (total content of polyphenolics, IC_{50} with DPPH, IC_{50} with ABTS, content of chemical elements) are shown in Table 5.

The Spearman rank correlation test showed a correlation between total polyphenolics and antioxidant activity, as found by means of DPPH and ABTS. For total polyphenolics, very high correlation coefficients in the range of $0.7 \leq r < 0.9$ were observed for copper and potassium, and full correlations $r = 1$ were observed for sulfur. This indicates that the presence of polyphenolics in the fruiting bodies of fungi found on birch trees is correlated with the two chemical elements. Antioxidant capacity (IC_{50} measured with DPPH and ABTS) indicates a very high correlation with aluminum, rubidium, sulfur and an almost full correlation with potassium ($0.9 \leq r < 1$). Moreover, the very high correlation between antioxidant properties and total polyphenolics was confirmed experimentally (correlation coefficient in the range of $0.7 \leq r < 0.9$).

The cluster analysis in the form of a hierarchy dendrogram is shown in Figure 1.

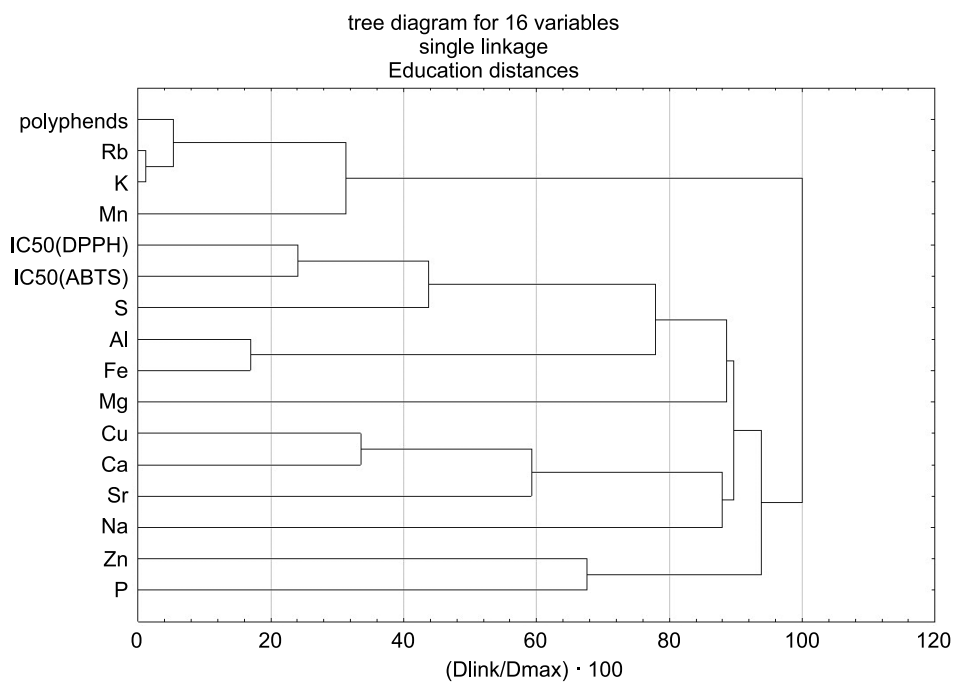

Fig. 1. Data clustering – a hierarchy tree diagram

The diagram above indicates that the value of total polyphenolics is most highly correlated with the presence of rubidium and potassium, although not so highly with manganese; only then is it correlated with antioxidant activity indicated by IC_{50} and with the other chemical elements. Antioxidant properites (IC_{50} as found with the use of DPPH and ABTS) are most highly correlated with the presence of sulfur, less highly with iron and aluminum, followed by magnesium.

Relationships resulting from the cluster analysis may or may not agree with the coefficients obtained by the Spearman rank method. This is explained by the fact that data clustering analyses are carried out in multi-dimensional areas, whereas the Spearman rank method describes linear relationships between the data analyzed.

CONCLUSIONS

In the tests involving fungal fruiting bodies growing on birch, all of the tested samples were found to contain polyphenolics in aqueuous extracts.

Each of the fungal species appears to have antioxidant properties, although their actual capacity varies.

Inonotus obliquus appears to have the highest content of polyphenolics. It also showed the highest free-radical scavenging potential, compared with the other test species.

The highest content of harmful metal contaminants was determined in *Trametes versicolor*, found on a birch trunk in Puszcza Notecka, near Chojno.

The fruiting body of *Inonotus obliquus* was characterized by the lowest level of iron, zinc, sulfur and strontium, while having the highest level of manganese, potassium, rubidium and boron.

Antioxidant properties of the tested fungal species may or may not result from the enzyme systems [referred to in the theoretical part] which account for wood decay and rotting. The enzymes also contain chemical elements such as manganese, copper and iron, which were determined in the test samples.

The Spearman rank correlation was used to show relationships between total polyphenolics and the presence of sulfur, copper and potassium in the test samples.

A correlation between antioxidant activity and the content of metals was also confirmed, mainly with respect to potassium, aluminum, rubidium and sulfur.

Conflict of interest

The authors declare that there are no conflicts of interest.

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