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Studies on the Antineoplastic Components of Korean Basidiomycetes*

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韓國產 擔子菌類의 抗癌 成分에 관한 研究*

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Abstract: To investigate antitumor components of Korean higher fungi, the carpophores of *Ganoderma lucidum* (Fr.)Karst. were collected at Gal-mae-ri in Gyeong-gi Province and extracted with 0.1N sodium hydroxide solution. The extract was purified by dialyzing through Visking tube and a dark brownish power was obtained. The antitumor activity of the fraction was tested against sarcoma 180 implanted in mice. The tumor inhibition ratio of the fraction against the tumor was 87.6% in doses of 50 mg/kg/day for the period of ten days. The tumor in five of 15 mice was completely regressed.

The antitumor fraction was chemically analyzed and found to be a complex of polysaccharide and protein, from which four monosaccharides and eighteen amino acids were identified.

In the last decade, investigations were carried out on polysaccharides, which had been neglected as pharmacologically inert compounds, for their newly recognized bioactivity. As a result, some useful microbial polysaccharides such as antitumor polysaccharides, anti-inflamatory polysaccharides and others were found.

Polysaccharides (Chihara *et al.*, 1969; Fukuda *et al.*, 1975; Ikekawa *et al.*, 1969; Nakahara *et al.*, 1964 and Komatsu *et al.* 1969, 1967; Nakanish *et al.* 1963; Sasaki *et al.* 1971; Shimura *et al.* 1978; Tanaka *et al.* 1965 and 1967) from some members of the class Basidiomycetes, that is, *Lentinus edodes*, *Pleurotus ostreatus*, *Elfvingia applanata*, and *Coriolus versicolor*, showed high antitumor activities *in vivo*. However, no examination for antitumor polysaccharides of Korean higher fungi has been made except that of Kim (Choi *et al.*, 1975; Kim *et al.*, 1970, 1971, 1973, 1976[a], 1977, 1976[b], 1978[a], 1978[b], 1978[c], 1978[d], 1978[e], 1978[f], 1978[g], 1979[a] and 1979[b]; Kwon *et al.*, 1980; Lee *et al.*, 1979[a], and 1978[b]; Min *et al.*, 1980; Shin et al., 1978; Park *et al.*,, 1979; Park *et al.*, 1979) in 1978. To examine antitumor polysaccharides of Korean higher fungi, the authors collected *Ganoderma lucidum* which has long been used as one of folk remedies and whose Korean name is "*Young Ji*" or "*Bul-no-cho*" and examined its antitumor activities.

Materials and Methods

1. Fungal Material

The carpophores of *Ganoderma lucidum* (Fr.) Karst., a member of the family *Polyporaceae*, were collected at Gal-mae-ri in Gyeong-gi Province during the period from September to October 1977.

There are several different types of this species all over the world(Imazcki and Hongo, 1957; Rinaldi and Tyndalo, 1974; Singer, 1975). In Korea, the authors found two different types which can

*Part XVI, Studies on the Constituents of the Higher Fungi of Korea.

easily be distinguished by the surface of the pileus. One of them has a shiny surface as if varnished and the other has a coarse suface. The fungal material used in this study belongs to the latter. The mushrooms were dried in open shadow for two days and in an oven at 50° for five hours and kept in a glass bottle. Some of the dried carpophores were preserved as voucher speciemens at the Department of Microbial Chemistry, College of Pharmacy, Seoul National University. The characteristics of *Ganoderma lucidum* collected are as follows: cap circular or flabellate, 3~10cm; generally horizened but sometimes slanted; surface of the cap divided into concentric steps, centered on the top of the stem; covered with a reddish brown or reddish ochreous crust. Stem 5~13 ×0.6~1.2cm; more dark colored than the cap; underground part more swollowed showing white mycelium. Flesh elastic. Pores (Fig. 3) white; 30~40/mm^2. Spores brownish; 10~11×6.5~7.5μm; ovoid; of characteristic *Ganoderma* type (Fig. 4).

2. Extraction and Isolation

One-hundred grams of the dried carpophores of *Ganoderma lucidum* were homogenized with 1000 ml of 0.1N sodium hydroxide solution for five minutes in a Waring blendor (Scheme I). Extraction was performed by stirring and refluxing at 97° for eight hours. After filtration, the residue was extracted under the same condition with 800ml and then 700ml of the same solvent for eight hours, respectively. All filtrates were combined and condensed in a rotary vacuum evaporator to 800ml. The condensed filtrate was dialyzed at 5° for seven days with Visking tube. After dialysis this extract was condensed into 350ml and centrifugated for 40 minutes at 6000 g at 20° (Beckman model J-21 centrifuge). The supernatant containing polysaccharide was dried at −65° in a lyophilizer (Edwards high vacuum model No. EF03). An odorless and tasteless dark brownish powder was obtained. This powder was used as the polysaccharide preparation in the following experiments.

3. Antitumor Test

(1Tumor cells

Sarcoma 180 cells were implanted into the intraperitoneal cavity of A-strain mice of female sex weighing about 20g (Scheme II). After cultivation for ten days, the animals were killed and sarcoma 180 ascites fluid was collected with a syringe in an ice-cold bath. Ascites fluid was diluted with saline to adjust the tumor cell concentration at 1×10^7 cells/ml.

2) Preparation of test solution

Two hundred milligrams of the dark brownish powder which was obtained from the carpophores of *Ganoderma lucidum* were dissolved in 20 ml of saline. For control, physiological saline was used. These solutions were autoclaved and stored in a refrigerator.

3) Animal test

For tumor implantation, 0.1 ml of ascites fluid (1×10^7 cells/ml) was injected into the right groin of each mouse. As a test group of 15 mice, each mouse was injected intraperitoneally with 50 mg of the test compound per killogram body wight everyday. The injection was initiated on the fifth day after tumor implantation. As a control group, nine mice were taken and each of them was injected with physiological saline by the same method as the test group. Average tumor weight was estimated at the end of 28 days and inhibition ratio was determined by comparing with tumors of the control mice. Complete regression was also examined.

4. Chemical Analysis of the Extract

The concentration of test solutions containing the polysaccharide of *Ganoderma lucidum* used in the reactions was 1% (w/v) in all cases.

1) Molish test

Two drops of α-naphthol reagent (5.0% ethanol solution) were added to two milliliter test solution. After shaking, one milliliter of conc. sulfuric acid was carefully poured.

2) Anthrone test

Two milliliters of anthrone reagent (0.2% conc. sulfuric acid solution) were added to two milliliter test solution and mixed completely.

3) Xanthoprotein test

One milliter of conc. nitric acid was added to one milliliter of test solution.

4. Iodine test

One drop of dilute hydrochloric acid was added to two milliliter test solution and two drops of iodine solution were addd.

5) Tryptophan test

Seven milliliters of 77% sulfuric acid were added to one milliliter test solution and cooled to 10～15° with water. One milliliter of fresh 1% tryptophane solution was added to the above solution and heated on a boiling water bath for 20 minutes and cooled to room temperature.

6) Ninhydrin test

Two milliliters of 1% ninhydrin solution were added to two milliliter test solution (neutral pH) and heated on a boiling bath for two minutes.

7) Ninhydrin test on hydrolysate

The sample was hydrolyzed with 6N-hydrochloric acid at 110° for 24 hours in an ampoule filled with nitrogen gas. After filtration ninhydrin test was conducted.

8) Biuret test

Three drops of cupric sulfate solution were added to two milliliter test solution. After two milliliters of 10% sodium hydroxide solution were added and mixed thoroughly, the color change was observed.

9) Lowry-Folin test

One hundred milliliters of fresh alkaline copper reagent were prepared in order of one ml of 1% cupuric sulfate, one ml of 2% sodium tartrate and 98 ml of 2% sodium carbonate in 0.1N sodium hydroxide solution. Three solutions were thoroughly mixed in a 100-ml Erlenmyer flask immediately. Five milliliters of alkaline copper reagent were added to one milliliter test solution. After ten minutes, 0.5ml of Folin-Ciocalteu reagent was added and mixed thoroughly. After 30 minutes the color change was observed.

5. Assay for Polysacchride of the Extract

1) Polysaccharide content

Polysaccharide content of the extract was calculated by anthrone method using glucose as a standard sugar with Hitachi Recording Spectrophotometer at 625 nm.

2) Sugar analysis

Five milligrams of the extract were dissolved in two ml of 3% hydrochloric acid-methanol in an ampoule.

The ampoule was filled with nitrogen gas and sealed. Methanolysis was carried out at 100±5° for 20 hours. The methanolysate was filtered and evaporated to dryness in a rotary vacuum evaporator. After dissolving in one milliliter of pyridine, trimethylation was carried out using 0.2ml of hexamethyldisilazane and 0.1ml of trimethylchlorosilane.

Gas liquid chromatography was performed under the condition in Table I, using authentic sugars as standards.

Table I. Measurement condition (G.L.C.)

Column	3% O V-17 (80～100 mesh Shimalite) 3mm ID × 2m boronsilicate glass column.
Temperature	Column: 140°C Detector: 240°C
Flow rate	N_2: 50ml/min H_2: 60ml/min(0.8kg/cm^2) Air: 88ml/min(1.2kg/cm^2)
Attenuation	16×10^2 a.f.s.(ampere full scale)

6. Assay Protein of the Extract

1) Protein content

Protein content of the extract was calculated by Lowry-Folin method[26] using albumin as a standard protein with Hitachi recording spectrophotometer at 750 nm.

2) Amino acid analysis

Two hundred milligrams of the extract were dissolved in 50 ml of 6N-hydrochloric acid and divided into 13 ampoules. The ampoules were filled with nitrogen gas and sealed. Hydrolysis was carried out at 110° for 24 hours. The hydrolysate was filtered and evaporated to dryness in a rotary vacuum evaporator. Dry substance was dissolved in 10 ml of 0.1N-hydrochloric acid solution and diluted with 0.2M sodium citrate buffer (pH 2.2). Under the condition of Table II, amino acids were analyzed with Hitachi amino acid autoanalyzer Model KLA-5.

Table II. Measurment condition (amino acid analyzer)

Column size	9mm ID×550mm 9mm ID×100mm
Ion exchange resin	For AN analysis: Hitachi custom ion exchange resin No. 2613.. 50g For B analysis: Hitachi custom ion-exchange resin No. 2611. 10g
Flow rate	Buffer solution: 60 ml/hr Ninhydrin reagent: 30 ml/hr
Wave length	15mm tubular flow cell, 570 nm(red) 440 nm(green)
Buffer solution	pH 3.25, pH 4.25, pH 5.28 Na citrate buffer solution
Column temperature	55°C
Reaction bath	Reaction coil: 0.5 mm ID×28.8 m Temp.: 100°C
Recorder	Chart speed: 90 mm/hr
Volume of sample coil	0.5 ml ± 1 %

Standard amino acids were also analyzed under the same condition and a chromatogram was also obtained. The amino acid mixture used for standardization contained 0.1μ mol of each amino acid (in case of proline, 0.2μ mol) dissolved in 0.5ml of 0.2M sodium citrate buffer (pH 2.2). Contents of each amino acid were calculated from chromatograms by H W law.

Results and Discussion

1. Yield of the Polysaccharide Fraction

From 100g of the dried carpophores of *Ganoderma lucidum*, 9.92g polysaccharide fraction was obtained (yield 9.92%).

2. Antitumor Test

Antitumor effect of the polysaccharide fraction from *Ganoderma lucidum* on sarcoma 180 in mice is shown in Table III. Complete regression was also examined on twenty-eighth day after the tumor implantation. The tumor in five of the 15 mice was completely regressed.

4. Chemical Assay for the Extract

The results in Table IV showed that the extract consisted of protein and polysaccharide.

5. Assay for Polysaccharide of the Extract

1) Polysaccharide content

Th polysaccharide content was 27% of the extract.

2) Sugar analysis

The component sugars of the extract were determined by Shimadzu gas chromatograph GC-4BM.

Glucose, mannose, glactose and xylose were detected as component sugars of the polysaccharide(Table V). Fig. 6 shows G.L.C. pattern of the sugars.

6. Assay for Protein of the Extract

1) Protein content

The protein of the extract was 72%.

2) Amino acid analysis

The chromatograms of amino acids of the sample are shown in Fig. 7.

Table III. Antitumor effect of the polysaccharide fraction from *Ganoderma lucidum* on sarcoma 180 in mice

	Average tumor weight(g)	Inhibition ratio(%)	100% regression
Control	4.52		0/9
50mg/kg/day	0.56	87.6%	5/15

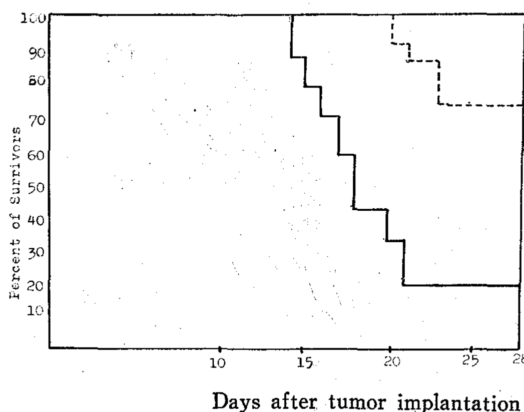

Fig. 5. Effect of the polysaccharide fraction on survival of mice implanted with sarcoma 180.

Table IV. Results of various color reactions on the extract from *Ganoderma lucidum*

Method	Result	
Molish test	purple	++
Anthrone test	dark green	++
Xanthoprotein test	yellow	++
Iodine test		−
Tryptophan test	violet-brown	++
Ninhydrin test	blue-violet	+
Ninhydrin test after acid hydrolysis	violet	++
Biuret test	purple-blue	+
Lowry-Folin test	dark blue	++

Table V. Contents of the sugars of the polysaccharide fraction of *Ganoderma lucidum*

Monosaccharide	Content(%)
Xylose	3.4
Mannose	15.8
Galactose	8.3
Glucose	72.5

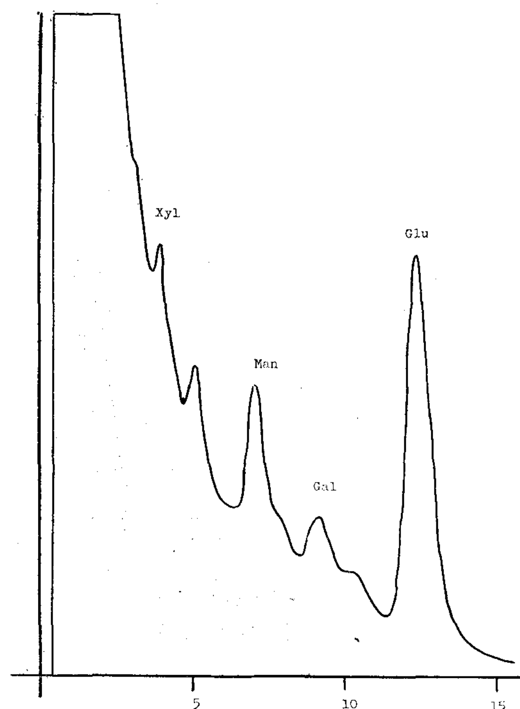

Fig. 6. G.L.C. pattern of monosaccharides of the extract of *Ganoderma lucidum*.

Table VI. The contents of amino acids in the protein fraction of the extract of *Ganoderma lucidum*

Amino acid	Content(mg/g)	Per cent
Lysine	11.40	4.59
Histidine	4.60	1.85
Arginine	2.84	1.14
Aspartic acid	22.56	9.06
Threonine	6.69	2.69
Serine	3.30	1.33
Glutamic acid	30.40	12.21
Proline	5.71	2.29
Glycine	16.80	6.75
Alanine	18.77	7.54
Cysteine	63.42	25.47
Valine	0.79	0.32
Methionine	0.75	0.30
Isoleucine	18.00	7.23
Leucine	25.95	10.42
Tyrosine	2.75	1.10
Phenylalanine	13.83	5.55

*Ammonia was also detected.

Aspartic acid, glutamic acid, cysteine and leucine were major amino acids. Especially, cysteine content was 25% of the total amino acid contents (Table VI).

Conclusion

1. The polysaccharide-protein fraction of the water extract of *Ganoderma lucidum* of Korea showed a high antitumor activity against sarcoma 180 in mice.
2. The dialyzate consisted of polysaccharide fraction (27%) which includes glucose, galactose, mannose and xylose, and protein fraction (72%) which has 18 amino acids. It is particularly noted that cysteine content is the highest.

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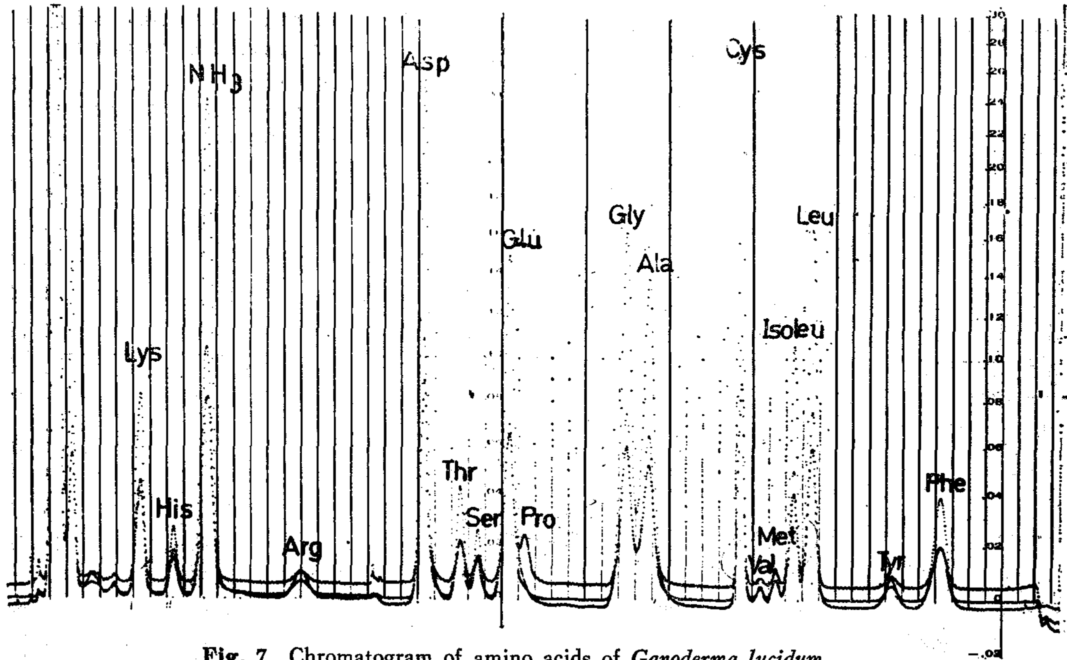

Fig. 7. Chromatogram of amino acids of *Ganoderma lucidum*.

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References

Choi, E.C., and Kim, B.K. (1975): *Korean J. Pharmacogn.* **6**, 49.

Chihara, G., Maeda, Y., Gamuro, J., Sasaki, T. and Fukuoka, F. (1969): *Nature*, **222**, 687.

Fukuda, K., Uematsu, T., Hamada, A., Akiya, S., Komatsu, N. and Okubo, S. (1975): *Chem. Pham. Bull.* **23**, 1955.

Ikekawa, T., Uehara, N., Maeda, Y., Nakanishi, M., and Fukuoka, F. (1969): *Cancer Res.* **29**, 734.

Imazeki, R., and Hongo, T. (1957): *"Coloured Illustration of Fungi of Japan,"* Vol. 1, p. 142, Hoikusha Publ. Co., Osaka.

Kim, B.K., Kim, N.D., Choi, N.J., and Lee, Y.N. (1970): *Yakhak Hoeji*, **14**, 15.

Kim, B.K., Lim, J.H., Yoon, I.H., and Kim, H.S. (1971): *Korean J. Pharmacogn.* **2**, 31.

Kim, B. K., Hwang, S. H., Auck, S., and Lee, E.K. (1973): *Korean J. Pharmacogn.* **4**, 23.

Kim, B. K., Choi, H. K., and Choi, E. C. (1976): *J. Natl. Acad. Sci. Republ. Korea* **15**, 212.

Kim, B.K., Lee, Y.S., Choi, E.C., Shim, M.J., and Lee, Y.N. (1977): *Korean Biochem. J.* **19**, 47.

Kim, B.K., Kang, C.Y., Choi, E.C., and Kim, K.H. (1976): *Korean J. Mycol.* **4**, 27.

Kim, B. K., Jang, S. Y., and Shim, M. J. (1978): *Korean J. Mycol.* **6**, 1.

Kim, B. K., Lee, M. H., and Shim, M. J. (1978): *Korean J. Mycol.* **6**, 5.

Kim, B. K., Shim, M. J., and Sohn, J. S. (1978): *Korean J. Mycol.* **6**, 53.

Kim, B. K., Chung, K. S., and Shim, M. J. (1978): *Arch. Pharm. Res.* **1**, 33.

Kim, B. K., and Kwon, Y. (1978): *Korean J. Mycol.* **6**, 2.

Kim, B. K. (1978): *Yakhak Hoeji* **22**, 91.

Kim, B. K., Park, E. K., and Shim, M. J. (1979): *Arch. Pharm. Res.* **2**, 145.

Kim, B.K., Lee, D.K., Lee, C.O., and Han, S.S, (1979): *Korean J. Mycol.* **7**, 83.

Kwon, T.J., Park, D.W., Lee, C.O., Kang, C.Y., and Kim, B.K. (1980): *Korean J. Mycol.* **8**, 25.

Lee, M. H., Choi, E. C., and Kim, B. K.(1979): *Arch. Pharm. Res.* **2**, 133.

Lee, S. A., Min, H. K., Chung, K. S., and Kim, B.K. (1979): *Korean J. Mycol.* **7**, 87.

Min, H. K., Choi, E. C., and Kim, B. K.(1980): *Korean J. Mycol.* **8**, 13.

Komatsu, N., Okubo, S., Kikumoto, S., Kimura, K., Saito, G. and Sakai, S. (1969): *Gann* **60**, 137.

Nakahara, W., Fukuoka, F., Maeda, Y. and Aoki, K. (1664): *Gann* **55**, 283.

Nakahara, W., Tokuzen, R., Fukuoka, F., and Wistler, R. L.(1967): *Nature* **216**, 374.

Nakanish, K., Tada, M., Yamada, Y., Ohashi, M., Komatsu, N., and Terakawa, H. (1963): *Nature* **197**, 292.

Rinaldi, A., and Tyndalo, V. (1974): "*The Complete Book of Mushrooms*," Crown Publishers, Inc., New York, p. 213.

Sasaki, T., Arai, Y., Ikekawa, T., Chihara, G. and Fukuoka, F. (1971): *Chem. Pharm. Bull.* **19**, 821.

Shimura, K., Ito, H. and Naruse, S. (1978): *Mie Medical J.* **26**, 147.

Shim, M.J., Lee, S.I., and Kim, B.K. (1978): *Seoul Univ. J. Pharm. Sci.* **3**, 65.

Singer, R. (1975): "*The Agricales in Modern Taxonomy*," 3rd ed., 912pp., J. Cramer, Liechtenstein.

Tanaka, T., Fukuoka, F. and Nakahara, W. (1964): *Gann*, **55**, 529.

Tanaka, T.(1967): *Gann*, **58**, 451.

Park, D. W., Shim, M. J., and Kim, B. K.(1979): *Seoul Univ. J. Pharm. Sci.* **4**, 19.

Park, E. K., Choi, E. C., and Kim, B. K.(1979): *Arch. Pharm. Res.* **2**, 153.

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