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Bioactivities of β-Glucan and Tannin Extracted with Superheated Water by Using a Macchinetta Extractor

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Abstract. This work investigated the use of a macchinetta extractor to obtain β-glucan and tannin from bark and fungi, respectively. Normally a macchinetta extractor is used to extract espresso coffee through employment of a high extraction temperature (100–150 ℃ of superheated water), rapid cooling after emission from the extractor (below 100 ℃), and a very short extraction time (1-5 minutes). β-Glucan obtained from the edible fungus *Hypsizygus marmoreus* by macchinetta extraction, had antitumor activity toward the Caco-2 cancer cell at concentrations above 100 mg/L. Autoclave extraction from fungi was effective for providing a high extraction yield, but this solution showed no antitumor activity. Macchinetta extracts from various edible fungi possessed higher antioxidant activity than those obtained from hot water and autoclave extractions. The yield of tannin from bark obtained from macchinetta extraction was about half that obtained from autoclave extraction. However, the protein adsorption activity of the tannin from the macchinetta extract was 2-fold higher than that from the autoclave extract. Macchinetta extracted tannin exhibited high antimicrobial activity toward *Staphylococcus aureus*.

Keywords: macchinetta extractor; β-glucan; tannin; bioactivity; superheated water.

1. Introduction

Fungi and bark contain the bio-active components β-glucan and tannin, respectively. β-Glucan has antitumor and immune boosting activities [1]. However, β-glucan is poorly soluble in water because of its highly polymerized triple helical structure [2-4]. To obtain a β-glucan solution, slight depolymerization by hydrolysis is necessary. It is well known that tannin possesses antioxidant activity, adsorbs to protein and heavy metal ions and is poorly soluble in water. Both β-glucan and tannin are comparatively more soluble in hot water. However, use of superheated water induces excessive pyrolysis or condensation because of the exposure time and/or temperature.

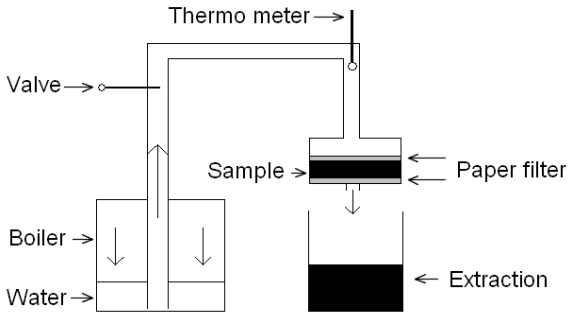

Fig. 1: Schematic diagram of a macchinetta extractor.

To avoid excessive exposure of samples to heat, a macchinetta extractor, which is normally used to extract espresso coffee, was investigated. This extractor employs a high temperature (100–150 ℃ of

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superheated water), with rapid cooling after emission from the extractor (below 100 ℃), and a very short extraction time (1-5 minutes) [5]. These extractor characteristics should make it advantageous for use in the extraction of β-glucan and tannin [6,7].

2. Materials and Methods

2.1. Materials

Various fungi were supplied or purchased from a market in Fukuoka, Japan. The fungi were air dried for 2 days and then were grinded using a coffee mill. The Japanese cedar bark was supplied by Kyushu University Forest. The bark was powdered using a ball mill and sieved through a 200 mesh. Gallotannin (95% purity) was used as a standard tannin and was purchased from Cosmo Bio Co., Ltd.

2.2. Extraction method

An EP930 (Electrolux) macchinetta extractor was used. The structure and mechanism of the macchinetta extractor is shown in Fig.1. Several grams of powdered sample were placed between the top and bottom filter paper in the basket and compressed by hand pressing. After connecting the sample basket with a holder to the macchinetta body, about 100 ml of water was placed in the boiler and heated to 100 ℃ and then to about 140 ℃. This is possible because the paper filters and the compressed sample help to contain the pressure. Superheated water was passed through the sample and water-soluble extracts were produced by hydrolysis. As the extract was immediately cooled at the collector to below 100 ℃ under atmospheric pressure, the decomposition of the extracts was avoided. Finally, the extract was centrifuged to remove insoluble material.

2.3. Analytical methods

β-Glucan content: The β-glucan content was measured by the method of Yuan and Yu [8]. The extracted solution was first diluted with 1 N NaOH. Next, 6 N NaOH was added and the solution was incubated at 80 °C for 30 min. The denatured β-glucan conformers were immediately put in an ice bath. A dye mix was mixed, and then incubated at 50 ℃ for 30 min to form a β-glucan-fluorochrome complex. The unbound fluorescent dye was further decolorized at room temperature for 30 min, and the fluorescence intensity was measured using a fluorescence spectrophotometer.

Total sugar content: Total sugars were determined according to a phenol-sulfuric acid colorimetric method.

Protein content: The protein content was determined according to the Bradford method using Coomassie Brilliant Blue (CBB) as the reagent for detecting the protein.

Antioxidant activity: The antioxidant activity of the extract was measured by quantitating the radical scavenging activity using diphenylpicrylhydrazyl (DPPH) [9]. The activities were compared with a Trolox standard.

Anti-tumor activity assay: The extract was exposed to a human colon cancer cell line (Caco-2) pre-cultured for 24 h. At 72 h after exposure, the anti-tumor activity of the extract was determined by counting the number of surviving cancer cells.

Tannin content: Tannin was determined according to the Folin-Denis method. This method is based on the color reaction yielded by a phenolic hydroxyl group that is reduced by reaction with the Folin reagent.

Protein adsorption activity: The tannin and bovine serum albumin (BSA) solutions were mixed at the same volumes. A total of 1 h after mixing, the tannin-BSA complex was centrifuged. Then, the free BSA in the supernatant was measured by the Bradford method.

Antimicrobial activity: The tannin solution was added to a solution containing *Staphylococcus aureus*, and cultured for 1 day at 30 ℃. Then, the numbers of surviving cells were counted.

3. Results and Discussion

3.1. Temperature control of the macchinetta extractor by using different filter papers

First, the influence of different filter papers in the basket on the extraction temperature was investigated. As shown in Fig. 2, the maximum temperature of the extraction water reached over 100 ℃ and depended on the kind of filter paper employed. Use of No.4A filter resulted in the highest temperature. This is because the filter paper works as a pressure holding material, and of the papers tested, No.4A was the one with the finest particle retention. No.4A filter paper was used in following experiments so that a higher temperature could be employed.

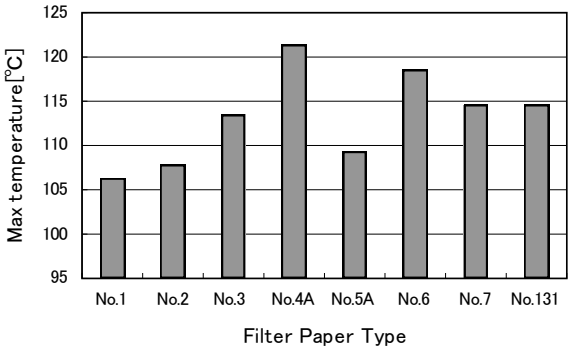

Fig. 2: Maximum temperature achievable versus filter paper type when it is used as a pressure holding material in a macchinetta extractor (in the absence of powder sample).

3.2. Extraction of β-Glucan from fungi

Table 1 shows the component yield extracted from the various fungi. Overall, autoclave extraction was more effective for the extraction of sugars, β-glucan and protein, compared with macchinetta extraction. However, the use of macchinetta extraction is still useful because the extraction only takes a few minutes.

Table 1: Component yield extracted from various edible fungi.

Material	Extraction method	Total sugar [wt%]	β-Glucan [wt%]	Protein [wt%]
Hypsizygus marmoreus (Bunashimeji)	Macchinetta	1.0	0.3	0.3
	Autoclave	2.5	0.8	1.2
Flammulina velutipes (Maitake)	Macchinetta	0.5	0.3	0.3
	Autoclave	1.6	0.6	0.8
Grifola frondosa (Enokitake)	Macchinetta	0.9	0.5	0.3
	Autoclave	2.1	1.1	0.9
Pleurotus ostreatus (Hiratake)	Macchinetta	1.0	0.3	0.3
	Autoclave	3.1	0.9	0.8

Antioxidant activity: Table 2 shows the antioxidant activities of the extract from various edible fungi obtained using different extraction methods. Overall, macchinetta extracts have higher antioxidant activities compared with those from other extraction methods. The higher antioxidant activities of macchinetta extracts may be caused by the prevention of thermal decomposition of polyphenols through the use of a shorter extraction time.

Anti-tumor activities of the fungi extract: Figure 3(a) shows the dependence of Caco-2 cell viability on the concentration of exposed β-glucan contained in various fungi extracts and has been reported previously [10]. The extract from *Hypsizygus marmoreus* was the most effective at reducing Caco-2 cell viability. Fig. 3(b) shows the dependence of the Caco-2 cell viability on the concentration of β-glucan extracted from *Hypsizygus marmoreus* using different extraction methods. No antitumor activity was observed for the extract obtained by autoclaving. This is likely because the β-glucan extract was excessively degraded by thermal decomposition during the extended time it was treated in the autoclave. On the other hand, the extract obtained from the macchinetta extractor had antitumor activity.

Table 2: Antioxidant activity of extract from various edible fungi using different extraction methods.

Material	Antioxidant activity [μmol-Trolox/mg-extract]		
	Hot water at 90 ℃	Autoclave at 121 ℃	Macchinetta at over 140 ℃
Hypsizygus marmoreus (Bunashimeji)	2.7	4.0	5.5
Flammulina velutipes (Maitake)	3.0	5.5	6.9
Grifola frondosa (Enokitake)	4.2	4.8	4.7
Pleurotus ostreatus (Hiratake)	4.1	4.7	7.1
Hericium erinaceum (Yamabushitake)	5.0	6.9	8.0
Agaricus bisporus (White Mushroom)	4.3	4.0	9.7

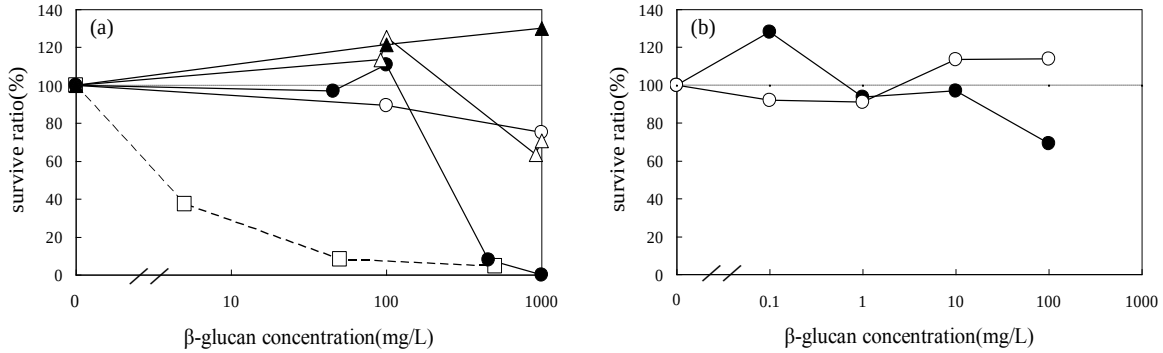

Fig. 3: Antitumor activity of β-glucan extracted from various fungi toward Caco-2 cancer cells. (a) Macchinetta extract from *Hypsizygus marmoreus* (●), *Poria sclerotium* (○), *Lentinula edodes* (▲), *Pleurotus eryngii* (△). Anti-cancer chemotherapy drug, oxaliplatin (□); (b) Extract from *Hypsizygus marmoreus* by a macchinetta (●) and an autoclave at (○).

3.3. Extraction of tannin from bark

Table 3 shows the results of the tannin extraction from each method and its corresponding protein adsorption ability. From the boiling extraction, the tannin yield was very small. From the macchinetta extraction, the yield from the extraction was increased at 130 ℃. Approximately twice the amount of tannin was extracted using the autoclave extraction method as the macchinetta extraction method.

Table 3: Extraction yield of tannin from bark, and its protein adsorption ability.

Extraction condition					Protein adsorption ability	
Method	Extraction temperature	Extraction time	Supplied bark	Tannin yield	Concentration of tannin	Adsorption ratio
	[℃]	[min]	[g]	[g/g]	[g/L]	[g/g]
Boiling	100	60	1	0.001	-	-
Macchinetta	112	2.7	1	0.127	0.1	0.072
	118	2.6	1	0.123	0.1	0.084
	135	0.8	1	0.153	0.1	0.189
Autoclave	121	120	0.5	0.297	0.1	0.042
Gallotannin	-	-	-	-	0.1	0.500

Protein adsorption of tannin: As shown in Table 3, the protein adsorption ability of macchinetta extracted tannin was higher than that obtained from the autoclave extraction method. The low adsorption ability of the tannin from the autoclave extraction method may be caused by the condensation of the tannin during the long extraction time. However, the protein adsorption ability of tannin from bark was half that of gallotannin. Since bark is an inexpensive raw material, the utility value of bark tannin from macchinetta extraction is high.

Antimicrobial activity: Figure 4 shows the antimicrobial activity of tannin obtained from the macchinetta extractor toward *Staphylococcus aureus*. The number of surviving cells was decreased with an increase in the added tannin concentration. At 5 g/L of tannin, the growth of *Staphylococcus aureus* was almost completed abolished. Therefore, the tannin extracted by this method has high antimicrobial activity.

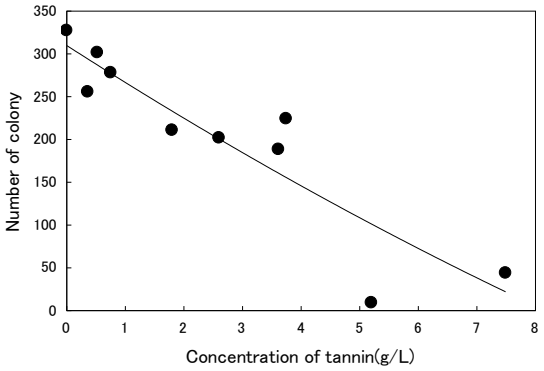

Fig.4: Antimicrobial activity of tannin extracted using a macchinetta extractor toward *Staphylococcus aureus*.

4. Conclusion

The macchinetta extraction of β-glucan from the edible fungus *Hypsizygus marmoreus*, showed antitumor activity toward Caco-2 cancer cell at concentrations above 100 mg/L. Autoclave extraction was effective for producing a high extraction yield, but this solution showed no antitumor activity. The reason may be the excess decomposition occurred in the autoclave during the long 2 h extraction time. Overall, the macchinetta extractor was useful for obtaining antioxidant extracts from edible fungi.

The tannin yield from the macchinetta extraction of the bark of Japanese cedar was about half compared with that obtained by autoclave extraction. However, the protein adsorption activity of the tannin from the macchinetta extract was 2-fold higher than that from the autoclave extract. Furthermore, it was found that the macchinetta extracted tannin had high antimicrobial activity.

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