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Studies on the Antitumor Components of Korean Basidiomycetes(Ⅳ)*

Antitumor Components of *Naematoloma fasciculare* (Fr.) Karst.

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Abstract□ The carpophores of a mushroom, *Naematoloma fasciculare* (Fr.) Karst. were extracted with 0.2 N NaOH and the extract was dialyzed through visking tube. It was found to contain an antitumor activity against sarcoma 180 implanted in mice. The components of this aqueous extract were found to be a polysaccharide and a protein by color reactions including Anthrone and Lowry-Folin tests. The hydrolysis of the polysaccharide with 3% HCl-MeOH and trimethylsilylation of the hydrolysate yielded five monosaccharides, *i. e.*, glucose, fructose, mannose, galactose and xylose, which were detected and analyzed by GLC. After hydrolysis of the protein with 6N HCl, 15 amino acids, including aspartic acid and glutamic acid, were detected and analyzed by an auto amino acid analyzer.

Keywords□ *Naematoloma fasciculare*-antitumor components-sarcoma 180.

Various kinds of Basidiomycetes have been used as edible and medicinal materials. Among the Korean mushroom components, some alkaloids, fatty acids, amino acids, steroids and antibacterial substances were reported. Recently the polysaccharides that were obtained from *Lentinus edodes, Pleurotus ostreatus, Coriolus versicolor* and *Ganoderma lucidum* are known to exhibit high antitumor activity *in vivo*[1,2]. These polysaccharides are considered to exert their antitumor activity by potentiation of host animals' defense mechanism rather than direct inhibition of tumor growth.

From this point of view, the author attempted to examine antitumor polysaccharides of one of Korean higher fungi, *Naematoloma fasciculare* (Fr.) Karst.. In this paper, the author reports the extraction, isolation and chemical analysis of the polysaccharide of *N. fasciculare* and its antitumor effects in tumor-bearing animals.

MATERIALS AND METHODS

Fungal Material

Naematoloma fasciculare (Fr.) Karst., a representative of xylophilous Basidiomycetes which belongs to the family Strophariaceae, is one of bitter and poisonous mushrooms[3-5]. The carpophores of this fungus used in this study were collected in natural habitats at Su-weon in Gyeong-gi Province during the period from July to August 1979.

Extraction and Isolation

The carpophores of *N. fasciculare* were

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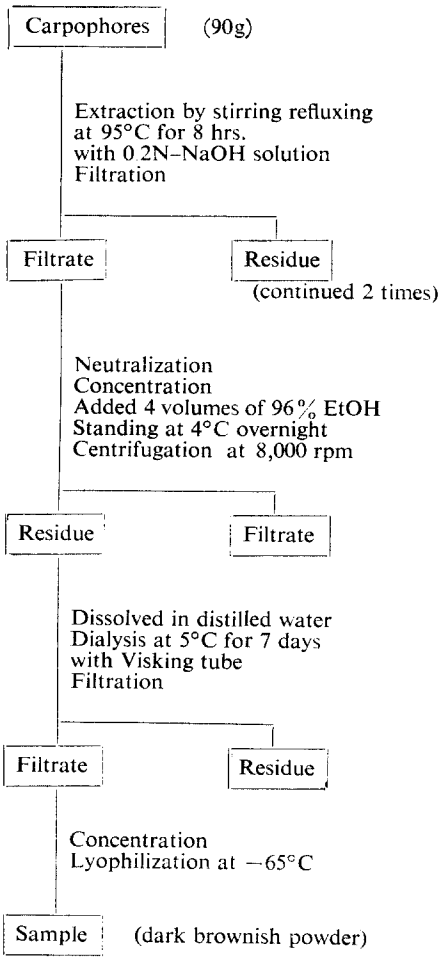
extracted and isolated by the method described in the previous reports[6-9]. Extraction was performed with 0.2N sodium hydroxide solution. After filtration, the filtrate was concentrated in a rotary vacuum evaporator. The concentrated filtrate was mixed with four volumes of 96% ethanol. The precipitate was collected by centrifugation and dissolved in distilled water for dialysis. After dialysis, insoluble substances were removed by filtration. The filtrate was concentrated and dried at -65°C in a lyophilizer. An odorless and tasteless dark brownish powder was obtained (Scheme I). The powder was dissolved in the physiological saline and used as the sample solutions.

Antitumor Test

Sarcoma 180 cells were implanted into intraperitoneal cavity of A-strain mice of male sex weighing about 20g. After ten days, the animals were killed and sarcoma 180 ascitic fluid was collected with a syringe in an ice-cold bath.

To test antitumor effects of the extract, three groups of 10 mice were respectively inoculated with 0.1 ml of the ascitic fluid ($1x10^7$ cell/ml) into the right groin. The administrations of the sample solution were initiated on the fourth day after tumor implantation and continued once daily for 10 sucessive days. The first group of 10 mice was intraperitoneally injected with 0.1 ml of the sample solution at a dose of 20 mg/kg. The second group was injected with 0.1 ml of the sample solution at a dose of 50 mg/kg. The last group was injected intraperitoneally with 0.1 ml of physiological saline and used as control group. Tumor weights were measured on the 32nd day after implantation and inhibition ratio was determined by comparison with tumors of the control mice. Tumor inhibition ratio was calculated as follows;



Scheme I: Extraction and isolation of aqueous extract of *N. fasciculare*.

$$\text{Tumor inhibition ratio } (\%) = \frac{Cw - Tw}{Cw} \times 100$$

Tw=Average tumor weight of each treated group

Cw=Average tumor weight of control group

To test on prophylatic antitumor effects of the extract, one group of 10 mice was injected intraperitoneally with 0.1ml of the solution at a dose of 20 mg/kg and injected once daily for 7 seccessive days. On the eighth day after administration, the two groups of 10 mice were implanted with 0.1 ml of ascitic cells (1×10^7 cell/ml) into the right groin. Average tumor weights were measured on the 30th day after implantation and inhibition ratio was also determined by comparison with tumors of the control mice.

Assay for Polysaccharide of the Extract

Polysaccharide content of the extract was quantitatively calculated by Anthrone method using glucose as a standard sugar. After the extract and glucose were processed by Anthrone test, degree of absorption was measured by Hitachi Recording Spectrophotometer at 620 nm. Polysaccharide content was calculated from the calibration curve.

According to the methods described in the previous reports[6-9], the methanolysis of the polysaccharide with 3% HCl-MeOH was carried out at 100±5°C for 20 hours. The methanolysate was trimethylsilylated and examined by GLC. Several monosaccharides of the extract were identified by comparison with retention times of authentic standard sugars. The content of each monosaccharide was calculated from the chromatograms by HW law and planimetry[6-9].

Assay for Protein of the Extract

Protein content of the extract was determined by Lowry-Folin method using albumin as a standard protein with Hitachi Recording Spectrophotometer at 750 nm.

After the hydrolysis of the protein with 6N-HCl, amino acids were analyzed with Hitachi amino acid auto analyzer Model KLA-5. Contents of each amino acid were calculated from the chromatograms by HW law[6-9].

RESULTS

Antitumor Test

Antitumor effects of the extract of *N. fasciculare* on sarcoma 180 in mice were shown in Table I. The effects of the therapeutic and prophylatic administrations of the extract on the life span of mice with sarcoma 180 were shown in Fig. 1 and Fig. 2 , respectively. From these figures, the life span of the treated groups were longer than that of the control groups. In the therapeutic effects on sarcoma 180, 20 mg/kg *i. p.* administration of the

Table I: Antitumor effects of the extract of *N. fasciculare* on sarcoma 180 in mice.

Schedule of *i.p.* administration		Average tumor weight(g)	Inhibition ratio (%)	Complete regression
Prophylatic (10 mice/group)	Control (saline)	2.81±0.98*		0/10
	20mg/kg	0.91±0.63 ($P<0.01$)	67.6	4/10
Therapeutic	Control (saline)	4.35±1.71		0/10
	20mg/kg	0.69±0.23 ($P<0.01$)	84.2	4/10
	50mg/kg	1.65±0.85 ($P<0.01$)	62.1	2/10

* Values are means±standard deviation.

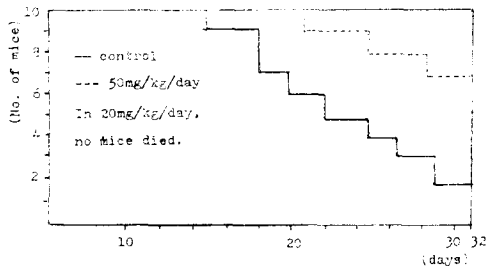


Fig. 1: Therapeutic effect of the extract on the life span of mice inoculated with sarcoma 180.

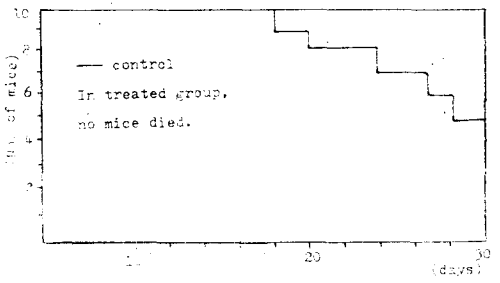


Fig. 2: Prophylatic effect of the extract on the life span of mice with sarcoma 180.

extract inhibited growth of the tumor more than 50 mg/kg administration. In case of administration of 20 mg/kg dose, none of the the 10 mice died for 32-day observation. Futhermore antitumor effects of the extract were observed in case of prophylatic as well as therapeutic administrations.

Assay for Polysaccharide of the Extract

The polysaccharide in the extract was 39.3% of the extract.

The monosaccharides of the extract were found to be glucose, fructose, mannose, galactose and xylose as shown in Table II. A GLC pattern of the monosaccharides of the extract was shown in Fig. 3.

Assay for Protein of the Extract

The results of Lowry-Folin test showed

Table II: The polysaccharide and the monosaccharide contents of the extract of *N. fasciculare*.

Polysaccharide content (%)*	39.3
Monosaccharide content (%)**	
Glucose	26.4
Fructose	28.8
Mannose	19.2
Galactose	24.0
xylose	1.4

* Percentage to the aqueous extract.

** Percentage to the polysaccharide portion of the extract.

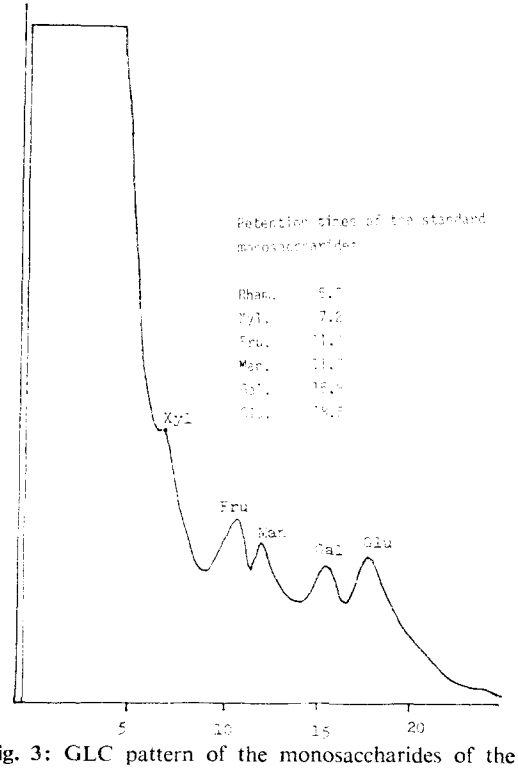


Fig. 3: GLC pattern of the monosaccharides of the extract of *N. fasciculare*.

that the protein content of the extract was 59.0%. The ratio of the amino acids of the protein fraction was shown in Table III. The chromatogram of the protein fraction of the extract was shown in Fig. 4. That of standard amino acids was omitted in this paper. The protein fraction of the extract was composed

Table III: The contents of amino acids in the protein fraction of the extract of *N. fasciculare*.

Amino acid	Content (%)*
Lysine	3.09
Histidine	2.19
Arginine	3.32
Aspartic acid	8.11
Threonine	3.04
Serine	2.81
Glutamic acid	12.02
Proline	4.63
Glycine	10.38
Alanine	10.24
Valine	6.60
Isoleucine	5.05
Leucine	8.47
Tyrosine	1.75
Phenylalanine	4.21

* Percentage to the protein portion of the extract.

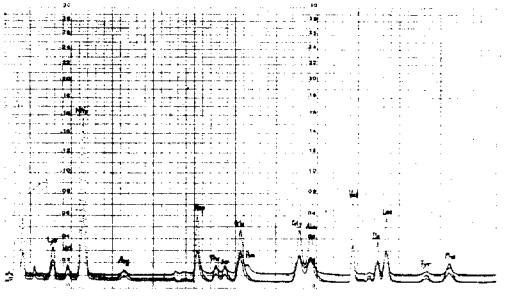


Fig. 4: Chromatogram of amino acids of *N. fasciculare*.

of 15 amino acids which included aspartic acid, glutamic acid, glycine and alanine as major amino acids. The ratio of the acidic amino acids was high, whereas that of the basic amino acids was low and sulfur-containing amino acids were not detected.

DISCUSSION

The extract of the carpophores of *N. fasciculare* had the biological activity of inhibiting tumor growth. The antitumor activity was demonstrated in case of prophylatic as well as therapeutic administration. The extract was a dark brownish amorphous powder, acting positively in the following chemical reactions: Molish, anthrone, ninhydrin, Lowry-Folin and biuret, but negatively in the iodine reaction. The polysaccharide fraction of the extract was shown to contain glucose, mannose, fructose, galactose and xylose. The protein fraction was composed of glutamic acid, aspartic acid, glycine, alanine and other amino acids.

Several high molecular weight substances isolated from Basidiomycetes such as lentinan, PSK and KS-2, appeared to have the biological activity of inhibiting rodent tumors. Some preliminary reports showed that the antitumor activity was no direct action on tumor cells, but that it was exhibited by immunopotentiation of the host against tumor cells. The antitumor mechanism has been recently proposed to be caused by activated macrophage and other lymphocytes.

CONCLUSION

The aqueous extract of *N. fasciculare* of Korea showed a high antitumor activity against sarcoma 180 in mice.

The antineoplastic extract was found to contain a polysaccharide and a protein. The polysaccharide fraction consisted of glucose, mannose, fructose, galcatose and xylose, and the protein fraction contained 15 amino acids, including aspartic acid and glutamic acid.

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