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Antigenotoxicity of extracts from *Pleurotus citrinopileatus*

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Abstract: While *Pleurotus citrinopileatus* is a widely used edible mushroom, little is known about its physiological effects. Extracts, including aqueous extract, water-soluble polysaccharide (WSP), crude protein solution (CPS) and residue from chloroform–ethyl acetate–methanol elution (CEM), were obtained first from fruiting bodies, through a solid-state culture, and then from the mycelium, through a submerged culture. This study explored the antigenotoxicity effects of these extracts from *Pleurotus citrinopileatus* via the Ames test and a spore rec-Assay. The results showed that, regardless of where the extract came from, the fruiting body or the mycelium, the antigenotoxicity effect was highest for CEM, followed by CPS, aqueous extract and WSP. The results of the Ames test indicated that, among several mutagens, CEM had the highest inhibition rate against AFB_1 in TA98 and TA100 and the lowest inhibition rate against NQNO. The concentrations of the various extracts were as follows: water extracts were 1 mg ml^{-1} and 5 mg ml^{-1} WSP, while CPS and CEM were 0.4 mg ml^{-1} and 2 mg ml^{-1}, respectively; the higher the concentration of the extract, the higher the antimutagenicity effect. The results of the rec-Assay indicated that CEM had the highest anti-DNA-damaging activity with or without the S9 mixture; the higher the concentration, the more significant the effect ($p < 0.05$). The anti-DNA-damaging activities were lower in the water extract concentrations, at 30 μg disc^{-1} dry weight^{-1}, while the WSP, CPS and CEM at 12, 150 and 60 μg disc^{-1}, respectively, were high.

Keywords: antigenotoxicity; genotoxicity; extract; *Pleurotus citrinopileatus*; Ames test; rec-Assay

INTRODUCTION

Genotoxic materials and mutagens are widespread. Raw materials, such as processed dairy products and food additives, are also genotoxic (eg safrole, coumarine, allyisothiocyanate, food colorings, etc[1,2]) and can be carcinogenic.[3] Certain defence mechanisms existing within the human metabolism are crucial in regulating and detoxifying these hazardous components and preventing them from entering and damaging the human body. Therefore, it is important to explore natural materials having antigenotoxicity effects, such as ingredients found in herbs and foods used on a daily basis that can increase resistance to disease and genotoxic hazards.[4]

The constituents of some natural products have antigenotoxicity effects. For example, cauliflower, carrots and other vegetables and fruits contain some mutagenic components.[5,6] Recent studies have been conducted on the physiological effects of edible and medicinal fungi, to find out which is the most powerful. It was found that some families of Polyporaceae, Tricholomataceae and Agaricaceae have physiological activities, such as *Agaricus blazei*,[7] *Hericium* spp[8] and *Pleurotus* spp.[9] *Pleurotus ostreatus* (oyster mushroom) has been most frequently investigated among *Pleurotus* spp, and has been found to cause many physiological effects on humans and other animals, such as hypocholesterolemic,[10] hypoglycaemic,[11] antiatherogenic[9] and antihyperlipidaemic activity,[12] as well as activation of non-specific phagocytes,[13] antitumour[14,15] and antifungal activity[16] and antioxidation.[17] *Pleurotus citrinopileatus* is another important edible mushroom, with a delicious taste, that is rich in nutrients.[18,19]

The Ames test[1,3] and rec-Assay[20,21] are rapid and effective methods of assessing antigenotoxicity. In this study, we obtained aqueous extracts, water-soluble polysaccharides (WSP), crude protein solutions (CPS) and residue from chloroform–ethyl acetate–methanol elution (CEM) from both the fruiting body and the mycelium of *P citrinopileatus*, to explore its antigenotoxicity using the Ames test and rec-Assay.

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MATERIALS AND METHODS

Organism

P citrinopileatus (supplied by You-Hao Mushroom Research Institute, China) was grown on Potato Dextrose Broth (PBD) (Difco, Detroit, USA) at 25 °C for regular subculture and maintained on PDA at 4 °C for a maximum of 3 months.

Cultivation of *Pleurotus citrinopileatus*

Liquid culture

P citrinopileatus was precultured on PDA at 25 °C and the mycelium harvested after 7 days. Mycelium fragments from one plate (9 cm) were transferred into 500 ml flasks containing 250 ml of medium (glucose 40 g l^{-1}, yeast extract 10 g l^{-1}, peptone 5 g l^{-1}, pH 5.5); the flasks were shaken at 120 rpm, 25 °C for 8 days on an orbital shaker. The liquid products of the mycelial culture were used directly as liquid spawn and mycelial source.

Solid-state culture

A polyethylene bag was filled with the sawdust substrate (sawdust 850 g kg^{-1}, wheat bran 30 g kg^{-1}, paddy husk 50 g kg^{-1}, sucrose 5 g kg^{-1}, $CaCO_3$ 2 g kg^{-1}, wt/wt, water-holding capacity 2.5) and sterilized at 121 °C for 60 min. After cooling to room temperature, the sterilized sawdust substract was inoculated with 5 ml of the liquid spawn (mycelium 50 mg, dry weight), and incubated at 24 ± 1 °C, 85% humidity, for 20 days. The fruiting body was then harvested.

Mutagens

Aflatoxin B1 (AFB_1), benzo(a)pyrene (B[a]P), *N*-methyl-*N*′-nitrosoguanidine (MNNG), 4-nitroquinoline-*N*-oxide (NQNO), mitomycin and kanamycin were purchased from Sigma (St Louis, MO USA). 3-Amino-1,4-dimethyl-5-pyrido (4,3-b) indole (Trp-P-1) and 2-amino-1-dimethyl-5H-pyrido (4,3-b) indole (Trp-P-2), were purchased from Wako Pure Chemical Co (Tokyo, Japan).

Sample preparation

The collected fruiting body and mycelium were washed three times with distilled water and then dried by lyophilization. Following this, the samples were subjected to testing as shown in Fig 1.

Samples were added to distilled water (1:15, wt vol^{-1}), blended and separated into three parts. One part was macerated overnight (at 18 °C) and then filtered. Other parts were heated (60 °C) for 10 or 30 min, and then filtered. The filtrates, aqueous extracts, were freeze-dried (Fig 1(A)).

Dried samples were placed into a pre-chilled mortar, covered with liquid nitrogen and then ground into a fine powder. This was allowed to warm to about 4 °C, before being added to distilled water (1:15, wt vol^{-1}), and macerated overnight (at 4 °C); it was then passed through filter paper (Whatman No 1, Whatman Int Ltd, Maidstone, Kent, UK) and the filtrate collected. Ammonium sulphate (Merck, Darmstudt, Germany) was added to the filtrate at a final concentration of 0.65 g ml^{-1}, and the extracts were centrifuged at 12 400 × *g*, 4 °C, for 15 min. The precipitates were collected and dissolved in 20 mM Tris–HCl buffer solution (pH 8.2). The solution was applied to an ion-exchange column (40 cm × 3 cm id) packed with DEAE–Sepharose (Amersham Pharmacia Biotech AB, Uppsala, Sweden). The column was eluted with 20 mM Tris–HCl buffer solution containing concentrations of NaCl elevated in a stepwise manner.[22] Eluate, with the absorption peak at 280 nm was collected, and dialysed overnight at 4 °C; it was then lyophilized. The freeze-dried samples were dissolved in an appropriate volume of distilled water, represented as CPS (crude protein solution) in this study (Fig 1(B)). The protein content was determined by Bradford assay.[23]

The water-soluble polysaccharide (WSP) was obtained by adding the correct volume of distilled water to the samples (1:10, wt vol^{-1}), boiling for 30 min, and then filtering. Ethyl alcohol, 95 and 75% was successively added to the filtrate, the precipitate collected and then WSP obtained by lyophilization (Fig 1(C)).[24]

The dried fruiting body and mycelium were extracted twice by methanol, concentrated by reduced vapor concentration, and the residues separated by silica gel column chromatography (Merck, Kiesegel 60, 230–400 mesh, 8 cm × 70 cm). This was eluted using mixtures of chloroform–ethyl acetate–methanol, of increasing polarities (1:0:0 to 0:0:1, vol vol^{-1}); fractions with an absorption peak of 254 nm were collected and concentrated. The residues were represented as CEM, and was dissolved in dimethyl sulphonic acid (DMSO, Merck) for testing (Fig 1(D)).

Genotoxicity test

The genotoxicity of samples was detected by the Ames test and spore rec-Assay. The Ames test was performed as follows:[1,25] 0.1 ml aliquots of different concentrations of the samples (aqueous extracts 5 mg ml^{-1}, CEM, CPS and WSP 2 mg ml^{-1} in each plate) were mixed with 0.1 ml of bacterial suspension (10^8 cells ml^{-1}) of *Salmonella typhimurium* (TA98, TA100, IFO 14193 and IFO 14194, Institute for Fermentation, Osaka, Japan) and 0.5 ml of S9 mixture (ICN Biochemicals Inc, CA, USA), and precultured at 37 °C for 20 min. Then 2 ml of 45 °C top agar (containing L-histidine 0.05 mM, biotin 0.05 mM, NaCl 0.09 M) was added and mixed homogeneously. The mixture was placed on a superficial layer of solidified glucose minimal agar, and incubated at 37 °C for 48 h. Finally, the number of his^+ revertants was counted. In addition, the phosphate buffer or DMSO was used as a control (spontaneous revertants). The number of revertants was more than double the number of spontaneous revertants, which showed that the samples had mutagenicity.

The spore rec-Assay was performed as follows.[20,21] Recombinationless M45 *rec*$^-$ (IFO 14192) and

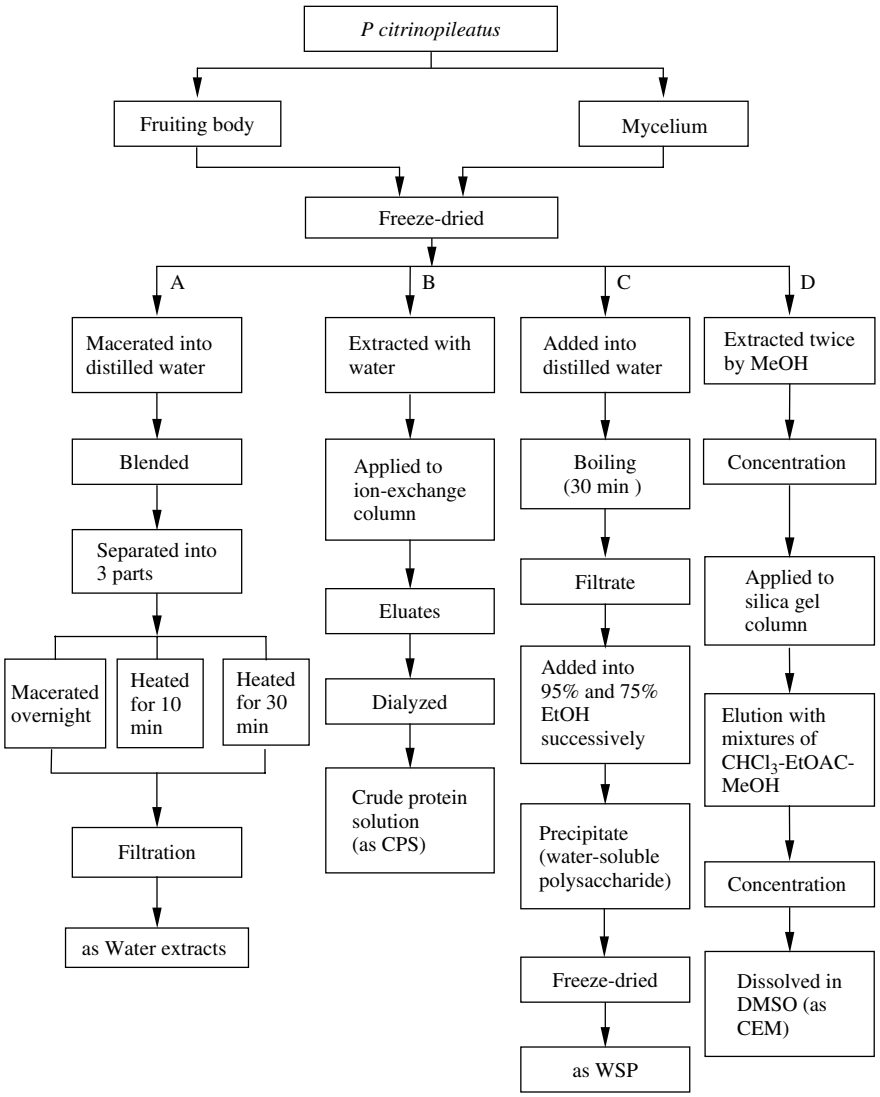

Figure 1. Flow diagram of the sample preparation.

wild-type strain H17 *rec*$^+$ (IFO 14191) of *Bacillus subtilis* were cultured in Schaeffer's medium (Difco nutrient broth 1.6 g, KCl 0.2 g, $MgSO_4 \cdot 7H_2O$ 0.05 g, $MnCl_2 \cdot 4H_2O$ 1.98 mg, $FeSO_4 \cdot 7H_2O$27.8 µg, $Ca(NO_3)_2 \cdot 4H_2O$ 23.6 mg, glucose 0.1 g, agar 1.5 g, and H_2O 100 ml) for 5 days, then prepared as a spore suspension (2×10^5 spores ml^{-1}). The above spore suspension (1 ml) was then placed on the surface of a solidified culture medium (Difco meat extract 1 g, Difco polypeptone 1 g, NaCl 0.5 g, H_2O 100 ml, pH 7.0, and containing 0.5 ml of S9 mixture), and smeared uniformly. Each paper disc (diameter 8 mm, Adventec Co, Tokyo, Japan) contained 30 µl of the test sample solution, indicating that the dry weight of each extract was 150 µg disc^{-1} for aqueous extract and 60 µg disc^{-1} for WSP, CPS and CEM. The tested paper disc was placed on the surface of the culture medium, and incubated at 37 °C for 36 h. The diameter of the inhibition zones, including the disc, was measured in mm. If the inhibition zone from same treatment in M45$^-$ than that of H17$^+$ was less than 2 mm or larger than 5 mm it implied that the sample either did not have or had, respectively, DNA-damaging activity.

Antigenotoxicity test

Antimutagenicity effects were detected by the Ames test method,[1,25,26] in which 0.1 ml of different concentrations of tested samples (aqueous extracts 1 or 5 mg ml^{-1}, CEM, CPS and WSP 0.4 or 2 mg ml^{-1} in each plate) were mixed with 0.1 ml of cell suspension (10^8 cells ml^{-1}) of *S typhimurium* (TA98 and TA100), 0.5 ml of S9 mixture and 0.1 ml of different mutagens (2 µg plate^{-1} for AFB_1, B[a]P, 4 µg plate^{-1} for MNNG and 0.5 µg plate^{-1} for Trp-P-1, NQNO), and then incubated at 37 °C for 20 min. Top agar, 2 ml, 45 °C, was then added, mixed homogenously, placed on a superficial layer of solidified glucose minimal agar, and incubated at 37 °C for 48 h. Finally, colony-forming units, expressed by the inhibition rate (%) were counted. The inhibition rate was calculated as $100 \times [(A - B)/(A - C)]$, where A is the number of revertants in the control group, B is the number of revertants in the test group with the mutagen and C is the number of spontaneous revertants.

A disc method was used in the spore rec-Assay to detect anti-DNA-damaging activity in the samples.[26,27] A spore suspension of two strains of

B subtilis was prepared and mixed with Trp-P-2 (1 mg ml^{-1}). Solution (1 ml) was placed on the surface of a solidified culture medium and smeared uniformly. Each paper disc (diameter 8 mm; Adventec Co) contained 30 µl of the tested sample solution, indicating that the dry weight of each extract was 30 or 150 µg disc^{-1} for the aqueous extract and 12 or 60 µg disc^{-1} for WSP, CPS and CEM. The tested paper disc, containing different strains of *B subtilis* (M45^{-} and H17^{+}), was placed on the surface of the culture medium and incubated at 37 °C for 36 h. The difference between the inhibition zones of the two bacteria was calculated. A difference in value of less than 2 mm, or greater than 5 mm, implied that the samples had or did not have anti-DNA-damaging activity.[28]

Statistical analysis

Each test was performed in triplicate. Data from each test were subjected to SAS for analysis of variance, and Duncan's multiple range test was used to determine any significant difference ($p < 0.05$) among treatments.

RESULTS AND DISCUSSION

Genotoxicity of extracts from *P citrinopileatus* by Ames test

Tests conducted with TA98 showed that the number of revertants in unheated water extracts of the fruiting body of *P citrinopileatus* was the highest among all tested samples, without the S9 mixture, but not greater than double the number of spontaneous revertants; the mutagenicity ratio (number of induced revertants/number of spontaneous revertants) was also less than 2 (Table 1). Tests conducted without the S9 mixture indicated that the mutagenicity ratios of aqueous extracts that had been heated for 10 and 30 min, were 88 and 84% of the unheated aqueous extracts, respectively. The mutagenicity ratio was lower if the aqueous extract was heated for longer, regardless of whether the S9 mixture was added or not. The TA100 tests, with or without the S9 mixture, showed that the number of revertants was highest in the unheated water extract and that CPS was lowest among all water-soluble samples. The difference in number of revertants was about 19%, and the mutagenicity ratio showed a significant difference between the two tested samples ($p < 0.05$). The mutagenicity ratio of CEM was the lowest among all the tested samples. None of the tested samples were significantly different, regardless of the presence or absence of the S9 mixture ($p > 0.05$). No mutagenicity was found in the aqueous extracts and methanol extracts of *Hericium erinaceus* or *H laciniatum*, with or without the S9 mixture.[8]

The total numbers of revertants in extracts from the mycelium of the two strains of *P citrinopileatus* were greater than those in the fruiting body, especially the differences between WSP and unheated aqueous extract. The test using TA98 showed that the mutagenicity ratios of WSP from the mycelium with or without the S9 mixture were 120 and 110% of the fruiting body ratio. The numbers of revertants from any sample with or without the S9 mixture vs any of the strains (TA98 or TA100) were not more than double the number of spontaneous revertants, implying that

Table 1. Mutagenicity of *Pleurotus citrinopileatus*

	Without S9		With S9	
Tested samples	TA98	TA100	TA98	TA100
Fruiting body[a]				
Aqueous extracts				
Unheated	41.0^{Ab}	115.1^{A}	58.9^{A}	176.9^{A}
Heated, 10 min	36.1^{BC}	102.3^{BC}	54.5^{AB}	157.6^{B}
Heated, 30 min	34.2^{CD}	97.5^{BC}	52.7^{ABC}	152.5^{B}
CEM	30.3^{D}	94.8^{BC}	47.2^{C}	144.3^{B}
CPS	32.1^{CD}	90.8^{C}	49.3^{BC}	148.4^{B}
WSP	38.6^{AB}	107.4^{AB}	56.3^{A}	160.8^{AB}
Mycelium[a]				
Aqueous extracts				
Unheated	47.2^{A}	130.2^{A}	70.9^{A}	199.7^{A}
Heated, 10 min	39.7^{BC}	116.3^{BC}	61.8^{B}	175.6^{BC}
Heated, 30 min	37.9^{CD}	106.5^{C}	59.4^{BC}	167.1^{BCD}
CEM	33.6^{D}	103.5^{C}	54.1^{C}	152.9^{D}
CPS	33.8^{D}	104.5^{C}	55.6^{BC}	158.6^{CD}
WSP	43.5^{AB}	126.2^{AB}	72.8^{A}	185.5^{AB}
Spontaneous revertants	30.5 ± 1.8	95.7 ± 5.8	45.6 ± 2.7	139.8 ± 8.7

[a] The tested samples were aqueous extracts, CEM, CPS and WSP of fruiting body or mycelium from *P citrinopileatus*. The concentration of aqueous extracts was 5 mg ml^{-1}, and that of CEM, CPS and WSP was 2 mg ml^{-1} in each plate.

[b] All data are represented as the number of his^{+} revertants per plate, and the data on fruiting body and mycelium in a column with different letters are significantly different at $p < 0.05$.

none of the tested samples of *P citrinopileatus* had mutagenicity.[29]

Genotoxicity of extracts from *P citrinopileatus* by rec-Assay

Table 2 shows that none of the tested samples of the fruiting body with the S9 mixture had inhibitory effects against $M45^-$ or $H17^+$, except for unheated aqueous extracts and WSP. The differences in the inhibition zones of CEM, CPS and aqueous extract heated for 30 min were less than those of the three other samples (WSP, aqueous extract heated for 10 min and unheated aqueous extract).

Some foodstuffs having no genotoxicity in the natural state will become genotoxic on interaction with other substances, or from the influence of external factors such as heat; for example, potassium sorbate and ascorbic acid are edible food additives that possess genotoxicity in the presence of ferric (or ferrous) salt.[30,31] The results of all tested samples of the mycelium without the S9 mixture were similar to those of the fruiting body. While with the S9 mixture, WSP, aqueous extract heated for 10 min and unheated aqueous extract had an inhibitory effect against $M45^-$, the other three samples (CPS, CEM and aqueous extract heated for 30 min) did not have an inhibitory effect against $M45^-$ or $H17^+$. The differences in the inhibition zones of all tested samples against the two strains were about 0.5–1.2 mm, which was greater than that of the fruiting body, but still less than 2.0 mm.

Antimutagenicity of extracts from the fruiting body of *P citrinopileatus*

The antimutagenicity effects of extracts from the fruiting body of *P citrinopileatus* are shown in Table 3. When the concentrations in each plate of unheated water extract were 1 mg ml^{-1}, neither TA98 nor TA100 had inhibitory effects against B[a]P or NQNO. When the concentrations were 5 mg ml^{-1}, they possessed the highest antimutagenicity against AFB_1, followed by Trp-P-1. The inhibition rates of the two water extracts, heated for 10 and 30 min, in the presence of three mutagens, Trp-P-1, B[a]P and NQNO, were not significantly different for the

Table 2. DNA-damaging activity of *P citrinopileatus* in *B subtilis*

	Inhibition halo diameter (mm)[a]					
	Without S9			With S9		
Tested samples	$M45^-$	$H17^+$	DNA-damaging activity $M45^- - H17^+$	$M45^-$	$H17^+$	DNA-damaging activity $M45^- - H17^+$
Fruiting body						
Aqueous extracts						
Unheated	11.5	9.5	2.0^A	10.0	8.9	1.1
Heated, 10 min	11.0	9.4	1.6^B	—	—	—
Heated, 30 min	10.4	9.2	1.2^C	—	—	—
CEM	10.0	9.0	1.0^D	—	—	—
CPS	10.4	9.4	1.0^D	—	—	—
WSP	11.4	9.3	2.1^A	10.1	9.2	0.9
Control[b]						
Mitomycin	26.3	14.5				
Trp-P-2				14.8	—	
Kanamycin	16.5	15.9				
DMSO	—	—		—	—	—
Mycelium						
Aqueous extracts						
Unheated	11.8	9.7	2.1^{AB}	10.3	9.1	1.2
Heated, 10 min	11.4	9.5	1.9^B	10.1	9.0	1.1
Heated, 30 min	10.5	9.0	1.5^C	—	—	—
CEM	10.8	9.3	1.5^C	—	—	—
CPS	11.0	9.5	1.5^C	—	—	—
WSP	11.8	10.7	2.2^A	10.0	9.2	0.8
Control[b]						
Mitomycin	29.5	13.7				
Trp-P-2				15.7	—	
Kanamycin	17.7	16.9				
DMSO	—	—		—	—	

[a] Each paper disc contained 30 µl of the tested sample solution, indicating that the dry weight of each extract was 150 µg disc^{-1} for water extract, 60 µg disc^{-1} for WSP, CPS and CEM, respectively. (—) No inhibition zone. The data for fruiting body and mycelium in the same column with different letters are significantly different at $p < 0.05$.

[b] The dose of mitomycin, Trp-P-2, and kanamycin were 0.04, 7 and 20 µg disc^{-1}, respectively, in the control group.

Table 3. Antimutagenicity on extracts of fruiting body from *P citrinopileatus*

	Trp-P-1		B[a]P		MNNG		AFB_1		NQNO	
Tested samples	TA98	T100	TA98	T100	TA98	T100	TA98	T100	TA98	T100
Low concentration[a]										
Aqueous extracts										
Unheated	4.0^{Db}	8.1^{D}	—[c]	—	—	5.7^{D}	—	15.2^{D}	—	—
Heated, 10 min	37.1^{B}	39.6^{B}	18.5^{C}	21.6^{C}	24.2^{C}	27.6^{B}	34.1^{C}	37.8^{C}	3.2^{C}	8.4^{C}
Heated, 30 min	41.2^{B}	42.7^{B}	19.7^{C}	21.5^{C}	25.6^{BC}	32.7^{A}	33.1^{C}	42.6^{B}	—	22.5^{A}
CEM	49.2^{A}	49.3^{A}	32.8^{A}	36.7^{A}	34.2^{A}	34.7^{A}	50.7^{A}	52.8^{A}	13.5^{A}	15.6^{B}
CPS	47.0^{A}	48.8^{A}	26.6^{B}	28.3^{B}	27.6^{B}	29.6^{B}	40.7^{B}	42.5^{B}	8.4^{B}	9.5^{C}
WSP	16.2^{C}	19.8^{C}	—	4.2^{D}	7.0^{C}	16.2^{C}	12.6^{D}	15.2^{D}	—	5.7^{D}
High concentration[a]										
Aqueous extracts										
Unheated	30.7^{C}	36.2^{C}	22.7^{D}	23.6^{D}	17.6^{C}	31.2^{D}	40.7^{C}	47.1^{D}	11.8^{E}	14.3^{E}
Heated, 10 min	68.1^{A}	69.2^{A}	47.5^{C}	47.8^{C}	53.7^{A}	56.5^{BC}	62.2^{B}	65.3^{B}	25.2^{C}	28.5^{CD}
Heated, 30 min	70.3^{A}	71.5^{A}	48.2^{C}	49.6^{C}	56.1^{A}	61.8^{AB}	67.6^{B}	72.2^{AB}	27.3^{C}	30.3^{C}
CEM	73.6^{A}	74.5^{A}	60.7^{A}	66.8^{A}	58.8^{A}	64.7^{A}	78.7^{A}	79.3^{A}	49.8^{A}	50.9^{A}
CPS	70.5^{A}	75.8^{A}	53.3^{B}	58.6^{B}	55.6^{A}	57.1^{BC}	65.2^{B}	71.2^{AB}	37.2^{B}	38.8^{B}
WSP	50.7^{B}	54.3^{B}	26.4^{D}	28.2^{D}	40.2^{B}	51.7^{C}	46.2^{C}	57.1^{C}	19.2^{D}	25.6^{D}
Control[d]	1321	1561	1189	1307	458	637	758	925	587	701

[a] Low concentration was 1 mg ml^{-1} in each plate for aqueous extracts, and 0.4 mg ml^{-1} for CEM, CPS and WSP, while high concentration was 5 mg ml^{-1} in each plate for aqueous extracts, and 2 mg ml^{-1} for CEM, CPS, and WSP.
[b] All data are represented as the inhibition rate (%), and the data for low and high concentrations in the same column with different letters are significantly different at $p < 0.05$.
[c] No revertants.
[d] The spontaneous revertants were 32.5 ± 2.1 for TA98, 45.8 ± 2.9 for TA100.

two strains (TA98 and TA100; $p > 0.05$); however, there was a significant difference ($p < 0.05$) against MNNG and AFB_1 in TA100. CPS had the highest antimutagenicity effect at a concentration of 2 mg ml^{-1} against Trp-P-1 in TA100, whereas it had the lowest inhibition rate against NQNO in TA98. The result was similar to that of the aqueous extracts (except for the unheated aqueous extract). The inhibition rate of WSP against five mutagens, in TA98 and TA100, was slightly higher than that of unheated aqueous extract, but was lower than that of the aqueous extracts heated for 10 and 30 min. In terms of the inhibitory effect of TA98 revertants, the inhibition rate of WSP against Trp-P-1 at a higher concentration (2 mg ml^{-1}) was 170% of the inhibition rate of the unheated water extract, and 70% of the inhibition rate of CPS. There was no inhibitory effect in TA 98 revertants when the concentration of WSP was 0.4 mg ml^{-1} in the presence of B[a]P and NQNO.

The antimutagenicity effects of CEM against five mutagens were higher than those of other tested samples. The methanol extract from Korean soybean paste indicated its anti-AFB_1 effect in the TA100 test.[26] The inhibition rate of CEM against AFB_1 in TA100 was the highest among all the mutagens. The next highest inhibition rate was against Trp-P-1, B[a]P, MNNG and NQNO. These findings were different from those of other tested samples. The inhibition rate of CPS against Trp-P-1 in TA 98 was the highest, followed by AFB_1, MNNG, B[a]P and NQNO, in that order. The inhibition rate of WSP and the three aqueous extracts against five mutagens had a similar order. These findings showed that the components of the *P citrinopileatus* fruiting body, having antimutagenic effects, might be contained in both water-soluble and water-insoluble parts. The findings of other studies have also illustrated that substances with antimutagenic effects could have different chemical properties. For example, magnesium salt induced an antimutagenicity effect[32] and selenium compounds of yeast cells had the same effect;[33] aqueous extracts and the lipid soluble chemicals of teas also had inhibitory effects against Trp-P-1, Glu-P-1, B[a]P and AFB_1.[34]

Antimutagenicity of extracts from the mycelium of *P citrinopileatus*

The inhibition rate of unheated aqueous extract, from the mycelium of *P citrinopileatus,* against five different mutagens, was the lowest among all tested samples (Table 4). Comparing the five mutagens, the inhibition rate of water extract heated for 30 min was highest against Trp-P-1, or 290 and 220% of the inhibition rate against NQNO in TA98 and TA100, respectively. The inhibition rate of WSP against all five mutagens was just slightly higher than that of unheated aqueous extract, when compared with other tested samples of mycelium ($p < 0.05$). When the concentration of WSP was 0.4 mg ml^{-1} against B[a]P and NQNO, it did not have an inhibitory effect in TA98. The unheated aqueous extract showed no inhibitory effects against NQNO and B[a]P at a lower concentration of 1 mg ml^{-1}. NQNO was a direct

Table 4. Antimutagenicity on extracts of mycelium from *P citrinopileatus*

	Trp-P-1		B[a]P		MNNG		AFB_1		NQNO	
Tested samples	TA98	T100	TA98	T100	TA98	T100	TA98	T100	TA98	T100
Low concentration[a]										
Aqueous extracts										
Unheated	—	6.8^{Db}	—[c]	—	—	—	4.6^{F}	10.6^{D}	—	—
Heated, 10 min	33.6^{C}	34.8^{B}	16.7^{D}	—	21.4^{C}	24.5^{B}	29.8^{C}	29.9^{C}	—	5.1^{B}
Heated, 30 min	35.6^{BC}	38.7^{AB}	17.3^{C}	18.3^{C}	20.1^{C}	28.2^{A}	24.8^{D}	28.7^{C}	—	16.8^{A}
CEM	40.4^{A}	42.5^{A}	30.9^{C}	33.6^{A}	28.6^{A}	31.2^{A}	47.4^{A}	48.1^{A}	11.6^{A}	15.4^{A}
CPS	38.8^{AB}	41.2^{A}	23.1^{B}	25.7^{B}	25.6^{B}	29.7^{A}	36.8^{B}	38.7^{B}	3.5^{B}	5.2^{B}
WSP	14.7^{D}	17.5^{C}	—	—	4.2^{D}	12.8^{C}	9.6^{E}	11.7^{D}	—	—
High concentration[a]										
Aqueous extracts										
Unheated	26.5^{C}	33.4^{D}	20.5^{D}	21.5^{D}	14.6^{C}	28.6^{C}	36.3^{C}	41.7^{D}	8.5^{E}	17.2^{E}
Heated, 10 min	63.2^{A}	65.7^{B}	45.8^{C}	47.8^{C}	51.2^{A}	54.2^{A}	56.4^{B}	57.8^{C}	22.4^{C}	28.9^{C}
Heated, 30 min	65.3^{A}	67.6^{AB}	46.1^{BC}	47.6^{C}	52.4^{A}	60.1^{A}	60.5^{B}	66.5^{B}	22.6^{C}	30.2^{C}
CEM	70.2^{A}	73.8^{A}	58.3^{A}	62.6^{A}	55.5^{A}	58.8^{A}	74.3^{A}	77.7^{A}	46.5^{A}	49.9^{A}
CPS	66.8^{A}	70.6^{AB}	51.1^{B}	54.2^{B}	52.6^{A}	54.9^{A}	60.4^{B}	69.5^{B}	35.4^{B}	40.6^{B}
WSP	46.2^{B}	52.5^{C}	23.8^{D}	27.6^{D}	37.5^{B}	45.3^{B}	41.6^{C}	52.3^{C}	15.4^{D}	22.4^{D}
Control[d]	1857	1902	1427	1563	976	1026	1187	1325	926	1009

[a,b,c] The descriptions are as in Table 3.

[d] The spontaneous revertants were 31.3 ± 1.9 for TA98, 41.6 ± 2.7 for TA100.

mutagen, so this result may imply that *P citrinopileatus* has desmutagenic action.[35]

The inhibition rate of aqueous extract heated for 30 min was the highest, among the three aqueous extracts of mycelium, against all mutagens. This differed from the findings that the higher the heating temperature and the longer the heating time for Indonesian jasmine tea, or *Hericium* spp, the higher the mutagenicity.[8,36] This may be related to different degrees of heating or to the optimal extract antimutagenicity effects of the water-soluble constituents of *P citrinopileatus* existing at 60 °C.

The polyphenolic compounds were extracted by alcohol from sweet potato leaf and were found to have antimutagenicity effects against Trp-P-1 in TA98.[37] The inhibition rate of CEM against AFB_1 was the highest among the five mutagens, followed by Trp-P-1, B[a]P, MNNG and NQNO, in that order. However, the inhibition rates of water extracts, WSP and CPS against the different mutagen were in a different order from those of CEM. The results were the same as for the fruiting body. The CEM showed the highest inhibition rates among all tested samples in TA98 and TA100. The inhibition rate of CEM at a low concentration ($0.4\,mg\,ml^{-1}$) against B[a]P was 130% of the inhibition rate of CPS; the inhibition rate at a high concentration ($2\,mg\,ml^{-1}$) was 110% of inhibition rate of CPS. This indicated that higher concentrations significantly increased the inhibition rate.

The inhibition rates of different extracts of mycelia against the different mutagens in TA98 and TA100 were lower than those of the fruiting bodies, which were similar to the antimutagenicity findings of *Hericium* spp.[8] The ranges of the inhibition rates of the six samples at higher concentrations against AFB1 in TA98 were 4.3–7.2% lower than the rates of the fruiting bodies. Under the same conditions, the differences of inhibition rate between all mycelium extracts and fruiting bodies were close, whether at low or high concentrations.

Anti-DNA-damaging activity of extracts from *P citrinopileatus*

The results of anti-DNA-damaging activity against *B subtilis* of six samples of the fruiting body of *P citrinopileatus*, in the presence of Trp-P-2, are shown in Table 5. The inhibition zones of CEM were the smallest at both high and low concentrations, followed by CPS, aqueous extract heated for 30 min, aqueous extract heated for 10 min, unheated aqueous extract and WSP. The inhibition zones of the latter two samples in $M45^{-}$ were similar.

The study also showed that CEM had higher anti-DNA-damaging activity. In the previous report, a 50% EtOH extract of *Smilax rhizome, Mulberry bark* and *Berchemia racemosa* had anti-DNA-damaging activity on Trp-P-1 and Trp-P-2.[26] The physical and chemical properties or the sample dose may be the influencing factor behind the antigenotoxicity effect, owing to metabolism modulation, block or suppression or modulation of the DNA replication and repair effect.[38] Among all tested samples of the fruiting body at lower concentrations ($30\,\mu g\,disc^{-1}$ for aqueous extracts and $12\,\mu g\,disc^{-1}$ for both CEM and CPS), the inhibition zones of unheated aqueous extracts and WSP in the two strains of *B subtilis* were the highest (1.9 and 2.0 mm, respectively). When concentration of the samples reached $150\,\mu g\,disc^{-1}$ for aqueous extracts or $60\,\mu g\,disc^{-1}$ for CEM, CPS and WSP), the inhibition zones in $M45^{-}$ were significantly lower than for the low concentrations. The difference in anti-DNA-damaging activities

Table 5. Anti-DNA-damaging activity on extracts from fruiting body of *P citrinopileatus*

Tested samples	Inhibition halo diameter (mm)		
	M45$^-$	H17$^+$	Anti-DNA-damaging activity[a] M45$^-$ − H17$^+$
Low concentration			
Dose of 30 μg disc^{-1}			
Aqueous extracts			
Unheated	11.1	9.2	1.9^A (+)
Heated, 10 min	10.6	8.9	1.7^B (+)
Heated, 30 min	10.1	8.7	1.4^C (+)
Dose of 12 μg disc^{-1}			
CEM	9.7	8.5	1.2^D (+)
CPS	9.7	8.5	1.2^D (+)
WSP	10.9	8.9	2.0^A (+)
High concentration			
Dose of 150 μg disc^{-1}			
Aqueous extracts			
Unheated	10.3	9.0	1.3^A (+)
Heated, 10 min	10.1	8.8	1.3^A (+)
Heated, 30 min	9.6	8.5	1.1^B (+)
Dose of 60 μg disc^{-1}			
CEM	9.0	8.5	0.5^C (+)
CPS	9.1	8.5	0.6^C (+)
WSP	10.4	9.0	1.4^A (+)
Control[b]	14.2	0	

[a] Values less than 2 mm or larger than 5 mm implied that the sample had or did not have, respectively, anti-DNA-damaging activity. The data for low and high concentrations in the same column with different letters are significantly different at $p < 0.05$.
[b] The dose of Trp-P-2 was 1 mg ml^{-1} in the control group.

between high and low concentrations among all tested samples was significant ($p < 0.05$), implying that an increase in sample concentrations would enhance antigenotoxicity effects.

The anti-DNA-damaging assay of mycelium extracts showed that the differences in inhibition zones between the two *B subtilis* strains were greater than those of the fruiting bodies (Table 6). The anti-DNA-damaging activity was shown to have no significant difference ($p > 0.05$) among the unheated aqueous extract, aqueous extract heated for 10 min and WSP of the fruiting body and mycelium at low concentrations. The differences in the inhibition zones of CEM and CPS between the fruiting body and the mycelium were significant ($p < 0.05$). The difference in inhibition zones between CEM and CPS of the fruiting bodies at a low concentration (12 μg disc^{-1}) was 75% that of mycelium, while at a high concentration (60 μg disc^{-1}) it was 73% of the mycelium. The differences in the inhibition zones of all samples were less than 2.0 mm, indicating anti-DNA-damaging activity. The anti-DNA-damaging activity of extracts from fruiting bodies was superior to that of extracts from mycelium, and the anti-DNA-damaging activity of the higher concentrations of water extracts (150 μg disc^{-1}), CEM, CPS and WSP (60 μg disc^{-1}) had a positive correlation with dose.

Table 6. Anti-DNA-damaging activity on extracts from mycelium of *P citrinopileatus*

Tested samples	Inhibition halo diameter (mm)[a]		
	M45$^-$	H17$^+$	Anti-DNA-damaging activity
Low concentration			
Dose of 30 μg disc^{-1}			
Aqueous extracts			
Unheated	11.5	9.5	2.0^A (+)
Heated, 10 min	11.1	9.2	1.9^{AB} (+)
Heated, 30 min	10.9	9.2	1.7^{BC} (+)
Dose of 12 μg disc^{-1}			
CEM	10.6	9.0	1.6^C (+)
CPS	10.6	9.0	1.6^C (+)
WSP			
High concentration			
Dose of 150 μg disc^{-1}	11.6	9.6	2.0^A (+)
Aqueous extracts			
Unheated	10.8	9.3	1.5^{AB} (+)
Heated, 10 min	10.4	9.0	1.4^B (+)
Heated, 30 min	9.9	8.7	1.2^C (+)
Dose of 60 μg disc^{-1}			
CEM	9.2	8.5	0.7^D (+)
CPS	9.3	8.5	0.8^D (+)
WSP	10.8	9.2	1.6^A (+)
Control[b]	15.1	0	

[a,b] The descriptions are as in Table 5.

CONCLUSIONS

The results of this study revealed that some extracts from *P citrinopileatus* had an antigenotoxicity effect, while the largest effect was found in the cem eluate. Further study of the components responsible for this antigenotoxicity effect is required, in order to investigate their feasibility as a food supplement.

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