International Journal of Medicinal Mushrooms, Vol. 1, pp. 345–349 (1999)

Some Biologically Active Substances from Medicinal Mushroom *Pleurotus ostreatus* (Jacq.: Fr.) P. Kumm. (Agaricomycetideae)

Valentina G. Babitskatya,[1] Nina A. Bisko,[2] Victor V. Scherba,[1] Nadezda Yu. Mitropolskaya,[2] and Tatjana A. Puchkova[1]

[1]Institute of Microbiology of the National Academy of Sciences of Byelorussia, Minsk, Byelorussia; [2]M. G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine, Tereschenkivska Str. 2, Kiev, 01601–GSP, Ukraine

ABSTRACT: Antioxidant activity, fatty acid composition and ultraviolet and infrared (IR) spectra of fruiting bodies, mycelium, and cultured liquid ethanol extracts of *Pleurotus ostreatus* (Jacq.: Fr.) P. Kumm. were studied. It was demonstrated that the extracts of fruiting bodies had higher antioxidant activity than mycelium and cultured liquid extracts. It has been estimated that the content of unsaturated fatty acids in fruiting body extracts was higher than in mycelium and cultured liquid ones. IR spectra of some biologically active substances from fruiting bodies and mycelium extracts showed that they include mainly phenolic, carbonyl, and methoxyl groups.

KEY WORDS: biologically active substances, cultured liquid, fruiting bodies, mycelium, *Pleurotus ostreatus*.

INTRODUCTION

Pleurotus ostreatus (Jacq.: Fr.) P. Kumm. is one of the edible higher Basidiomycetes mushrooms with medicinal value. Thus this species may be considered a wholesome food or natural plant-derived biological response modifier (Gunde-Cimerman, 1999; Wasser and Weis, 1999).

It is known that besides polysaccharides a number of low molecular weight organic substances such as terpenoids, steroids, and phenols inhibited cancer cell growth (Mizuno, 1996; Wasser and Weis, 1999).

The study of some biologically active substances from fruiting bodies, mycelium, and cultured liquid of *Pleurotus ostreatus* including phenols was the aim of our work.

MATERIALS AND METHODS

The selected strain of *Pleurotus ostreatus* for this study was obtained from the culture collection of the Institute of Microbiology of the National Academy of Sciences of Byelorussia, Minsk.

Mycelium of this strain was grown in submerged culture on glucose–peptone medium (g/liter): glucose, 10; peptone, 3; $K_2HPO \cdot 3\,H_2O$, 1; KH_2PO, 1; $MgSO_4 \cdot 7H_2O$, 0.25; corn extract, 20 ml; deionized water, 1,000 ml; pH 5.5.

Mycelium was grown in fermenters (volume 3 liters and 20 liters). The cultivation temperature was 26–28°C; intensity of the aeration, 0.3–0.5 l/l/min, and rate of the mixer rotation, 100 rotations/min (rpm). The biomass was ready to harvest after 5 days.

Mycelium was separated from medium by filtration, washed with distilled water, dried to a constant weight at 60°C, and pounded. Fruiting bodies of *P. ostreatus* were grown on wheat straw (Zadrazil, 1978). They were harvested, dried at 60°C, and pounded.

In fruiting bodies, mycelium and cultured liquid ethanol extracts of fatty acids were estimated using a Chrom-5 gas–liquid chromatograph with 15% polyethylenglycol succinate as liquid (tem-

1521-9437/99/$5.00

perature of the column, 160°C; temperature of the evaporation, 210°C) (Vereschagin et al., 1963).

Extracts of the mycelium and fruiting bodies of *P. ostreatus* were obtained after freezing in quartz sand, and extraction was accomplished with 70% ethanol for 0.5 h. These extracts were centrifuged (8,000 rpm) for 15 min.

These extracts were fractionated according to the method of Pisarnickij et al. (1986).

Thin-layer chrofnatography was performed on Silufol plates using various solvents (Kirchner, 1981). The antioxidant activity of the fruiting bodies, mycelium, and cultured liquid ethanol extracts was determinated according to the method described by Kapich et al. (1991). Ultraviolet (UV) spectra of substances in these extracts were determined on spectrophotometer Specord UV VIS; infrared (IR) spectra using Spectrometer UR-20.

RESULTS AND DISCUSSION

The data obtained in our work indicated that the extracts of fruiting bodies had a strong antioxidant effect (Fig. 1). The increase of concentration of dry substances did not result in an increase of antioxidant activity. However, the increase of concentration of dry substances in cases of mycelium and cultured liquid extracts promoted the accumulation of the antioxidizers (Fig. 2). The

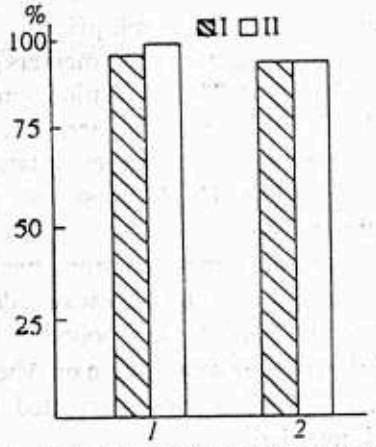

FIGURE 1. Antioxidant activity (%) of fruiting body extracts of *Pleurotus ostreatus* before (1) and after (2) processing with chloroform. I, The content of dry substances is 0.005%; II, the content of dry substances is 0.01%.

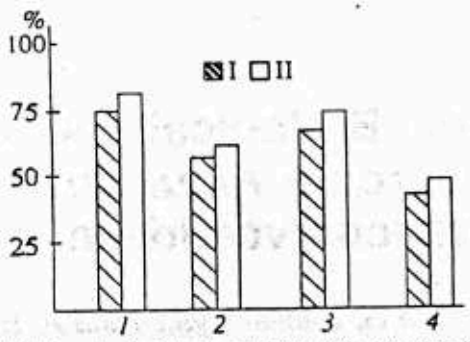

FIGURE 2. Antioxidant activity (%) of mycelia extracts of *Pleurotus ostreatus* before (1) and after (2) processing with chloroform; cultured liquid extracts before (3) and after (4) processing with chloroform. I, The content of dry substances is 0.005%; II, the content of dry substances is 0.01%.

processing of the chloroform extraction increased the antioxidant activity for all studied samples, including the extracts of the fruiting bodies, mycelium, and cultured liquid (Figs. 1 and 2).

The highest antioxidant activity was characterized for extracts of *P. ostreatus* fruiting bodies as compared to mycelia and cultured liquid extracts (Figs. 1 and 2). Activity was also determined for fruiting body extracts of *Lentinus edodes* (Berk.) Sing. (Scherba et al., 1999). The effect of the fruiting body extracts of *P. ostreatus* is similar to the antioxidant activity of ascorbic acid and niacin.

Differences in antioxidant activity between samples of extracts from *P. ostreatus* may be related to different fatty acid composition of their lipids (Table 1). Thus the content of unsaturated fatty acids in fruiting body extracts was higher than in mycelium and cultured liquid extracts.

The investigation of antioxidant activity of different extract fractions—acidic, phenolic, alkaline, neutral—showed that the highest activity level was characterized for the phenolic fraction.

It has been known that the phenolic substances are active antioxidizers (Bioantioxidizers I, 1992). This effect may be related to their ability to neutralize active oxygen forms and bind iron ions (Potapovich et al., 1988).

It has been demonstrated that the content of phenolic substances in fruiting body extracts of *P. ostreatus* (1,660 mg/dl) was higher in comparison with mycelia and cultured liquid extracts.

Nine substances in the phenolic fraction of fruiting body extracts and seven in the fraction of

TABLE 1
Content of Fatty Acids in the Extracts of Cultured Liquid, Fruiting Bodies, and Mycelium of *Pleurotus ostreatus*, (% of Total)

Fatty acid	Fruiting bodies	Mycelium	Cultured liquid
C 14:0	1.11	—	—
C 15:0	1.39	1.38	—
C 16:0	12.85	14.94	18.12
C 17:0	0.88	1.66	—
C 17:1	—	—	53.75
C 18:0	0.55	1.50	2.32
C 18:1	22.70	4.27	4.62
C 18:2	60.47	76.25	21.19

the mycelia and cultured liquid extracts were determined by two-dimensional chromatography. The preliminary identification of the substances was carried out on the basis of change of fluorescence in UV light after the processing of specific solvents and on the determination of their mobility (R_f) in the different solvent systems (Table 2).

Some substances from extracts with the highest fraction content separated by the method of preparative thin-layer chromatography are presented in Table 3.

The spectral characteristics of some substances obtained in our work indicated that fruiting body and mycelia extracts have simple phenols and flavones (Figs. 3 and 4).

The substances with $R_f = 0.94$ and $R_f = 0.43$ predominated in the fruiting body and mycelia extracts accordingly.

The IR spectrum of the substance with $R_f = 0.94$ shows that in their composition phenolic groups (the absorption band 1,600 cm^{-1}), carbonyl groups (the absorption band 1,660–1,700 cm^{-1}) and methoxyl groups (the absorption band 1,150 cm^{-1}) are present (Fig. 5).

TABLE 2
Characteristics of the Fruiting Bodies, Mycelium, and Cultured Liquid Extracts of *Pleurotus ostreatus* (R_f) in Different Solvents on Filtrac FN 12 Paper

Fruiting bodies		Mycelium		Cultured liquid	
B:A:W = 4:1:5	5% Acetic acid	B:A:W = 4:1:5	5% Acetic acid	B:A:W = 4:1:5	5% Acetic acid
0.22	0.52	0.45	0.93	0.23	0.89
0.22	0.59	0.61	0.93	0.27	0.89
0.25	0.90	0.71	0.93	0.30	0.84
0.26	0.66	0.78	0.90	0.40	0.91
0.27	0.90	0.82	0.92	0.95	0.47
0.28	0.83	0.93	0.58	0.96	0.55
0.32	0.76	0.94	0.53		
0.34	0.91				
0.89	0.68				

B, butyl alcohol; A, acetic acid; W, water.

TABLE 3
Characteristics of the Fruiting Bodies and Mycelia Extracts of *Pleurotus ostreatus* (R_f) in Different Solvents on Silufol Plates

Fruiting bodies			Mycelia		
E:P:A = 90:70:70	C:E:F = 50:40:10	B:M:A = 90:16:8	E:B:A = 90:70:40	C:E:F = 50:40:10	B:M:A = 90:16:8
0.23	0.03	0.12	0.29	0.02	0.03
0.43	0.05	0.26	0.37	0.04	0.05
0.90	0.08	—	0.43	0.05	0.09
0.94	0.18	—			

E, ethyl acetate; P, propyl alcohol; A, ammonia; C, chloroform; F, formic acid; B, benzene; M, methanol; A, acetic acid.

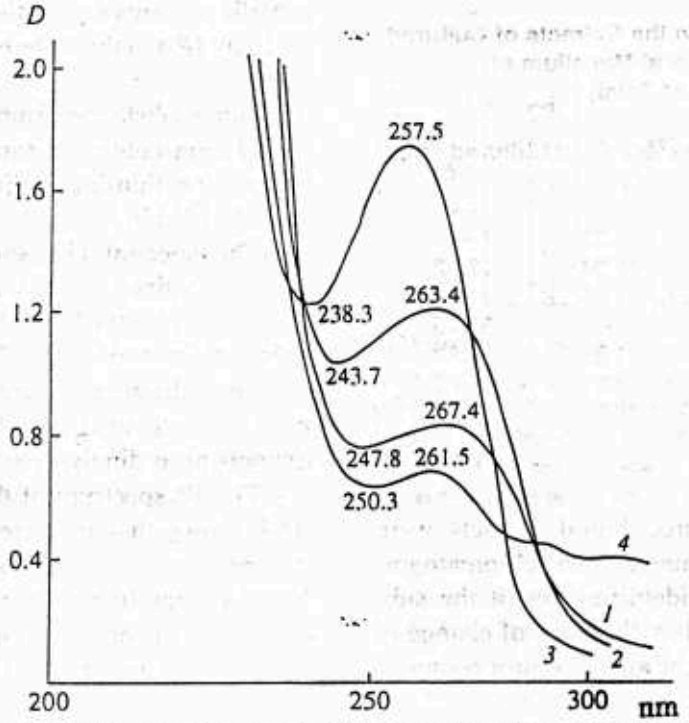

FIGURE 3. UV spectra of substances from the fruiting body extracts of *Pleurotus ostreatus*. 1, $R_f = 0.23$; 2, $R_f = 0.43$; 3, $R_f = 0.90$; 4, $R_f = 0.94$.

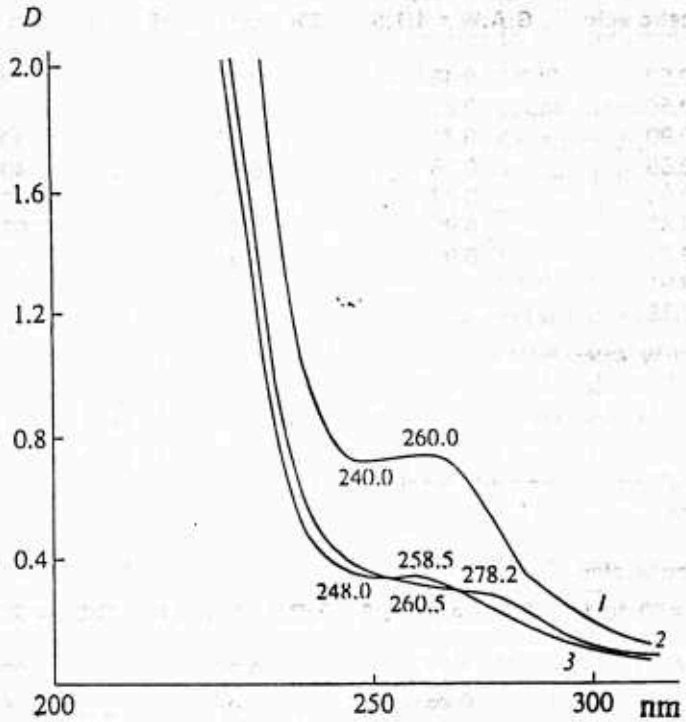

FIGURE 4. UV spectra of substances from the mycelia extracts of *Pleurotus ostreatus*. 1, $R_f = 0.29$; 2, $R_f = 0.37$; 3, $R_f = 0.43$.

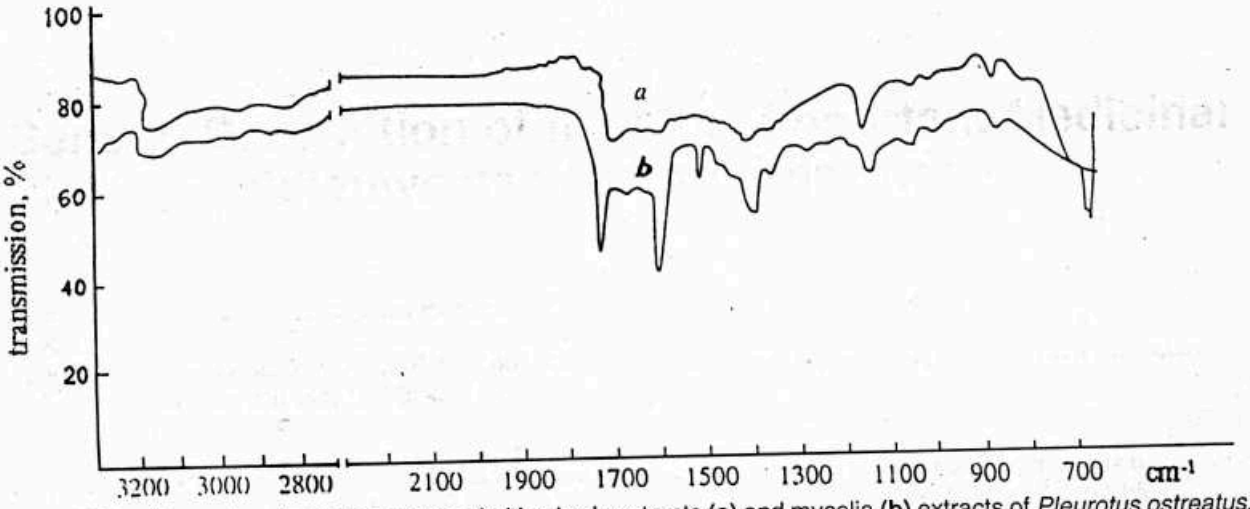

FIGURE 5. IR spectra of substances from fruiting body extracts (**a**) and mycelia (**b**) extracts of *Pleurotus ostreatus*.

The IR spectrum from mycelium extracts (R_f = 0.43) insignificantly differs from the spectrum of the substance from fruiting body extracts (Fig. 5). Thus, the substance from both mycelia and fruiting bodies was found to have a pronounced absorption zone at 1,510 cm^{-1}, which is characteristic for aromatic ring spectrum.

REFERENCES

Bioantioxidizers in regulation of metabolism in standard and pathology. **1992.** Moscow, Nauka, 240 pp.

Gunde-Cimerman N. 1999. Medicinal value of the genus *Pleurotus* (Fr.) P. Karst. (Agaricales s.l., Basidiomycetes). *Int J Med Mushr,* **1**(1):69–80.

Kapich A. N., Leontjev U. M., Konoplja E. F., and Vojt S. P. 1991. The antioxidative activity of mycelium extract *Fomes fomentarius* (Fr.) Fr. Vesti AN Byelorussia, **5**, 58–62.

Kirchner Y. 1981. Chromatography in thin layer. Moscow, Mir, 616 pp.

Mizuno T. 1996. A development of antitumor polysaccharides from mushroom fungi. *Food Food Ingred J (Jpn),* **167,** 69–85.

Pisarnickij A. F., Egorov I. A., Koptava T. G., and Lashchi A. D. 1986. The aromaforming components of fig fruits. *Appl Biochem Microbiol,* **22**(2), 281–285.

Potapovich A. I., Kostuk V. A., Tereschenko S. M., and Afanacjev I. B. 1988. Oxygen radicals in chemistry, biology and medicine. Riga, RMI, 25 pp.

Scherba V. V., Babitskaya V. G., Truchonovec V., Fomina V., Bisko N. A., and Mitropolskaya N. Yu. 1999. The influence of the cultivation conditions on the chemical composition of *Pleurotus ostreatus* (Jacq.: Fr.) Kumm. and *Lentinus edodes* (Berk.) Sing. *Int J Med Mushr,* **1**(2), 181–186.

Vereschagin A. G., Scvorcova S. V., and Ishakov N. I. 1963. The composition of threeglicerids of the cotton oil. *Biochemistry,* **28**(5), 868–878.

Wasser S. P. and Weis A. L. 1999. Medicinal properties of substances occurring in higher Basidiomycetes mushrooms: Current Perspectives (Review). *Int J Med Mushr,* **1**(1), 31–62.

Zadrazil F. 1978. Cultivation of *Pleurotus.* In The biology and cultivation of edible mushrooms, Chang S. T. and Hayes W. A., eds., Academic Press, New York, 521–558.