

Screening Antitumor Activity of Low-Molecular-Weight Compounds Obtained from the Fruit Bodies of Family Agaricaceae Chevall. (Higher Basidiomycetes)

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Numerous higher Basidiomycetes are an important source of medicinal substances, applied in the treatment of a wide range of ailments. The anticancer activity of mushroom-derived components has long been known and is well documented. The majority of active substances are high-molecular-weight (HMW) (200–400,000 Da) polysaccharides and polysaccharide complexes, active hexose correlated compounds (AHCC), polysaccharide-peptides, nucleosides, triterpenoids, complex starches, and other metabolites. Of the known 918 Agaricaceae species (Kirk et al., 2001), only 37 species of eight genera were screened on HMW antitumor active compounds (Ohta et al., 1999), and three species (*Agaricus langei* (F.H. Møller et Jul. Schäff.) Maire, *Leucoagaricus carneifolius* (Gillet) S. Wasser, *L. leucothites* (Vittad.) M.M. Moser ex Bon), were screened for low-molecular-weight (LMW) antitumor-active substances (Yassin et al., 2003). While activity of HMW substances is attributed to the immune-modulating function, LMW substances might show a direct antitumor effect against cancer cells.

The current study examined the anticancer activity of extracts, obtained using different organic solvents (dichloromethane: DCHM, ethyl acetate: EtAc, and methanol: Meth). Overall, 13 species (eight *Agaricus*, three *Macrolepiota*, one *Leucoagaricus*, and one *Lepiota*) were screened. Six lines of three

types of cancer were studied: prostate cancer cell lines LNCaP (androgen-dependent prostate cancer; the cell line carries mutation T877A in the androgen receptor); DU145; PC3 (both lines androgen-independent); breast cancer cell line MDA-kb2 (MDA-MB-453); patient-derived chronic myelogenous leukemia cell (CML) lines (K562); and laboratory model of CML, Baf3/p185 Bcr-Abl. All cell lines were subjected to 36 extracts, each of which was tested in two concentrations. When setting up the experiment, attention was focused on extracts able to inhibit nonselectively all cell lines used, as well as extracts that would inhibit growth of a specific type of cancer selectively.

Extracts showing significant growth inhibition (50% or more) against all cell lines used were considered to be nonselective. Only *Macrolepiota excoriata* (Schaeff.) M.M. Moser extract inhibited growth of all cancer cell lines by more than 50%.

Extracts that inhibited tumor growth of each cancer cell line by 50% or more and showed minimal activity (30% or less) against other cell lines used were considered as selective mushroom extracts. A total of 30 extracts were shown to be selective for the Baf3/p185 Bcr-Abl CML line. Out of these, 22 extracts were active at a concentration of 0.25 mg/mL and thus are considered most promising for further research, especially with *Agaricus xanthoderma* Genev.,

A. fissuratus (F.H. Møller) F.H. Møller, *A. arvensis* Schaeff., *A. cf. subrufescens*, and *Lepiota americana* (Peck) Sacc. extracts. Extracts from two latter species demonstrated the highest inhibition rate of the Baf3/p185 Bcr-Abl cell line (Table 1).

All of them are known medicinal species of the

family Agaricaceae (Didukh et al., 2003, 2004). *Agaricus xanthoderma* is a poisonous species of group I (causing gastrointestinal disorders) and is known to contain psalloytin, an antimicrobial substance of unknown nature, active against gram-positive bacteria *Salmonella* sp. (Shivrina, 1965). DCHM extracts

TABLE 1. Extracts of Agaricaceae Species Fruit Bodies Selectively Inhibiting Growth of Baf3/p185 Bcr-Abl Leukemia Cell Line

Species	EC, mg/mL	Solvent	Growth inhibition, %	
			Baf3/p185 Bcr-Abl	K562
<i>Agaricus arvensis</i>	1	DCHM	70.37	27.48
	0.25	DCHM	56.04	-3.54
	0.25	Etac	57.81	21.21
<i>Agaricus campestris</i>	1	Meth	74.95	23.22
	0.25	DCHM	60.73	20.14
<i>Agaricus fissuratus</i>	0.25	Etac	54.46	13.04
	1	Meth	70.86	16.11
<i>Agaricus pequinii</i>	0.25	DCHM	63.44	7.47
		Etac	59.89	-0.75
<i>Agaricus cf. subrufescens</i>	0.25	Etac	55.71	-5.00
	1	Meth	62.98	19.62
<i>Agaricus vaporarius</i>	0.25	DCHM	65.98	10.251
	0.25	Etac	62.66	2.20
	1	Meth	57.51	-6.57
<i>Agaricus xanthoderma</i>	0.25	DCHM	66.46	22.82
	0.25	Etac	75.93	15.23
	1	Meth	66.68	9.99
	0.25	Meth	62.08	-5.62
<i>Macrolepiota procera</i>	0.25	Etac	50.29	24.01
	0.25	Meth	54.66	26.15
<i>Macrolepiota rachodes</i>	0.25	Etac	63.18	8.30
	1	Meth	69.10	8.87
	0.25	Meth	62.02	-6.52
	0.25	Meth	65.79	29.45
<i>Leucoagaricus leucothites</i>	0.25	Etac	58.64	25.79
	0.25	DCHM	54.01	18.10
	1	Meth	63.15	0.253
	0.25	Meth	52.37	-6.40
<i>Lepiota americana</i>	0.25	Meth	53.43	21.08
	0.25	Etac	71.01	29.19

Note: EC: extract concentrations; SD: standard deviation; DCHM: dichloromethane; EtAc: ethyl acetate; Meth: methanol

are able to inhibit growth of *Escherichia coli*, *Biomphalaria glabrata*, and *Aedes aegypti*. Of the three *A. xanthoderma* extracts used in this study, EtAc extract was most active against Baf3/p185 Bcr-Abl.

Agaricus arvensis is known to help in the case of hypertension, in inhibiting cells of Sarcoma 180 and Erlich's carcinoma, and in inhibiting the growth of bacterial cells (Ying et al., 1989). *A. fissuratus* is very close to *A. arvensis*. Some authors (Nauta, 2001) consider these two species as conspecific. Despite their similarity, the species differed in extracts selective to Baf3/p185 Bcr-Abl: DCHM extract for *A. arvensis* and EtAc extract for *A. fissuratus*.

Agaricus cf. *subrufescens* extract 7a, extracted with DCHM, inhibited growth of cells by 89.76%. *A. cf. subrufescens* is closely related to the important culinary–medicinal species *A. brasiliensis* S.Wasser et al. The latter species is known for its anticancer, antiviral, and antimutagenic effects, as well as other activities.

Lepiota americana contains the aromatase-inhibiting fraction (Kim et al., 2000). However, active compounds of the fraction have not been elucidated. Aromatase-inhibiting function plays an important role in breast cancer cell proliferation.

None of the extracts were selective for other cell lines tested. Thus, the major part of active extracts revealed in the study was neither strictly selective nor strictly nonselective.

Based on the concentration at which an extract is active, the percent of growth inhibition, and standard deviation values, *Agaricus xanthoderma* and *Lepiota americana* extracts appear to be most promising in the study of the Baf3/p185 Bcr-Abl cell line.

Application of extracts against other cell lines seems to be less promising as growth of the prostate cancer cell lines (DU145, LNCaP, PC58) and breast cancer (MDA-kb2) were inhibited by extracts in high concentrations. PC58 line turned out to be most resistant to all kinds of extracts used.

Thus, the screened extracts turned out to be most active against chronic myelogenous leukemia cell lines. Most promising for further research are *Lepiota americana* and *Agaricus xanthoderma* extracts, selective to Baf3/p185 Bcr-Abl. Dichloromethane turned out to be the most efficient solvent for extraction of low-molecular-weight biologically active substances from fruit bodies.

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