

Brief Communication

Antitumor Activity of Aqueous Extracts of Edible Mushrooms¹Tetsuro Ikekawa,² Nobuaki Uehara, Yuko Maeda, Miyako Nakanishi, and Fumiko Fukuoka

National Cancer Center Research Institute, 5 - 1 Tsukiji, Chuo-ku, Tokyo, Japan

Summary. Aqueous extracts of seven species of edible mushrooms were tested for their antitumor activity against transplanted Sarcoma 180 in Swiss albino mice. All of the aqueous extracts except that of *Auricularia auricula-judae* (Bull. ex Fr.) Quél. highly inhibited the growth of transplanted tumors. The active antitumor component of *Lentinus edodes* (Berk.) Sing. was not adsorbed by the acetate form of a weakly basic ion-exchange resin. It was nondialyzable and precipitated with the addition of ethanol or acetone. The active antitumor material was suggested by chemical analysis to be a polysaccharide.

Introduction. The antitumor activities of high molecular weight substances of some wild Basidiomycetes for transplanted Sarcoma 180 in Swiss albino mice were reported in previous papers (1, 4). The suggestion was made that the antitumor activities were due to indirect host-mediated effects (2, 3, 5-7).

In this study we are using the same bioassay method that was described previously (1) to determine the antitumor activities of aqueous extracts of seven species of edible mushrooms occurring in Japan.

Materials and Methods. The method of Nakahara and co-workers (2, 3) was used as an assay for antitumor activity. Seven-day-old Sarcoma 180 ascites tumor cells were implanted subcutaneously into the right groin of Swiss albino mice. After 24 hours, these mice were injected intraperitoneally with 200 mg/kg/day doses of sample materials for 10 days. After observing the tumor growth for 5 weeks, the tumor weights of treated mice were compared with those of untreated mice.

One kg of mushrooms was extracted for 4.5 hours with two liters of water maintained at 95-97°C by a boiling water bath, and the extracts were evaporated under reduced pressure and then freeze-dried. The yield was 5.4% in *Lentinus edodes* (Berk.) Sing. Ten gm of the extract of *Lentinus edodes* (Berk.) Sing. was dissolved in about 200 ml of water and purified by passage through a 6 x 50 cm column of Duolite A-7 or S-30 (acetate form) ion-exchange resin. The column was eluted with 3 liters of water, and the eluate was evaporated to a volume of

approximately 500 ml. After adding 10 volumes of acetone, the precipitate was centrifuged, washed with acetone several times, and dried. A 5% yield of white powder was obtained. The column was then eluted with 3 liters of 0.25 N sodium hydroxide. After neutralization the eluted solution was evaporated under reduced pressure, and 3 volumes of ethanol were added to obtain the precipitate.

Results and Discussion. As seen from the tumor-inhibiting activities shown in Table 1, all the aqueous extracts except that of *Auricularia auricula-judae* (Bull. ex Fr.) Quél. highly inhibited the growth of the transplanted tumor.

A component of *Lentinus edodes* (Berk.) Sing. extract, eluted from a Duolite column with water, showed a 94.8% tumor inhibition at a 200 mg/kg/day dosage, but the alkaline-eluted component gave only 62.5% tumor inhibition at the

Table 1

Samples	Complete regression	Average tumor weight (gm)	Inhibition (%)
<i>Lentinus edodes</i> (Berk.) Sing.	6/10	2.2	80.7
Control	0/10	11.4	
<i>Flammulina velutipes</i> (Curt. ex Fr.) Sing.	3/10	2.1	81.1
Control	0/10	11.4	
<i>Pleurotus ostreatus</i> (Jacq. ex Fr.) Quél.	5/10	2.3	75.3
Control	0/10	9.4	
<i>Pleurotus spodoleucus</i> (Fr.) Quél.	0/8	2.3	72.3
Control	0/9	8.3	
<i>Pholiota nameko</i> (T. Ito) S. Ito et Imai	3/10	1.4	86.5
Control	0/10	10.4	
<i>Tricholoma matsutake</i> (S. Ito et Imai) Sing.	5/9	0.76	91.8
Control	0/9	9.3	
<i>Auricularia auricula-judae</i> (Bull. ex Fr.) Quél.	0/9	4.9	42.6
Control	0/9	8.3	

Antitumor activity of aqueous extracts of some edible mushrooms against Sarcoma 180. Swiss albino mice were given intraperitoneal injections of 200 mg/kg/day of Sarcoma 180 cells for 10 days. The vehicle was distilled water.

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²Present address: Department of Biochemistry, Purdue University, Lafayette, Indiana 47907.

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same dosage. A white powder was obtained through acetone precipitation of the fraction eluted with water. The inhibition ratio of tumor growth by the white powder was 97.3%; the tumor completely regressed in six of nine mice at a 200 mg/kg/day dosage. A loss of body weight in treated mice, which might cause tumor-growth inhibition, was not observed.

The level of tumor inhibition of this material towards Sarcoma 180 shows that it is the most effective of the materials produced by *Lentinus edodes* (Berk.) Sing.

The powder thus obtained was further purified in two ways. A portion was dialyzed for 3 days against deionized water and then lyophilized. A second portion was dissolved in water, 4 volumes of ethanol were added, and the precipitate was isolated by centrifugation. Both the nondialyzable and the ethanol-precipitated materials showed tumor-growth inhibition, but the dialyzable and supernatant did not show inhibition.

The active antitumor material appears to be a polysaccharide in nature as indicated by color reactions, IR and NMR spectral data, and elemental analysis. The nitrogen percentage was under 1.0%. The material migrated as a single spot with slight tailing on a thin-layer chromatogram using a crystalline cellulose layer. Gas chromatographic analysis of the acid hydrolysate of the material showed it to be made up mainly of D-glucose and small amounts of D-galactose and D-fructose.

The active antitumor components of other species are believed to be compounds related to this material. Further chemical and biologic studies are now in progress.

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