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Article

Wild and Cultivated Mushrooms Exhibit Anti-Inflammatory Effects Including Inhibition of Platelet Aggregation and Interleukin-8 Expression

Hiroaki Yoshimoto [1]*, Noriko Miyazawa [2] and Fumio Eguchi [3]

[1] Minami Kyushu University, Kirishima 5-1-2, Miyazaki-shi, Miyazaki, Japan; h-ysmt@nankyudai.ac.jp
[2] Kagawa Nutrition University, Chiyoda 3-9-21, Sakado-shi, Saitama, Japan; miyazawa.noriko@eiyo.ac.jp
[3] Tokyo University of Agriculture, Sakuragaoka 1-1-1, Setagayaku, Tokyo, Japan; f1eguchi@nodai.ac.jp
* Correspondence: h-ysmt@nankyudai.ac.jp; Tel.: 81-985-83-2111

Abstract: There are approximately 130 reported medicinal effects of mushrooms. We investigated the anti-inflammatory effects of hot-water extracts of 66 wild and cultivated fungi species (both edible and poisonous) by analyzing inhibition of platelet aggregation and interleukin (IL)-8 gene expression induced by sodium arachidonate (A-Na), platelet-aggregating factor (PAF), and adenosine diphosphate (ADP). All species exhibited inhibitory effects: 38.3%–98.1%, 37.3%–96.8%, and 41.0%–96.6% species inhibited platelet aggregation induced by A-Na, PAF, and ADP, respectively, while 17.0%–97.0% inhibited *IL-8* expression. *Gyromitra esculenta* showed the highest inhibition rate in all assays. High inhibition (≥80%) of A-Na-, PAF-, and ADP-induced platelet aggregation was observed in 29 (43.9%), 29 (43.9%) and 31 (47.0%) species, respectively. Half (33) of the species exhibited high inhibition of *IL-8* expression. Four (6.1%), five (7.6%), and seven (10.6%) species exhibited inhibition rates of <50% for A-Na-, PAF-, and ADP-induced platelet aggregation, while nine (13.6%) exhibited low inhibition of *IL-8* expression. The majority (87.5%–100%) of poisonous species exhibited high inhibition. Our findings suggest that anti-inflammatory effects are universal among fungi, with poisonous species showing particular potential as raw materials for drug discovery. It can be inferred that many fungi contain universal or pleiotropic compounds with anti-inflammatory activities.

Keywords: anti-inflammatory; medicinal mushrooms; poisonous mushrooms; wild edible mushrooms

1.Introduction

Mushrooms have been used as herbal medicines in China, Japan, and other countries since ancient times [1,2]. In Japan, there are a number of commercially available medicines made from mushrooms; for example, krestin from *Coriolus versicolor*, lentinan from *Lentinula edodes*, and schizophyllan from *Schizophyllum commune* [3]. Currently, there are 2.2–3.8 million species of fungi on Earth. Of these, only 120,000 (3%–8%) species have been accepted in Species Fungorum [4], and very few have been studied for their pharmacological effects or edibility.

The potential of mushrooms with as-yet unknown medicinal properties as raw materials for drug discovery is widely accepted, and these putative properties are an important focus of current research. Poisonous mushrooms in particular are suspected to contain useful medicinal components, despite their toxicity.

There are approximately 130 reported medicinal effects of mushrooms and fungi; these include antitumor, immunomodulatory, antioxidant, radical scavenging, cardiovascular, antihypercholesterolemic, antiviral, antibacterial, antiparasitic, antifungal, detoxifying, hepatoprotective, and antidiabetic effects [5,6]. Among these, anti-inflammatory activity is a key functionality, which explains the diverse pharmacological effects of mushrooms. Thus, examining the anti-inflammatory effects of mushrooms could provide important insight for future drug-

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discovery research. Although many studies have compared the anti-inflammatory effects of a few mushroom species, few have examined the effects across wide range of species.

The preset study provides a comprehensive comparison of 66 species of wild and cultivated mushroom, focusing on their inhibition of platelet aggregation and interleukin (*IL*)-*8* expression.

2. Materials and Methods

2.1. Obtaining and Storage of Fruiting Bodies

Table 1 presents the samples used in this study. Wild mushrooms were collected from mountains and fields in Japan—mainly in the eastern region from Hokkaido to northern Kanto—between 2010 and 2017. Artificially cultivated fruiting bodies were provided by Murata Shiitake Honpo, co. ltd., Miyazaki, Myogi Kinoko, Gunma, JA Nakano, Nagano and Takeuchi Kinoko, Nagano. The obtained fruiting bodies were using a mill, and stored at −25°C. Fungi were classified as edible, inedible, and poisonous based on a previous study [7]. Of the 66 mushroom species, there were 39 Agaricale, ten Polyporale, four Boletale/Pezizale, two Cantharellale/Russulale, and one Aphyllophorale/Auriculariale/Hymenochaetale/Thelephorale/Tremellale species. All samples were taken from mature fruiting bodies that had opened.

Table 1. Species used in the present study.

	Species	Japanese name	Wild/Cultivated
Poisonous	*Amanita muscaria*	Benitengutake	W
	Amanita pantherina	Tengutake	W
	Amanita virosa	Doutsurutake	W
	Chlorophyllum molybdites	Ooshirokarakasatake	W
	Gyromitra esculenta	Shagumaamigasatake	W
	Pleurocybella porrigens	Sugihiratake	W
	Psilocybe argentipes	Hikageshibiretake	W
	Tricholoma flavovirens	Kishimeji	W
Not edible	*Antrodia Cinnamomea*	Benikusunokitake	W
	Fomes fomentarius	Tsuriganetake	W
	Fomitopsis pinicola	Tsugasarunokoshikake	W
	Ganoderma lucidum	Mannentake	C
	Helvella crispa	Noboriryutake	W
	Helvella lacunosa	Kuronoboriryu	W
	Mycena haematopoda	Chishiotake	W
	Phellinus linteus	Meshimakobu	W
	Polyporus umbellatus	Choreimaitake	W
	Trametes versicolor	Kawaratake	C
	Wolfiporia cocos	Bukuryo	W
Edible	*Agaricus bisporus var. albidus*	Tsukuritake	C
	Agaricus bisporus var. brunnescens	Tsukuritake (Brown)	C
	Agaricus subrufescens	Himematsutake	C
	Agrocybe cylindracea	Yanagimatsutake	C
	Amanita hemibapha	Tamagotake	W
	Armillaria mellea	Naratake	W
	Auricularia auricula	Kikurage	C
	Boletus aereus	Susukeyamadoritake	W
	Boletus reticulatus	Yamadoritakemodoki	W
	Calvatia nipponica	Onihusube	W
	Cantharellus cibarius	Anzutake	W
	Clavaria zollingeri	Murasakihoukitake	W

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Clitocybe nebularis	Haiiroshimeji	W
Coprinus atramentarius	Hitoyotake	C
Craterellus cornucopioides	Kurorappatake	W
Flammulina velutipes	Enokitake	C
Flammulina velutipes var. brunnea	Enokiake (Brown)	C
Grifola frondosa var. alba	Maitake (White)	C
Grifola frondosa var. brunnea	Maitake (Brown)	C
Hericium erinaceum	Yamabushitake	C
Hypsizigus marmoreus	Bunashimeji	C
Lactarius laeticolorus	Akamomitake	W
Lactarius volemus	Chichitake	W
Laetiporus sulphureus	Masutake	W
Leccinum extremiorientale	Akayamadori	W
Leccinum scabrum	Yamaiguchi	W
Lentinula edodes	Shiitake	C
Lyophyllum decastes	Hatakeshimeji	C
Lyophyllum shimeji	Honshimeji	W
Morchella conica	Togariamigasatake	W
Naematoloma sublateritium	Kuritake	C
Panellus serotinus	Mukitake	C
Pholiota nameko	Nameko	C
Pleurotus abalonus	Kuroawabitake	C
Pleurotus cornucopiae var. citrinopileatus	Tamogitake	C
Pleurotus eryngii	Eringi	C
Pleurotus eryngii var. tuoliensis	Bairingu	C
Pleurotus ostreatus	Hiratake	C
Pleurotus pulmonarius	Usuhiratake	C
Pleurotus salmoneostramineus	Tokiirohiratake	C
Pleurotus sp.	Agitake	C
Rhodophyllus clypeatus	Harushimeji	W
Sarcodon aspratus	Koutake	W
Sparassis crispa	Hanabiratake	C
Suillus grevillei	Hanaiguchi	W
Tremella fuciformis	Shirokikurage	C
Tricholoma matsutake	Matsutake	W

* Statistically different from Gyromitra esculenta, $p < 0.05$.

2.2. Preparation of Samples for Assay

Dried fruit bodies were ground using a Wonder Blender WB-1 (Osaka Chemical Co., Ltd.; Osaka, Japan), then sieved through a 1000-μm mesh. The resulting powder was used for analyses. To evaluate inhibition of platelet aggregation and *IL-8* expression, the powder was extracted by incubating in 10 volumes of hot water (80°C) for 2 h, after which the hot-water extract was concentrated under reduced pressure after filtration using Advantec No. 2 filter paper (Toyo Roshi Kaisha, Ltd.; Tokyo, Japan).

2.3. Platelet Aggregation Assay

We evaluated the inhibition of platelet aggregation induced by sodium arachidonate (A-Na), platelet activating factor (PAF), and adenosine diphosphate (ADP). Human peripheral blood was

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collected from the median cubital vein of a medication-free healthy adult for at least 2 weeks. The blood was centrifuged (200 × g for 20 min at room temperature) and the upper layer collected to obtain platelet-rich plasma (PRP). The lower layer was then centrifuged (200 × g for 5 min at room temperature) and collected to obtain platelet-poor plasma (PPP).

PRP and PPP (223 μL each) were preheated at 37°C. The 5% hot-water extract was dissolved in 2% dimethyl sulfoxide (DMSO) solution and 2 μL was added to PRP and PPP. The resulting mixtures were incubated for 3 min at 37°C, then 25 μL of an aqueous solution of either AA, PAF, ADP, or ion-exchanged water (control) was added to induce platelet aggregation. The concentrations of AA, PAF, and ADP were 500 nM. Aggregation was measured using an aggregometer (MCM Hema Tracer 313M; MC Medical Co., Ltd. Tokyo, Japan) and inhibitory effects evaluated by comparing the maximum aggregation rate (calculated as the maximum value of the aggregation curve for each sample by normalizing the value of the PPP sample to 100) with the control. Inhibition rates were normalized to the control to calculation of the efficacy of the test agents [8].

2.4. Inhibition of Interleukin-8 Expression

Normal human dermal fibroblasts were cultivated in Dulbecco's Modified Eagle Medium with 10% fetal bovine serum until the growth was confluent (6 cm in diameter). The 5% hot-water extract was dissolved in 2% DMSO solution and 2 μL was added to the dish (final concentration: 0.01% [dry mass]). For the positive control, 10^{-7} M hydrocortisone was added in place of the hot-water extract. Next, 1 ng/mL of tumor necrosis factor alpha (TNF-α, which promotes chemokine gene expression) was added, and samples were incubated for 6 h at 37°C. A sample-free control was prepared without TNF-α, and incubated under identical conditions.

Total RNA was isolated using the ISOGEN reagent (Nippon Gene Co., Ltd.; Tokyo, Japan) according to the manufacturer's instructions. Total RNA (1 μg) was reverse transcribed to cDNA using M-MLV reverse transcriptase (Life Technologies Co. Ltd.; Maryland, USA) according to the manufacturer's instructions. Expression of *IL-8* was measured using quantitative real-time polymerase chain reaction. cDNA was prepared using TaqMan reverse transcription reagent, and samples were quantified using TaqMan universal PCR master mix and the ABI Prism 7,700 sequence detection system (Applied Biosystems; Foster City, CA, USA). Nucleotide sequences for PCR primers and probes were as follows: *IL-8* forward primer, 5′-TCAGAGACAGCAGAGCACACA-3′; reverse primer, 5′-CTTGGCAGCCTTCCTGATT-3′; probe, 5′-AACATGACTTCCAAGCTGGCCA-3′; GAPDH forward primer, 5′-GAAGGTGAAGGTCGGAGTC-3′; reverse primer, 5′-GAAGATGGTGATGGGATTTC-3′; probe, 5′-AGGCTGAGAACGGGAAGCTTG-3′. The glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) gene was used as an internal standard gene. Inhibition rates were normalized to that of TNF-α for calculation of the efficacy of samples [9].

2.5. Statistical Analyses

Data are expressed as means ± standard deviation of at least three replicates for each sample. Statistical analyses were performed using Microsoft 365 Excel with Statcel4 add-in software (OMS; Tokyo, Japan). Data were compared using one-way analysis of variance followed by the Tukey–Kramer post-hoc test for multiple comparisons. P values of <0.05 were considered statistically significant.

3. Results

3.1. Platelet Aggregation Assay and Inhibition of Interleukin-8 Expression

Table 2 shows the rates of inhibition of platelet aggregation and *IL-8* expression. All samples exhibited inhibitory effects. Regarding platelet aggregation inhibition, the highest mean value for A-Na was 98.1% ± 0.21%, whereas *Helvella crispa* had the lowest of 38.3% ± 0.47%. In terms of PAF, the highest mean value was 96.8% ± 0.74%, whereas *Mycena haematopoda* had the lowest of 37.3% ± 1.25%. In terms of ADP, the highest mean value was 96.6% ± 0.36%, whereas *Helvella crispa* had the lowest of 41.0% ± 3.74%. In terms of the *IL-8* gene, the highest mean value was 97.0% ± 1.41%, whereas *Agaricus bisporus* var. albidus had the lowest of 17.0% ± 2.45%. *Gyromitra esculenta* exhibited the

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highest inhibition rate among all assays. There were no significant differences in the *IL-8* expression among all poisonous mushrooms, including *Gyromitra esculenta*, *Amanita muscaria*, *Amanita pantherina*, *Pleurocybella porrigens*, *Tricholoma flavovirens*, *Amanita virosa*, *Psilocybe argentipes*, and *Chlorophyllum molybdites*. The nonedible mushrooms; *Trametes versicolor*, *Ganoderma lucidum*, *Wolfiporia cocos*, and *Polyporus umbellatus* did not statistically differ. In the edible mushrooms; *Tricholoma matsutake*, *Pleurotus ostreatus*, *Lyophyllum decastes*, *Hericium erinaceum*, and *Sparassis crispa*, the inhibition rates were not statistically significant.

Table 2. Inhibition rate of platelet aggregation and Interleukin-8 gene expression of 66 wild and cultivated mushrooms.

	Species	A-Na			PAF			ADP			IL-8		
		Mean ± S. D.		Rank	Mean ± S. D.		Rank	Mean ± S. D.		Rank	Mean ± S. D.		Rank
Poisonous	*Gyromitra esculenta*	98.1	± 0.21	**1**	96.8	± 0.74	**1**	96.6	± 0.36	**1**	97.0	± 1.41	**1**
	Amanita muscaria	96.7	± 0.94	**2**	92.7	± 2.62	**3**	93.3	± 1.70	**3**	97.0	± 1.41	**1**
	Amanita pantherina	94.0	± 1.63 *	**3**	89.0	± 1.63 *	**6**	90.7	± 1.25 *	**6**	92.0	± 2.45	12
	Pleurocybella porrigens	91.5	± 0.62 *	**7**	92.4	± 0.70	**4**	92.4	± 1.58	**4**	91.0	± 1.41	13
	Tricholoma flavovirens	90.7	± 0.33 *	**10**	86.8	± 1.02 *	13	87.0	± 0.75 *	20	94.0	± 1.41	**5**
	Amanita virosa	90.0	± 0.29 *	11	95.7	± 0.66 *	**2**	94.8	± 1.49	**2**	94.0	± 1.41	**5**
	Psilocybe argentipes	86.1	± 0.16 *	15	83.5	± 0.79 *	21	87.3	± 1.08 *	17	90.0	± 1.41	16
	Chlorophyllum molybdites	68.5	± 0.33 *	51	61.7	± 0.63 *	54	57.4	± 1.27 *	58	91.0	± 1.41	13
Not edible	*Trametes versicolor*	94.0	± 2.45 *	**3**	92.3	± 1.25	**5**	90.7	± 1.25 *	**6**	97.0	± 1.41	**1**
	Ganoderma lucidum	93.7	± 2.49 *	**5**	87.3	± 2.87 *	**10**	86.7	± 3.09 *	21	95.0	± 2.45	**4**
	Wolfiporia cocos	91.1	± 0.59 *	**9**	88.4	± 1.38 *	**7**	87.3	± 2.01 *	18	94.0	± 1.41	**5**
	Polyporus umbellatus	86.4	± 0.19 *	14	86.6	± 0.68 *	15	88.2	± 0.97 *	**10**	94.0	± 1.41	**5**
	Phellinus linteus	85.9	± 0.26 *	16	82.1	± 0.41 *	27	81.2	± 0.29 *	31	81.0	± 1.41 *	32

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	Fomitopsis pinicola	85.7	± 0.94	*	17	85.3	± 3.09	*	17	87.7	± 2.87	*	13	88.0	± 1.41	*	20
	Antrodia Cinnamomea	82.0	± 1.35	*	26	80.6	± 0.42	*	29	81.4	± 0.87	*	28	94.0	± 1.41	*	**5**
	Fomes fomentarius	71.7	± 2.05	*	43	65.0	± 1.63	*	52	56.0	± 0.82	*	59	93.0	± 1.41	*	**10**
	Mycena haematopoda	46.7	± 1.25	*	63	37.3	± 1.25	*	66	43.3	± 2.62	*	63	63.0	± 6.16	*	50
	Helvella lacunosa	46.0	± 1.63	*	64	43.7	± 3.40	*	62	41.3	± 2.05	*	65	63.0	± 6.16	*	50
	Helvella crispa	38.3	± 0.47	*	66	39.7	± 2.62	*	64	41.0	± 3.74	*	66	67.0	± 3.74	*	46
Edible	*Tricholoma matsutake*	92.3	± 1.25	*	**6**	88.3	± 2.05	*	**8**	92.0	± 2.16		**5**	89.0	± 2.45		18
	Pleurotus ostreatus	91.3	± 2.05	*	**8**	85.3	± 1.25	*	17	89.0	± 1.63	*	**9**	90.0	± 1.41		16
	Sarcodon aspratus	88.0	± 1.24	*	12	88.1	± 0.16	*	**9**	87.6	± 1.06	*	15	87.0	± 3.74	*	23
	Suillus grevillei	87.0	± 0.82	*	13	82.7	± 0.94	*	25	86.3	± 2.05	*	22	77.0	± 2.45	*	38
	Lyophyllum decastes	85.4	± 0.70	*	18	87.0	± 0.22	*	11	87.6	± 0.69	*	14	89.0	± 2.45		18
	Lactarius laeticolorus	85.3	± 1.25	*	19	82.7	± 1.25	*	25	84.0	± 2.45	*	25	81.0	± 1.41	*	32
	Boletus reticulatus	84.5	± 0.66	*	20	81.1	± 0.17	*	28	84.1	± 1.63	*	24	59.0	± 2.45	*	55
	Flammulina velutipes var. brunnea	84.4	± 0.75	*	21	86.9	± 0.71	*	12	87.7	± 0.26	*	12	82.0	± 2.83	*	28
	Agaricus subrufescens	84.2	± 0.59	*	22	85.9	± 0.31	*	16	87.6	± 0.78	*	15	82.0	± 1.41	*	28
	Pleurotus cornucopiae var. citrinopileatus	84.0	± 2.94	*	23	86.7	± 1.25	*	14	81.3	± 2.87	*	29	85.0	± 1.41	*	25
	Hericium erinaceum	82.8	± 0.96	*	24	84.5	± 2.02	*	20	90.1	± 0.53	*	**8**	91.0	± 1.41		13

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Flammulina velutipes	82.7	± 0.33	*	25	83.3	± 0.43	*	22	78.6	± 0.52	*	34	84.0	± 1.41	*	26
Pleurotus eryngii var. tuoliensis	81.9	± 0.96	*	27	83.1	± 0.57	*	24	87.9	± 0.85	*	11	87.0	± 1.41	*	21
Pleurotus abalonus	81.9	± 0.31	*	28	83.2	± 0.29	*	23	85.5	± 1.45	*	23	78.0	± 1.41	*	35
Panellus serotinus	80.9	± 0.93	*	29	78.6	± 1.37	*	30	77.5	± 1.38	*	36	78.0	± 1.41	*	35
Amanita hemibapha	78.9	± 1.01	*	30	85.3	± 0.21	*	17	87.1	± 0.37	*	19	86.0	± 2.45	*	24
Laetiporus sulphureus	78.0	± 0.82	*	31	75.7	± 2.62	*	36	82.0	± 1.41	*	27	79.0	± 2.83	*	34
Leccinum scabrum	77.7	± 1.25	*	32	72.7	± 2.62	*	42	76.0	± 1.41	*	39	78.0	± 1.41	*	35
Leccinum extremiorientale	76.7	± 1.47	*	33	76.4	± 1.52	*	35	73.3	± 0.62	*	47	76.0	± 1.41	*	39
Lyophyllum shimeji	76.7	± 0.42	*	34	72.3	± 0.45	*	43	75.9	± 1.44	*	40	59.0	± 2.45	*	55
Morchella conica	75.8	± 1.28	*	35	77.2	± 0.28	*	31	73.3	± 2.23	*	46	72.0	± 1.41	*	40
Pleurotus pulmonarius	75.2	± 1.98	*	36	71.8	± 0.78	*	44	79.2	± 1.14	*	32	70.0	± 1.41	*	43
Rhodophyllus clypeatus	74.5	± 2.64	*	37	76.7	± 0.54	*	32	79.1	± 0.46	*	33	40.0	± 1.41	*	59
Sparassis crispa	74.4	± 1.05	*	38	75.2	± 0.49	*	37	76.9	± 0.73	*	37	93.0	± 1.41		10
Boletus aereus	73.8	± 3.21	*	39	76.7	± 0.66	*	32	76.7	± 1.79	*	38	40.0	± 1.41	*	59
Craterellus cornucopioides	73.7	± 0.70	*	40	76.5	± 1.38	*	34	75.7	± 0.49	*	41	54.0	± 3.74	*	57
Lentinula edodes	72.8	± 0.69	*	41	70.8	± 0.31	*	46	74.5	± 1.43	*	44	70.0	± 1.41	*	42
Grifola frondosa var. alba	72.5	± 0.42	*	42	74.9	± 1.32	*	38	78.3	± 0.43	*	35	87.0	± 1.41	*	21

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Grifola frondosa var. brunnea	71.6	±	0.94	*	44	74.0	±	0.34	*	39	75.4	±	0.96	*	42	84.0	±	1.41	*	26
Agrocybe cylindracea	71.3	±	0.76	*	45	67.4	±	0.34	*	50	73.4	±	0.62	*	45	63.0	±	3.74	*	52
Auricularia auricula	71.3	±	0.46	*	46	73.9	±	1.25	*	40	81.3	±	0.86	*	29	71.0	±	2.45	*	41
Tremella fuciformis	71.2	±	0.73	*	47	70.8	±	0.37	*	45	75.0	±	1.69	*	43	62.0	±	2.45	*	54
Naematoloma sublateritium	70.2	±	0.90	*	48	57.2	±	1.18	*	61	57.8	±	0.77	*	57	63.0	±	1.41	*	52
Pleurotus eryngii	69.3	±	1.72	*	49	70.3	±	0.92	*	47	68.7	±	0.54	*	51	37.0	±	1.41	*	62
Lactarius volemus	69.0	±	1.41	*	50	64.3	±	3.86	*	53	70.3	±	3.40	*	50	70.0	±	1.41	*	43
Armillaria mellea	67.7	±	0.47	*	52	73.7	±	1.25	*	41	83.0	±	1.41	*	26	82.0	±	2.83	*	28
Cantharellus cibarius	66.9	±	2.19	*	53	68.1	±	0.60	*	49	68.0	±	0.66	*	52	65.0	±	4.24	*	48
Clitocybe nebularis	63.3	±	0.41	*	54	67.0	±	1.24	*	51	70.8	±	0.37	*	49	40.0	±	1.41	*	59
Pleurotus salmoneostramineus	62.9	±	0.45	*	55	68.2	±	0.90	*	48	71.1	±	0.36	*	48	48.0	±	1.41	*	58
Pleurotus sp.	62.9	±	0.14	*	56	57.8	±	1.72	*	60	48.0	±	0.70	*	60	27.0	±	1.41	*	64
Coprinus atramentarius	62.1	±	0.50	*	57	57.8	±	0.38	*	59	60.4	±	0.62	*	56	34.0	±	3.74	*	63
Pholiota nameko	61.9	±	1.43	*	58	58.2	±	0.79	*	57	67.8	±	0.24	*	53	66.0	±	3.74	*	47
Hypsizigus marmoreus	61.4	±	0.57	*	59	60.7	±	0.99	*	55	65.9	±	2.04	*	54	64.0	±	1.41	*	49
Calvatia nipponica	58.9	±	0.16	*	60	58.3	±	0.56	*	56	62.3	±	1.19	*	55	82.0	±	1.41	*	28
Clavaria zollingeri	57.5	±	0.47	*	61	58.0	±	2.94	*	58	45.3	±	1.25	*	61	69.0	±	2.83	*	45

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Agaricus bisporus var. brunnescens	51.2	± 0.74	*	62	39.6	± 0.57	*	65	42.4	± 1.11	*	64	21.0	± 3.74	*	65
Agaricus bisporus var. albidus	42.6	± 0.76	*	65	40.2	± 0.90	*	63	44.7	± 2.10	*	62	17.0	± 2.45	*	66

* Statistically different from Gyromitra esculenta, $p < 0.05$. Significant differences were detected with respect to *Gyromitra esculenta*, which showed the highest inhibition rate, and are marked with an asterisk.

Inhibition rates of 80% or more are considered empirically applicable for the purposes of drug discovery. Twenty-nine (43.9%) of the 66 species that we tested achieved this rate for aggregation induced by A-Na, while 31 (47.0%) and 31 (47.0%) species achieved high rates for inhibition of aggregation induced by PAF and ADP, respectively. In inhibition of *IL-8* gene expression, 33 (50.0%) species achieved high rates.

Of the samples that exhibited "low" inhibition rates (50% or lower), four (6.1%), five (7.6%), and seven (10.6%) showed low inhibition of A-Na-, PAF-, and ADP-induced platelet aggregation, respectively; while nine (13.6%) exhibited low inhibition of *IL-8* expression (Table 3). 87.5%–100% of poisonous mushrooms exhibited high inhibition rates of 80% or more through four assays.

Table 3. Summary of anti-inflammation of 66 mushrooms.

		A-Na		PAF		ADP		IL-8	
		species	%	species	%	species	%	species	%
All	>80%	29	43.9%	29	43.9%	31	47.0%	33	50.0%
	<50%	4	6.1%	5	7.6%	7	10.6%	9	13.6%
Poisonous	>80%	7	87.5%	7	87.5%	7	87.5%	8	100.0%
	<50%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
Not edible	>80%	7	63.6%	7	63.6%	7	63.6%	8	72.7%
	<50%	3	27.3%	3	27.3%	3	27.3%	0	0.0%
Edible	>80%	15	31.9%	15	31.9%	17	36.2%	17	36.2%
	<50%	1	2.1%	2	4.3%	4	8.5%	9	19.1%

3.2. Taxonomical Classification

Our examination of the taxonomic commonality of species revealed the variance of specimens in each order to be large, ranging from 23.3 for Cantharellale to 760.7 for Pezizale, and no trends were identified (Table 4).

Table 4. Summary of the taxonomical classification of species used in the present study.

Family	Number of samples	Average	S. D.	Variance	Min.	Max.
Agaricales	39	74.7	13.4	185.4	42.6	96.7
Polyporales	10	81.9	8.8	86.1	71.6	94.0
Boletales	4	81.5	4.4	25.5	76.7	87.0
Pezizales	4	64.6	23.9	760.7	38.3	98.1
Cantharellales	2	70.3	3.4	23.3	66.9	73.7

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Russulales	2	75.9	6.9	94.8	69.0	82.8
Aphyllophorales	1	82.0	—	—	—	—
Auriculariales	1	71.3	—	—	—	—
Hymenochaetales	1	85.9	—	—	—	—
Thelephorales	1	88.0	—	—	—	—
Tremellales	1	71.2	—	—	—	—
Total	66	75.9	13.3	180.7	38.3	98.1

Only A-na is described; other assays are omitted.

4. Discussion

Inhibition of platelet aggregation is an important aspect of anti-inflammatory activity. Platelets aggregate at the site of vascular injury to stop bleeding, as well as to induce an inflammatory response through the release of inflammatory mediators including prostaglandin E2 (PGE2), thromboxane A2 (TXA2), histamine, serotonin, and PAF. Anti-inflammatory drugs suppress inflammatory responses by inhibiting platelet aggregation; for example, nonsteroidal anti-inflammatory drugs inhibit cyclooxygenase and suppress the production of PGE2 and TXA2. In contrast, inflammatory mediators released from platelets induce the expression of inflammatory chemokine genes such as IL-8, promoting inflammation [10]. Inhibition of platelet aggregation and chemokine expression can have effects both upstream and downstream of the inflammatory response. The present study supports previous reports of the anti-inflammatory effects of fungi [11–13]. Inhibition of cyclooxygenase 2 has been confirmed in several fungi [14,15]. Although COX inhibition was not examined in this study, we expect a COX2 inhibitory effect, and we would like to consider this as a topic for future study.

Our results suggest that many species of fungi have anti-inflammatory properties and that poisonous species in particular have great potential value as raw materials for drug discovery. Our findings highlight the high number of poisonous species with medicinal properties.

Isolation and/or identification of specific bioactive compounds responsible for the observed activities of the tested species was beyond the scope of the present study; however, polysaccharides, proteins, fatty acids [16–18], sterols and polyisoprene polyols [19,20], and carbohydrate-protein complexes [21] have been suggested to be involved in the bioactivity.

We investigated the inflammatory effects using hot-water extracts, which may lead to different results compared with other extraction methods. Further detailed studies are required to identify the active compounds. Furthermore, pharmacological effects cannot be determined on the basis of in-vitro tests alone; thus, further in vivo tests and human clinical trials are required to draw firm conclusions from the present findings, as well as identify the absence or involvement of cytotoxicity etc.

The commonality between genera could not be examined due to the small sample number, but we speculate that it may be due to the characteristics of each species. The pharmacological effects of mushrooms have been reported to be influenced by the composition of the medium [22], as well as by the strain and cultivation stage [23]. This suggests that the characteristics of fungi species may vary depending on the growth environment and stage.

5. Conclusions

The present examination of the anti-inflammatory effects of 66 wild and cultivated fungi species revealed approximately half of the mushrooms to have high rates of inhibition of inflammation. Our findings imply that the presence of substances with anti-inflammatory effects may be a universal property of fungi. Mushrooms have been reported to exhibit various pharmacological activities, and their anti-inflammatory effects are speculated to be involved in the underlying mechanisms. The results presented here highlight the potential of fungi as promising raw materials for drug discovery and open up new avenues for detailed research to identify the active pharmacological components in these species.

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Abbreviations

The following abbreviations are used in this manuscript:

ADP	adenosine diphosphate
A-Na	Sodium arachidonate
DMSO	dimethylsulfoxide
IL-8	interleukin-8
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
PAF	platelet activating factor
PPP	platelet-poor plasma
PRP	platelet-rich plasma
TNF-α	tumor necrosis factor-α

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