

Pro- and Antioxidative Properties of Medicinal Mushroom Extracts

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ABSTRACT: Hot water extracts of 2 groups of medicinal mushrooms have been tested from the genera *Agaricus*, *Antrodia*, *Auricularia*, *Coprinus*, *Cordyceps*, *Hericium*, *Grifola*, *Ganoderma*, *Lentinus*, *Phellinus*, and *Trametes* for ROS-generating activity in human cells and for DPPH-TEAC antioxidant activity. Group 1 comprised 39 commercial extracts (7 species), and group 2 comprised 12 fruiting body extracts made from 11 different species of culinary-medicinal mushrooms. For both groups, the ROS-generating activity and the antioxidant activity were strongly correlated, as were their respective polysaccharide and polyphenol contents. The extracts differ in their amounts of the latter components but not in the ratio of the two. The slopes of the correlation curves were different for both groups, which is related to the higher polyphenol content of the commercial extracts. It is suggested that possible excess cell defense-related intracellular ROS generated by mushroom extracts may be downregulated by the antioxidant components present in the same extracts.

KEY WORDS: β -glucan, polyphenol, ROS, antioxidant, pro-oxidant, medicinal mushrooms, *Agaricus bisporus*, *Agaricus brasiliensis*, *Antrodia camphorata*, *Auricularia polytricha*, *Coprinus comatus*, *Cordyceps* sp., *Trametes versicolor*, *Ganoderma lucidum*, *Grifola frondosa*, *Hericium erinaceus*, *Lentinus edodes*, *Phellinus linteus*

I. INTRODUCTION

Medicinal mushrooms, such as *Agaricus brasiliensis* S. Wasser et al. (= *A. blazei* Murrill s. Heinem.), *Coprinus comatus* (O. F. Müll.) Pers., *Trametes* (= *Coriolus*) *versicolor* (L.: Fr.) Lloyd, *Ganoderma lucidum* (W. Curt.: Fr.) P. Karst., *Lentinus edodes* (Berk.) Singer, *Phellinus linteus* (Berk et W. Curt.) Teng, and many others, have traditionally

been used as a health food or supplement for the prevention and cure of a range of diseases, including atherosclerosis, cancer, chronic hepatitis, and diabetes. The preventive and therapeutic effects of these mushrooms and their components have been well documented in mouse and rat model systems and in cancer cell lines.^{1,2} Many studies have led to important knowledge regarding the effects of mushroom extracts and their modes of action. This

ABBREVIATIONS

Afu: arbitrary fluorescence unit; **Apaf:** apoptosis-activation factor; **BSA:** bovine serum albumin; **CR3:** complement receptor type 3; **DCF-DA:** dichloro fluorescein diacetate; **DEAE:** diethyl diamino ethyl; **DPPH-TEAC:** diphenyl picryl hydrazyl-based trolox equivalent antioxidant activity; **FCS:** fetal calf serum; **GAE:** gallic acid equivalent activity; **HMW:** high molecular weight; **PBMC:** peripheral blood monocytic cell; **PBS:** phosphate-buffered saline; **R:** correlation coefficient; **ROS:** reactive oxygen species; **RPMI 1640:** Roswell Park Memorial Institute cell culture medium 1640; **RT:** room temperature; **TLR:** Toll-like receptor.

includes immunomodulatory effects in various forms, for example, proinflammatory activity or adjuvant effects, as well as apoptosis, mostly with the involvement of chemokines as mediators that are produced by the cells of the immune system.

Mushroom extracts contain several biologically or medicinally active components. The influence of mushroom extracts on oxygen metabolism, both *in vitro* and *in vivo*, may be a common denominator. Redox reactions strongly influence a majority of the physiological processes. Several higher Basidiomycetes contain antioxidant phenolic compounds.³ *Agaricus bisporus* (J. Lge) Imbach, which contains high amounts of antioxidants,⁴ has been reported to additionally harbor the pro-oxidative 4-(hydroxymethyl)-phenyl radical.⁵

Although phenolic compounds may generally have antioxidative properties, some are able to activate the intracellular formation of reactive oxygen species (ROS).^{6,7} ROS play an important role in the prevention of infection by destroying potential intracellular pathogens.⁸

The most fundamental role of ROS might, however, be signal transduction and regulation of diverse processes, including neurotransmission, phagocyte activation, cell proliferation, and apoptosis.⁹ Cellular generation of ROS is central to redox signaling.¹⁰ Molecular damage caused by ROS in normal cells challenges the cells to repair the damage. Abnormal or affected cells are activated toward the cell death program, that is, to either apoptosis^{11,12} or autophagous cell death.¹³ Tumor cell lines appear to be particularly sensitive to ROS-induced apoptosis,¹⁴ and many antitumor agents, in fact, exhibit their activity via ROS-dependent activation of apoptotic cell death.¹⁵ In cancer cells, apoptosis is thought to be generally suppressed¹⁶ because of K⁺ channel inhibition resulting from mitochondrial damage, which, in turn, leads to attenuated mitochondrial function and enhanced glycolysis dependency.^{17,18} If ROS and, especially, H₂O₂ are generated in a cancer cell, cytochrome c will be released from the intermembrane space into the cytosol where it binds to the apoptosis activation factor (Apaf-1) and activates the caspase cascade, leading to death.^{17,19}

On the other hand, the presence of intracellular ROS may also have adverse consequences. There is

accumulating evidence that ROS play major roles in the initiation and progression of cardiovascular dysfunction associated with diseases such as diabetes, hypertension, ischemic heart disease, and chronic heart failure.²⁰ Pancreatic β -cells are particularly vulnerable to oxidative stress, which may induce apoptosis and a decrease in β -cell mass, leading to dysfunction of insulin secretion and the onset of type 1 and 2 diabetes.²¹ In mast cells, high levels of ROS contribute to degranulation and to harmful effects in patients suffering from inflammatory disease.^{22,23} These harmful effects associated with prolonged exposure to ROS present an additional motive to study the properties of the different fractions of fungal cell wall extracts commonly sold as medicinal mushroom glucans.

In the present study, we describe the influence of medicinal mushroom extracts on ROS synthesis and on diphenyl picryl hydrazyl-based trolox equivalent antioxidant activity (DPPH-TEAC) antioxidation in the human leukemia cell line K562. Earlier, we demonstrated that polysaccharide extracts of various mushrooms are highly active in ROS generation.²⁴ When hot water-extracted polysaccharides of *Agaricus bisporus* were subjected to DEAE cellulose-adsorption chromatography, a nonadsorbed colorless high molecular weight (HMW) 1,4 glucan was obtained that was unable to generate ROS, suggesting the inability of some glucans to generate ROS. The DEAE-adsorbed fractions were eluted by salt gradient and were found to consist of a hazel-colored polysaccharide-polyphenol complex that was very active in ROS generation. This led us to the tentative conclusion that *A. bisporus* glucan needs to be associated with polyphenolic compounds to become an active ROS generator.²⁴

In the present article, we describe the ROS-generating capacity, the concentration of polysaccharides and polyphenols, and the antioxidant effects of extracts of a collection of commercially available medicinal mushroom preparations of several different species that were obtained from the P. R. China, Korea, Thailand, Europe, and the United States. Further, we report the ROS-generating activity, the polyphenol content, and the antioxidant activity of extracts of various cultivated medicinal mushrooms.

II. MATERIALS AND METHODS

A. Materials

RPMI-1640 medium with L-glutamine and sodium bicarbonate was obtained from Sigma Chemical Corp (St. Louis, MO, USA). Serum supreme and fetal bovine serum (of South American origin) were from BioWhittaker (Walkersville, MD, USA). 2',7'-dichlorofluorescein-diacetate and DPPH were obtained from Sigma. Trolox was obtained from Hoffmann-Laroche (Switzerland).

B. Cells

K562 cells²⁵ were cultivated as described before²⁴ and cultured in a humidified incubator with 5% CO₂ at 37°C in RPMI 1640 containing 10% fetal calf serum (FCS) and 100 U.mL⁻¹ penicillin + 100 µg.mL⁻¹ streptomycin.

C. Mushrooms

Fresh stipe tissues of cultivated *Agaricus bisporus* and fruitbodies of *A. brasiliensis* were obtained from Innerlife B.V. (Venlo, The Netherlands). *Coprinus comatus* strain S 435 and *Lentinus edodes* dried fruitbodies were obtained from the (former) Mushroom Experimental Station (Horst, The Netherlands). Dried hot water extract of wild-type *Phellinus linteus* was kindly provided by Amazing Grace (Thailand). *Ganoderma lucidum* spore extract Grade A was obtained from Fujian Xianzhilou Biological Science and Technology Co. Ltd. (P. R. China). Dried fruitbodies of *Auricularia polytricha*, *Trametes versicolor*, *Grifola frondosa*, and *Hericiium erinaceus* were from commercial sources, and *Cordyceps militaris* (L.) Link was a kind gift from Dr. J. M. Sung of Kangwon National University (Chuncheon, Korea).

D. Polysaccharide Extracts

The mushroom polysaccharide extract was obtained by hot water extraction and ethanol precipitation, as described previously.²⁴

A collection of 39 commercial medicinal mushroom preparations (Table 1) was obtained from dispensaries and health shops in the United Kingdom, P. R. China, Germany, Taiwan, Thailand, the Netherlands, and the United States. They consisted of 18 samples derived from *Ganoderma lucidum*, 5 from *Cordyceps* sp., 4 from *Trametes versicolor*, 4 from *Phellinus* sp., 2 from *Grifola frondosa*, 2 from *Agaricus brasiliensis*, 1 from *Antrodia camphorata* (M. Zhang & C.H. Su) Sheng H. Wu, Ryvarden et T.T. Chan, and 3 were mixtures of several mushrooms. Two of the *Phellinus* samples were water extracts; all the others were dry powders.

The dry samples were subjected to hot water extraction analogously to fruiting-body extraction and used without further alcohol precipitation.

E. Detection of Intracellular ROS

To detect intracellular ROS, 2',7'-dichlorofluorescein diacetate (DCF-DA) was used. This nonfluorescent-fluorescein analogue is oxidized to highly fluorescent 2',7'-dichlorofluorescein by intracellular oxidants.²⁶ Incubations were done 4-fold in Greiner black 96-well plates and repeated several times. The fluorescence was measured in a Tecan fluorescence spectrophotometer at excitation/emission = 485/535 nm. The results are shown as arbitrary fluorescence units (afu) at 535 nm (or as a percentage of change from baseline values). Values were corrected for autofluorescence, that is, reaction mixtures incubated with fluorescein but without cells, and also for the endogenous activity of the cells used. Routinely, 4 × 10⁵ cells.mL⁻¹ were incubated in PBS in the presence of 100 µg.mL⁻¹ polysaccharide and 25 µg.mL⁻¹ DCF-DA, unless stated otherwise.

F. Determination of Antioxidant Activity Using the DPPH Method

TEAC of extracts was measured as described by Sun et al.²⁷ Watery extracts were diluted 5-fold with methanol. The mixture was incubated for 15 minutes at 60°C in a 1.5-mL Eppendorf tube and centrifuged at 10,000 g for 5 minutes. A DPPH

TABLE 1
Contents of Polysaccharide, Protein, and Phenolic Compounds of Hot Water Extracts of Different Commercially Available Medicinal Mushroom Preparations and Their ROS-Activating Capacity and DPPH-TEAC Antioxidant Activity in K562 Cells

Units, species, and origin	Polysaccharide (mg glucose eq.mL ⁻¹)	Protein (µg BSA eq.mL ⁻¹)	Total Phenolics (µg gallic acid eq.mL ⁻¹)	ROS (AFU.µL ⁻²)	Antioxidant (10 µg TEAC.mL ⁻¹)
<i>Ganoderma lucidum</i>					
1. China	5.9 ± 1.4	58.2 ± 1.3	236.2 ± 0.4	1350 ± 73	6.3 ± 0.3
2. Korea	23.3 ± 0.5	224.3 ± 1.8	1191.3 ± 4.2	3006 ± 35	17.8 ± 0.6
3. China	24.2 ± 0.0	292.4 ± 10.7	1904.6 ± 35	9362 ± 51	22.8 ± 0.1
4. China	32.1 ± 6.5	187.2 ± 3.5	1470.1 ± 19	8542 ± 105	17.8 ± 0.6
5. China, Hong Kong	28.4 ± 1.4	168.6 ± 1.2	1333.6 ± 4.8	4037 ± 24	10.4 ± 0.5
6. China, Hong Kong	20.0 ± 0.4	49.9 ± 0.9	475.2 ± 1.9	1697 ± 17	5.8 ± 0.2
7. China, Hong Kong	20.9 ± 0.5	286.5 ± 4.7	1490.4 ± 18.8	4889 ± 74	17.2 ± 0.4
8. Thailand	19.2 ± 3.8	85.4 ± 0.4	177.9 ± 2.9	2319 ± 70	2.3 ± 0.3
9. Thailand	9.7 ± 1.0	33.8 ± 2.1	404.7 ± 0.6	892 ± 9	6.5 ± 0.5
10. Thailand	1.6 ± 0.1	0 ± 0	128.2 ± 0.8	291 ± 1	5.1 ± 0.2
11. Thailand	46.6 ± 0.0	0 ± 0	2641.9 ± 2.1	8170 ± 43	40.2 ± 0.2
12. Singapore	10.6 ± 3.2	73.9 ± 14.4	213.8 ± 1	851 ± 8	4.5 ± 0.1
13. Thailand	23.6 ± 0.4	328.1 ± 2.1	1828.4 ± 12.3	10539 ± 186	20.7 ± 0.8
14. Singapore	49.8 ± 2.8	372.8 ± 2.7	2787.5 ± 36.2	11319 ± 61	34.2 ± 0.8
15. China	19.2 ± 0.1	134.8 ± 4.1	2656.9 ± 34.4	1528 ± 8	45.8 ± 0.4
16. Taiwan	19.1 ± 0.1	421.9 ± 3.4	1813.5 ± 38.5	3727 ± 155	17.8 ± 0.8
17. Japan	24.4 ± 1.9	264.3 ± 4.7	1769.7 ± 10	7722 ± 424	20.3 ± 0.4
18. Thailand	6.1 ± 0.7	80 ± 5	234.4 ± 2.5	594 ± 23	4.6 ± 0.5
<i>Cordyceps sp.</i>					
19. United Kingdom	3.9 ± 0.2	75.3 ± 6.4	92.9 ± 3.8	426 ± 23	3.9 ± 0.3
20. China	6.7 ± 0.2	187.3 ± 3.6	814.2 ± 14.8	809 ± 36	7.1 ± 0.5
21. Netherlands	1.7 ± 0.4	52.3 ± 2.5	63 ± 3.5	245 ± 10	1.6 ± 0.2
22. China	6.1 ± 0.0	275.7 ± 2.8	956.4 ± 10.6	1068 ± 14	4.8 ± 0.6
23. China, Hong Kong	2.1 ± 0.3	284.8 ± 2.5	316.7 ± 6.9	353 ± 2	5.1 ± 0.8
<i>Trametes versicolor</i>					
24. United Kingdom	2.9 ± 0.5	73.2 ± 0.7	45.3 ± 0.8	425 ± 21	2.6 ± 0.2
25. China	40.8 ± 0.3	353.5 ± 1.4	2389.4 ± 30.8	8716 ± 76	22.1 ± 0.7
26. China, Hong Kong	58.0 ± 3.8	415 ± 1.1	2900.4 ± 3.8	16760 ± 23	42.4 ± 1.0
27. Germany	10.3 ± 0.7	42.6 ± 1.5	2562.1 ± 20.4	1121 ± 42	46.4 ± 0.1
<i>Phellinus sp.</i>					
28. Thailand	53.0 ± 2.8	2.3 ± 4.2	2657.3 ± 5.8	7730 ± 217	41.5 ± 0.4
29. Thailand	9.5 ± 0.1	52 ± 0.4	1452 ± 15	747 ± 35	27.8 ± 0.9
30. Thailand	43.3 ± 3.2	12.8 ± 2.3	2604 ± 41.5	7077 ± 132	41.2 ± 0.4
31. Thailand	14.2 ± 0.2	60 ± 21.4	2002.7 ± 10.4	1705 ± 0	28.7 ± 0.1
<i>Grifola frondosa</i>					
32. United Kingdom	3.2 ± 0.4	74.1 ± 5	69.8 ± 0	387 ± 23	2.4 ± 0.2
33. China	20.3 ± 2.5	362.9 ± 0.3	1784.6 ± 17.7	5866 ± 129	12.9 ± 0.4
<i>Agaricus brasiliensis</i>					
34. China	5.5 ± 0.8	165.8 ± 2.3	512.5 ± 14.6	825 ± 51	5.7 ± 0.2
35. Netherlands	3.9 ± 0.8	45.9 ± 3.7	85.3 ± 0.4	584 ± 17	2.6 ± 0.1
<i>Antrodia camphorata</i>					
36. China	1.9 ± 0.2	10.8 ± 3.0	191.6 ± 5.0	546 ± 5.0	1.9 ± 0.2
Mixtures					
37. China, Hong Kong	15.0 ± 0.7	77.6 ± 6.7	343.3 ± 2.7	2088 ± 93	5.2 ± 0.4
38. United States	1.2 ± 0.1	156.8 ± 4.1	213.8 ± 0.6	540 ± 1	1.7 ± 0.1
39. China, Hong Kong	6.2 ± 0.2	150.7 ± 3.6	647.8 ± 12.5	2088 ± 2	6.6 ± 0.3

(Sigma D 9132) solution of $25 \text{ mg}\cdot\text{L}^{-1}$ was prepared in 500 mL of methanol of which 1 mL of DPPH was mixed with 10-, 20-, and 40- μL sample mixtures. The solution was incubated for 30 minutes at room temperature (RT). Its absorbance was measured in a Tecan multifunctional fluorescence spectrophotometer at 485 nm. As a standard reference, Trolox (0.5 mM) was used. The inhibition percentage was calculated and compared with the corresponding volume, so that a regression line with a slope could be created. The ratio between the slope of Trolox and the sample was calculated and expressed as Trolox equivalent per milligram dry weight of mushroom extract.

G. Protein Determination

Protein concentrations were determined by Bradford's²⁸ method using bovine serum albumin (BSA) (Sigma A7030) as the standard. Total protein content is expressed as milligrams of BSA equivalents per gram of dry weight.

H. Total Phenolics Determination

Total phenolic content was determined using Folin-Ciocalteu reagent, as described in detail by Ainsworth and Gillespie²⁹ using gallic acid as the standard. Total phenolics are expressed as milligrams of gallic acid equivalents per gram of dry weight.

I. Polysaccharide Determination

Polysaccharide concentrations were determined by the phenol-sulphuric acid method, using D-glucose as the standard.³⁰ Total polysaccharide is expressed as milligrams of glucose equivalents per gram of dry weight.

J. Statistical Analysis

Instat+™ for Windows, an interactive statistical package from Reading University (UK), was used for all statistical analyses. Quantitative measure-

ments of samples were done 4-fold, and all experiments were repeated from two to six times.

III. RESULTS AND DISCUSSION

In the present study, we subjected 43 commercially available medicinal mushroom preparations to hot water extraction. We made 10% (w/v) solutions in water and measured the polysaccharide content, the protein and total phenolics content. ROS-generating activity was measured in the leukemia cell line K562 and the DPPH-TEAC antioxidant activity was determined *in vitro*. The results are given in Table 1. Statistical analysis of the correlation between the different collections of numbers, which was done using Instat+™, shows interesting results (Table 2).

The correlation coefficient of protein content and the other component activities, respectively, is rather small, indicating a limited or no role for protein in the generation of ROS ($R = 0.32$) and in scavenging activity ($R = 0.02$), respectively. This is in contrast to the correlation of the polyphenol content and the other component activities, respectively. Table 2 shows high correlation coefficients for the relationship of polyphenol content and ROS or TEAC, respectively. Figure 1 shows the relationship of polysaccharide content and the phenol content of the hot water extracts. The relationship appears linear and has a correlation coefficient of $R = 0.82$. The slope of the curve indicates that 1 mg of polysaccharide is associated with approximately 50 μg of polyphenols, measured as gallic acid equivalent (GAE) weight.

For comparison, we prepared semipurified fruiting-body extracts of a number of different medicinal mushrooms (Table 3). The polysaccharides in these fruiting-body extracts were precipitated with 70% ethanol and, after centrifugation, dissolved in water. The same parameters as for the commercial extracts were measured, and the same statistical relations were calculated. Semipurified extracts have a much lower polyphenol content, that is, 10 mg/g for the polysaccharide versus 50 mg/g for the commercial extracts.

We determined the correlation between the concentration of phenolic compounds and the

TABLE 2
Correlation of the Contents of Different Components of Medicinal Mushroom Extracts and Their Respective Activities

Relation	Correlation coefficient
Protein and polysaccharide content	R = 0.26
Protein content and ROS	R = 0.32
Protein content and TEAC	R = 0.02
Polyphenol and polysaccharide content	R = 0.82
Polyphenol content and ROS	R = 0.75
Polyphenol content and TEAC	R = 0.93
Polysaccharide content and ROS	R = 0.88
Polysaccharide content and TEAC	R = 0.73

ROS-generating activity for the commercial extracts and found $R = 0.75$ (Table 2). As there is a known linear relation between total phenolics content and antioxidant activity of mushroom extracts,^{3,31} we also determined this relation for the whole collection of commercial extracts, as well as for our own semipurified extracts. We found a linear relationship between the concentration of phenolic compounds and DPPH-TEAC antioxidant activity,

with a correlation coefficient of $R = 0.93$ and $R = 0.90$, respectively (Table 4).

The results further indicate that the ratio of polysaccharide and phenolics is approximately the same for all extracts of the particular group of medicinal mushrooms we measured. The correlation coefficient of ROS generation and concentration of phenolics seems lower than the correlation coefficient of ROS generation and polysaccharide concentration. ROS generation seems to be determined by the concentration of the mixture of polysaccharide and phenolics independent of the species of mushroom or the brand of preparation. Given the use of gallic acid (3,4,5 trihydroxy benzoic acid; FW = 170.2) as a standard in the Folin-Ciocalteu assay for phenolic compounds, the GAE we measured of the extracts vary with the composition of the phenolic compounds involved. The GAE weight is different for the different phenolics because the method applied (Folin-Ciocalteu) measures phenolic OH groups as a determinant of quantity. Moreover, the composition and contents of the medicinal mushroom samples we tested are disputable; all samples are of commercial origin, without independent proof of quality. This may

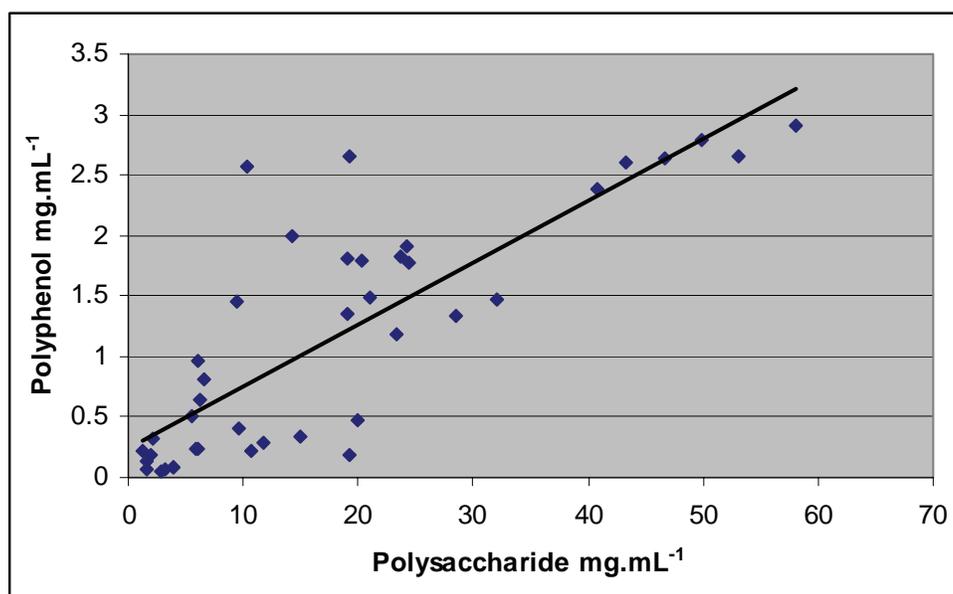


FIGURE 1. The relationship between the concentration of polysaccharide and phenolic compounds of extracts of a collection of medicinal mushrooms. Y axis: polyphenol content (mg.mL^{-1}) weight equivalents of gallic acid. X axis: polysaccharide content (mg.mL^{-1}).

TABLE 3
Polysaccharide Content, Total Phenol Content, and ROS-Generating and Antioxidant Activity (DPPH-TEAC) of Typical Hot Water Extracts of Different Medicinal Mushrooms

Species	Polysaccharide (mg.mL ⁻¹)	Total phenol (10 µg.mL ⁻¹)	ROS (AFU.nL ⁻¹)	DPPH-TEAC (10 µg. mL ⁻¹)
<i>Agaricus bisporus</i>	1.6 ± 0.0	2.0 ± 0.0	0.2 ± 0.0	2.3 ± 0.2
<i>A. brasiliensis</i>	36.4 ± 0.5	29.1 ± 0.1	4.1 ± 0.2	23.0 ± 0.2
<i>Auricularia polytricha</i>	1.3 ± 0.0	1.2 ± 0.1	0.4 ± 0.0	2.7 ± 0.1
<i>Coprinus comatus</i>	6.0 ± 0.3	14.1 ± 0.6	0.4 ± 0.0	9.0 ± 0.2
<i>Cordyceps militaris</i>	2.2 ± 0.5	5.0 ± 0.8	0.2 ± 0.0	5.5 ± 0.6
<i>Trametes versicolor</i> 1	34.6 ± 0.8	45.6 ± 0.8	2.6 ± 0.1	20.6 ± 0.3
<i>T. versicolor</i> 2	10.9 ± 0.1	15.6 ± 0.3	1.7 ± 0.0	6.1 ± 0.5
<i>Grifola frondosa</i>	10.8 ± 1.0	18.0 ± 0.1	1.8 ± 0.0	6.4 ± 0.9
<i>Ganoderma lucidum</i>	7.5 ± 0.1	11.4 ± 0.1	1.2 ± 0.0	8.3 ± 0.6
<i>Hericium erinaceus</i>	3.0 ± 0.5	1.7 ± 0.1	0.6 ± 0.0	3.5 ± 0.6
<i>Lentinus edodes</i>	7.5 ± 0.5	7.8 ± 0.3	0.4 ± 0.0	2.9 ± 0.6
<i>Phellinus linteus</i>	4.1 ± 0.0	6.4 ± 0.1	0.2 ± 0.0	4.2 ± 0.5

TABLE 4
The Statistical Relationships between the Concentrations of Polysaccharide (mg.mL⁻¹) and Phenolic (mg.mL⁻¹) Compounds in Extracts of Medicinal Mushrooms and Their ROS-Generating (AFU.mL⁻¹) and Antioxidant Activities (mg TEAC.mL⁻¹)

Units	Commercial extracts	Prepared extracts
Phenolics (Y) and polysaccharide (X)	Y = 0.05X + 0.23 R = 0.82 S.E. slope: 0.006 95% C.I. slope: 0.039–0.063	Y = 0.01X + 0.026 R = 0.93 S.E. slope: 0.001 95% C.I. slope: 0.072–0.130
ROS (Y) and phenolics (X)	Y = 3.03X + 0.08 R = 0.75 S.E. slope: 0.428 95% C.I. slope: 2.164–3.897	Y = 7.38X + 0.17 R = 0.81 S.E. slope: 1.709 95% C.I. slope: 3.57–11.19
ROS (Y) and polysaccharide (X)	Y = 0.22X – 0.41 R = 0.88 S.E. slope: 0.019 95% C.I. slope: 0.186–0.263	Y = 0.09X + 0.17 R = 0.93 S.E. slope: 0.012 95% C.I. slope: 0.067–0.119
TEAC (Y) and phenolics (X)	Y = 0.14X – 0.004 R = 0.93 S.E. slope: 0.0084 95% C.I. slope: 0.120–0.154	Y = 0.46X + 0.02 R = 0.90 S.E. slope: 0.0723 95% C.I. slope: 0.302–0.625
TEAC (Y) and ROS (X)	Y = 0.02X + 0.022 R = 0.60 S.E. slope: 0.005 95% C.I. slope: 0.013–0.031	Y = 0.05X – 0.02 R = 0.89 S.E. slope: 0.01 95% C.I. slope: 0.032–0.069
TEAC and polysaccharide	Y = 0.007X + 0.034 R = 0.72 S.E. slope: 0.001 95% C.I. slope: 0.005–0.009	Y = 0.005X + 0.02 R = 0.96 S.E. slope: 0.00 95% C.I. slope: 0.004–0.007

Note: R = correlation coefficient; S.E. = standard error; C.I. = confidence interval.

also be deduced from the correlation coefficients that are for all but one relationship lower for the commercial extracts than for our own fruiting-body extracts. Gallic acid itself could not generate ROS in K562 cells (data not shown).

Ethanol-precipitated polysaccharides of *Agaricus bisporus*, *A. brasiliensis*, *Trametes versicolor*, *Ganoderma lucidum*, *Grifola frondosa*, *Lentinus edodes*, and *Phellinus linteus* were hazel colored. *Hericium erinaceus* and *Coprinus comatus* yielded white polysaccharides. All ethanol-precipitated and water-dissolved polysaccharide extracts of higher basidiomycetous mushrooms induced ROS generation in K562 cells, although with lower activity than the nonpurified commercial extracts.

The results (Table 4) of a regression analysis show a linear relation between the concentration of phenolic compounds and the ROS activation, with a high correlation coefficient ($R = 0.81$) but lower than that found for the correlation between polysaccharide content and ROS ($R = 0.93$) and between polysaccharides and phenolic compounds ($R = 0.93$), respectively. The relation between ROS and antioxidant activity also appeared linear, with a correlation coefficient $R = 0.89$.

The commercial extracts have not been further purified by ethanol precipitation and are, therefore, rich in ethanol-soluble polyphenols. It is interesting to observe that the ROS-generating activity per milligram of phenolic compounds is two times lower for the commercial extracts than for fruiting-body extracts of our own laboratory, and that the ROS-generating activity per milligram of polysaccharides is two times lower. This is exactly as expected for a complex of polysaccharide and polyphenols that exerts the biological effects mentioned. When TEAC is considered for an analogous comparison of correlation coefficients of the two groups, it is shown that, again, the biological effect is much higher for the prepared extracts than for the commercial extracts per milligram polyphenols, but almost identical per milligram of the polysaccharide. This suggests that different polyphenols in the extracts have different functions, that is, either ROS generating or radical scavenging.

The apparent linear relationship between antioxidant activity and concentration of phenolics, having a correlation coefficient $R = 0.90$, is not

surprising. As demonstrated in earlier studies,^{30,32} phenols are the major antioxidant components of mushrooms; *Agaricus bisporus* is an exception, with ergothioneine.⁴

In an earlier study,²⁴ we have shown for an *A. bisporus* extract that purified glucan devoid of measurable phenolic compounds cannot activate ROS in peripheral blood monocyctic cells (PBMCs) and in K562 and Jurkat cells. The present data show that commercial medicinal mushroom preparations are able to generate ROS in the K562 cell line and that the ROS-generating capacity is correlated with the concentration of polysaccharides and phenolic compounds in the extract. Not surprisingly,³² we observed a strong correlation between the concentration of phenolic compounds in the extracts and the DPPH-TEAC antioxidant activity. The mushroom extracts we tested contain both oxidative and antioxidative compounds. Again, the commercial extracts demonstrate lower antioxidant activity per milligram of phenolic compounds than the fruiting-body extracts from our own laboratory. However, when calculated per milligram of polysaccharide, the value is almost the same.

Although phenolic compounds are widely believed to function as antioxidants, it is clear that they can also generate reactive oxygens. The latter was published previously for polyphenols when accompanied by Cu (II)³³ and Fe (II), respectively.³⁴ Recently Maeta et al.³⁵ also demonstrated that antioxidative green tea polyphenols function as prooxidants and activate oxidative stress-responsive transcription factors in yeast. The question remains, however, if the same polyphenol molecule encompasses both functions or if this reflects the presence of a mixture of polyphenols.

ROS generation is one of the causes of the apoptosis-increasing ability of extracts of *Phellinus linteus*, which consists mostly of polysaccharides, in the cases of lung³⁶ and prostate³⁷ tumor cells. Hypothetically, phenolic compounds may determine ROS activation, depending on their primary structure and conformation. From our studies, it seems likely that both polyphenols and polysaccharides are involved in ROS generation and possibly also in DPPH-TEAC antioxidant activity.

Phenolic compounds present in mushrooms may in fact be complexed to soluble β -D-glucan by weak

chemical linkages. It has already been found that polysaccharides are able to bind to colored phenolic compounds by interaction with their carbonyl groups³⁸ or by hydrophobic interaction.³⁹ Such conformational changes have been demonstrated for zearalenone complexation with β -(1,3)-D-glucans branched by β -(1,6)-D-glucans, which depends on the interaction between the glucan hydroxyl groups and the ketone and hydroxyl groups of the zearalenone.⁴⁰ Given the latter's structural analogy to the oxidized monophenol derivatives that occur as intermediary products in the tyrosinase-driven browning of mushroom extracts,⁴¹ it seems likely that the brown-colored mushroom extract consists of such a complex.

As can be seen from Table 4, the DPPH-TEAC antioxidant activity of the mushroom polyphenols in the extracts is quantitatively comparable to that of trolox itself. The amount of polysaccharide that is isolated from the different mushrooms and that complexes with small amounts of polyphenol components seems to be the major determinant of the ROS-generating and DPPH-TEAC antioxidative activity of medicinal mushroom extracts.

It is tantalizing to speculate on the possible role of mushroom extracts that combine pro- and antioxidative properties. A mushroom glucan-phenol complex may bind to one or several of the receptors TLR2, TLR4, CR3,⁴² and Dectin-1,^{43,44} which leads to the rapid generation of intracellular ROS and to the activation of cytokine gene expression. ROS may induce a protective oxidative state against intracellular bacteria and viruses but, if not reduced, is transported outside the cell where the receptor-bound glucan-phenol complex is able to exercise its antioxidative action. Several aquaporins⁴⁵ have been suggested to function in the transport of hydrogen peroxide through the cell membrane.⁴⁶ Cancer cells, with their proven mitochondrial defects in oxidative phosphorylation, may thus be driven into apoptosis and cell death.¹⁸

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