



Extracts of *Hypsizygus tessellatus* (white var.) caps inhibited MCF-7 and MDA-MB-231 cell lines proliferation

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Abstract

Cancer management is associated with serious side-effects due to the harmful nature of radiation and chemotherapy on the body cells. These side-effects have necessitated the need for diversifying the alternative or complementary sources of cancer therapy. Natural products have been on the front line as alternative sources of anticancer agents and have attracted much attention in recent times. In this study, the anticancer activity of *Hypsizygus tessellatus* (white var.) caps (also known as Bunapi shimeji) extracted with acetone and ethyl acetate was evaluated in vitro against MDA-MB-231 and MCF-7 (breast cancer cell lines) and MCF-10a (Vero or normal breast cells). Likewise, the free radical scavenging and metal reducing activities of the extract were evaluated through in vitro chemical-based methods. Furthermore, the phytochemical compositions of the extracts were determined through LC–MS–QTOF-assisted mass spectroscopy. The results of this study indicated that acetone fraction had better radical scavenging activity against DPPH ($IC_{50} = 0.76$ mg/mL) and H_2O_2 ($IC_{50} = 0.84$ mg/mL) than ethyl acetate fraction against DPPH ($IC_{50} = 1.10$ mg/mL) and H_2O_2 ($IC_{50} = 1.26$ mg/mL) ($p < 0.05$). Additionally, the acetone fraction was observed to have more antiproliferative effects against MCF-7 ($IC_{50} = 0.051$ – 0.055 mg/mL) and MDA-MB-231 ($IC_{50} = 0.122$ – 0.131 mg/mL) compared to the ethyl acetate fraction against MCF-7 ($IC_{50} = 0.075$ – 0.096 mg/mL) and MDA-MB-231 ($IC_{50} = 0.161$ – 0.164 mg/mL) ($p < 0.05$). Both extracts generally had less effect on MCF-10a cells. Thus, these results suggested that Bunapi shimeji caps is a potential good natural source of anticancer agents.

Keywords MCF-7 · MDA-MB-231 · Antioxidant · Antiproliferation · Bunapi shimeji · *H. tessellatus* · Mushroom phytochemicals

Introduction

Cancer, diabetes, immune-system decline, cardiovascular diseases, and brain dysfunction are some of the degenerative diseases associated with reactive oxygen species (ROS) and aging [1]. Their linkage to free radicals is associated with the postulation that ROS-mediated damages to macromolecules and deoxyribonucleic acid (DNA) tend to accumulate with

time and has been considered as a major form of endogenous damage that results to aging [2]. Mutagens such as hydroxyl radicals or hydrogen peroxide are also side-products of normal human body metabolism [3]. Humans have always embarked on the consumption of plant foods (fruits, red wines, fruits, and juices) and some mushrooms (to an extent) with the belief that they can confer them with some levels of protection against oxidative damages and diseases such as cancer [4]. This presumed protection was due to the ability of the antioxidants contained in these natural foods to scavenge the generated free radicals and keep their body healthy [5].

Before now, chemically-sourced antioxidants such as beta hydroxy acid (BHA) and butylated hydroxytoluene (BHT) have been commonly used in the stabilization of foods. However, these synthetic antioxidants were reported to be carcinogenic in experimental animals [6]. Consequently, efforts are geared towards finding alternative sources of natural antioxidants, especially from natural sources. In this respect,

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mushrooms have received much attention [7] considering that they have served as a major source of dietary nutrients and wholesome body benefits for ages due to their high phenolic contents, low fat and caloric contents, abundance in essential amino acids (leucine, cysteine, methionine) minerals (iron, zinc, manganese), and vitamins (vitamin C, folate, vitamin D, riboflavin, niacin, thiamin) [8]. Polyphenols are one of the non-essential dietary components with proven capabilities towards atherosclerosis and cancer inhibition [9]. The biological activity of polyphenols is related to their metal chelating, lipoxxygenase inhibiting, and free radicals scavenging activities. Some of the previously identified polyphenols in the edible mushrooms include gallic acid, quercetin, pyrogallol, homogentisic acid, 5-sulfosalicylic acid, protocatechuic acid, chlorogenic acid, *p*-coumaric acid, *o*-coumaric acid, myricetin, resveratrol [10].

Several mushroom species contain several compounds with both medicinal and nutritional values. Mushrooms also contain other biologically active secondary metabolites such as polyketides, steroids, and terpenes [11] whose contents vary across mushroom species. Solvent polarity also contributes to the variation in the quantity of the extractable compounds from several mushrooms due to the differences in the solubility of the compounds in various compounds. Acetone and ethyl acetate have previously reported as effective solvents for the extraction of phytochemicals from biological extracts [12]. The justification for this study is based on the recent US breast cancer statistics report 2018, where breast cancer was projected as a leading cause of death around the world. According to the report, 266,120 new breast cancer cases are expected in the United States of America (USA) by 2018, while 63,960 new non-invasive cases are anticipated. Mortality-wise, approximately 40,920 breast cancer-related deaths are expected in the USA in 2018 [13]. These are challenging findings that require expedient actions towards diversifying the natural sources of anticancer agents. Hence, in this study, the anticancer activities of acetone and ethyl acetate extracts of white *Hypsizygus tessellatus* caps were investigated against 2 human breast cancer cell lines and Vero cells. The white variant of *H. tessellatus* (commonly called Bunapi shimeji) was selected for this study because there is no supportive evidence on its anticancer activity against any human breast cancer cell line despite its availability in the study area.

Materials and methods

Mushroom sample

The mushroom (white *H. tessellatus*), henceforth referred to as Bunapi shimeji, was identified based on literature evidence and supplied by a local supplier of biological

materials (Orioner High-Tech Sdn. Bhd) in Kuantan, Pahang, Malaysia.

Chemicals and cell lines

Sodium carbonate anhydrous 99.99% purity trace metal basis (TMB), ferric chloride (>99% purity), 1,1-diphenyl-2-picrylhydrazyl (DPPH), hydrogen peroxide, aluminum chloride (99.99% purity TMB), gallic acid (>98% purity), Folin–Ciocalteu reagent, ascorbic acid (99% purity), potassium phosphate (monobasic and dibasic (99.95% purity)), quercetin (>95% HPLC grade), potassium ferricyanide (99.5% REACH compliance), trichloroacetic acid (TCA >99% purity CAS grade) Dulbecco's modified eagles medium (DMEM), penicillin/streptomycin solution, fetal bovine serum (FBS), and phosphate buffered saline were bought from Merck-Darmstadt, Germany. MCF-10a, MDA-MB-231, and MCF-7 were procured from the American Type Culture Collection (ATCC- Manassas, VA, USA). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide (MTT) kit was purchased from Solarbio-China.

Mushroom preparation and extraction

The caps of the mushrooms were first separated from the mushroom stems. The separated mushroom caps were dried to a regular weight in an incubator at 70 °C. Then, the dried caps were pulverized in a grinder, packaged in a dark plastic bag, and stored at 4 °C prior to usage. The pulverized mushroom caps (180 g) was weighed and added into two 500-mL flat bottom conical flasks. Then, 500 mL of either acetone or ethyl acetate (99%) was added to each flask. The flasks were sealed with aluminum foil and mounted on a shaker rotating at a speed of 250 revolutions per minute (rpm). The flasks were shaken on the shaker for 24 h at room temperature (25 ± 2.4 °C). After 24 h, the content of the flasks was filtered through several changes of Whatman no. 1 filter paper, then through a 0.45 µm syringe filter, concentrated in a rotary evaporator, and allowed to vaporize in a hood chamber. The dried extracts were collected in sterile universal bottles and preserved at –20 °C prior to characterization.

Total phenolic content (TPC)

The extracts were characterized for TPC using the method described by Seonwook et al. [14]. Briefly, the extracts were first reconstituted in distilled water at the concentration of 1 mg/mL. Then, 1 mL of the reconstituted extract was added to 100-mL volumetric flasks. Then, 45 mL of distilled water was added to the flasks before adding Folin–Ciocalteu phenol reagent (1 mL). The flasks were thoroughly mixed and incubated with mild shaking for 3 min at ambient temperature. Then, 2% sodium carbonate solution (3 mL) was

added to the flasks before further incubation for 2 h at ambient temperature with occasional shaking. After about 2 h of incubation, the absorbance of the solution was read in a spectrophotometer (Bibby Scientific Limited) at 760 nm. The analysis was performed in triplicate using gallic acid as a standard phenolic source. The results were presented as micrograms of gallic acid equivalents ($\mu\text{g GAE/mg}$) of the extract.

Total flavonoids content (TFC)

The flavonoids content of the extracts was determined using the method reported by Muhammad et al. [15]. In this method, 1 mL of 2% AlCl_3 (prepared in absolute methanol) was incubated in a glass tube with 1 mL of the extract solutions (1 mg/mL) for 10 min at room temperature. After the incubation period, the absorbance of the solution was read at 415 nm in a spectrophotometer. Quercetin was used to prepare the standard flavonoid curve ($R^2 = 0.98$) at a concentration range of 0.1–1.0 mg/mL. The results were presented as the mean \pm standard deviation (SD) of triplicate measurements in micrograms of quercetin equivalent ($\mu\text{g QE/mg}$) of extracts.

LC-MS-QTOF analysis

The phytochemical composition of the extracts was determined using an AGILENT 6560 UHPLC-IM-QTOF-MS/MS system. For the analysis, the extract (5 mg) was first solubilized in methanol (1 mL) before injection into the system. The separation of the constituents was achieved using a mobile phase made up of 0.1% formic acid in water and acetonitrile. The sample was injected into the machine at a pre-set volume of 5 μL , maintaining a flow rate of 0.2 mL/min. The identification of the compounds was achieved by comparing the determined molecular weight to the theoretical molecular weights (MS/MS) from a standard database (NIST Mass Spectrometry Data Center) and calculating the mass error (ppm). This mass comparison and mass error calculation were automatically performed by the machine.

In vitro antioxidant activity

Three in vitro-based assay methods were deployed to ascertain the antioxidant activities of the extracts. These assays demonstrate the ability of different concentrations of the extracts to scavenge free hydrogen peroxide and DPPH radicals, as well as to reduce ferric ions to a ferrous state.

Free DPPH radicals scavenging

The free radicals scavenging activities of the extracts was studied at different concentrations against free DPPH

radicals using a chemical method reported by Avni et al. [16]. In this method, DPPH solution (126 μM) was prepared in absolute methanol. Then, the extracts were prepared in distilled water at different concentrations (0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL). Then, 1 mL of each concentration of the extracts was transferred to 10 mL glass test tubes, followed by adding 2 mL of the prepared DPPH solution. The tubes were incubated in a dark shelve for 30 min at ambient temperature. A control tube was prepared in a similar way using 1 mL of distilled water in the place of the extracts. The absorbance of the mixtures was determined at 517 nm. During the analysis, ascorbic acid was used as a standard radical scavenger. The scavenging activities of the extracts at different concentrations were determined using Eq. 1 and presented as both percentages (with respect to the initial level of DPPH radicals) and as IC_{50} values (required extract concentration to scavenge 50% of the initial DPPH radical concentration).

$$\text{DPPH scavenging effect (\%)} = \left[\frac{A_0 - A_1}{A_0} \right] \times 100 \quad (1)$$

where A_0 is the control absorbance, A_1 is absorbance of test and standard.

Hydrogen peroxide radicals scavenging

The extracts were also evaluated for free hydrogen peroxide (H_2O_2) radicals scavenging using an in vitro method [17]. Prior to the analysis, a 40 mM H_2O_2 solution was prepared in phosphate buffer solution (50 mM, pH 7.4). The initial radical concentration of the H_2O_2 solution was determined against a phosphate buffer blank at 230 nm and recorded as A_0 . Then, 0.6 mL of different extract concentrations were mixed with the H_2O_2 solution (3.4 mL) in 10 mL glass test tubes. The tubes were incubated at ambient temperature for 10 min before determining their absorbances at 230 nm (recorded as A_1). The scavenging of H_2O_2 by the extracts was calculated using Eq. 2 and reported both as percentages (with respect to the initial radical concentration) and as IC_{50} values. Ascorbic acid was used as a standard radical scavenger.

$$\text{Hydrogen peroxide scavenged (\%)} = \left[\frac{A_0 - A_1}{A_0} \right] \times 100 \quad (2)$$

Ferric reducing antioxidant power (FRAP)

The extracts were also evaluated for metal-reducing activity using a chemical method as described by Maruthamuthu [18]. Here, different concentrations of the extracts (0.2–1.0 mg/mL) at a volume of 1 mL was added into test tubes containing 2.5 mL of 0.2 M sodium phosphate pH 6.6 buffer. Then, 2.5 mL of potassium ferricyanide solution

(1% concentration) was introduced into the tubes. At this moment, the tubes were vortexed and incubated for 20 min at 50 °C. After the incubation, protein precipitation was ensured by adding 2.5 mL of 10% TCA into the tubes. The tubes were vigorously shaken and centrifuged for 10 min at 3000 rpm to obtain clear supernatants. The supernatants (2.5 mL) were carefully transferred to new tubes containing deionized water (2.5 mL), followed by the addition of 0.1% ferric chloride solution (0.5 mL) to facilitate a redox reaction between the antioxidants in the extracts and the unstable ferric ions. This redox reaction culminated in the formation of a colored complex upon the addition of TCA. The absorbance of this colored complex was determined in a spectrophotometer at 700 nm. Higher absorbances indicate stronger FRAP. Ascorbic acid served as a reference antioxidant source. The observed FRAP of the extracts was presented as the mean \pm SD of triplicate absorbance measurements.

Cytotoxicity assay

MTT assay

The anticancer activity of the extracts was determined in vitro after the cultured cells had reached 70–80% confluence [19]. The extract stock solutions (50 mg/mL) were first prepared in dimethyl sulfoxide (DMSO). The required working concentrations (0.20–0.0125 μ g/mL) of the extracts were prepared through a double dilution of the stock with supplemented DMEM. To perform the cytotoxicity assay, the cultured cells at the concentration of 5×10^3 cells/mL (counted using a hemocytometer) were first seeded into 96-well transparent microtiter plates and incubated for 24 h at 37 °C with 5% CO₂ concentration. After 24 h, the old media in the wells were replaced with fresh media supplemented with different extract concentrations to final concentrations of 0.20, 0.10, 0.050, 0.025, and 0.0125 μ g/mL. The media in the positive and negative control wells were replaced with doxorubicin-supplemented media and fresh DMEM supplemented with only FBS and P-S, respectively. All the treatment groups were prepared in three 96-well transparent microtiter plates. One set of the plates was incubated for 24 h, another set for 48 h, and the last set for 72 h. After end incubation period, thawed MTT solution (10 μ L) was added all the well and further incubated for 4 h at 37 °C. After 4 h, 100 μ L of 10% DMSO was used to solubilize the formed formazan crystals in each well. The absorbance of each well was read in a microplate reader at 570 nm, and from the absorbance readings, the cytotoxicity of the extracts on the cells was determined by calculating the percentage of the viable cells as a function of the untreated control using Eq. 3. The extracts concentration required to reduce the initial viable cells population by 50% (IC₅₀ values) was determined from a concentration-cell death plot for each extract.

$$\text{Cytotoxicity effect (\%)} = \left[\frac{\text{OD of treated cell}}{\text{OD of control cell}} \right] \times 100\% \quad (3)$$

Data analysis

All the data acquired from the study were analyzed using the non-paired Student *t* test package of Microsoft Excel® 2016 (Microsoft, Redmond, WA, USA) at a significance level of $p < 0.05$.

Results and discussion

LC-MS-QTOF analysis

The identified phytochemicals in the acetone and ethyl acetate extracts of Bunapi shimeji caps are presented in Tables 1 and 2, respectively. The base peak intensity (BPI) chromatograph and structure of some identified compounds in the acetone fraction are shown in Figs. 1 and 2 respectively, while the BPI and structure of some identified compounds in the ethyl acetate fraction are shown in Figs. 3 and 4, respectively. Some of the compounds identified in the acetone extract of Bunapi shimeji caps are xanthoxylin, forsythoside D, 2,4,6-trihydroxyacetophenone-2,4-di-*O*- β -D-glucopyranoside, feralolide, and *p*-cresol, while some compounds identified in the ethyl acetate extract of Bunapi shimeji caps include kukomine A, (3R,4R)-3,4-trans-7,2'-dihydroxy-4'-methoxy-4-[(3R)-2',7-dihydroxy-4'-methoxyisoflavan-5'-yl]-isoflavan, cyclocurcumin, and 6-gingerol. The ability of xanthoxylin in combination with a newly isolated ruthenium complex to induce S-phase arrest in HepG2 cells through a p53-independent pathway has been reported [20]. In addition, 6-gingerol has been reported to have cytotoxic effect against HCT15, L929, and Raw 264.7 in a concentration-dependent manner [21–23]. The antioxidant, anticancer, and anti-inflammatory activities of cyclocurcumin have been reported [24].

Total phenolics content (TPC) and total flavonoids content (TFC) in the extracts

The TPC and TFC of acetone and ethyl acetate extracts of Bunapi shimeji caps expressed as μ g GAE/mg and μ g QE/mg of the dry extract are presented in Table 3. The result showed that the TPC and TFC of the acetone fraction of Bunapi shimeji caps were significantly higher compared to those of ethyl acetate fractions. Flavonoids are a subgroup of phenolic compounds; therefore, the observed value of the TFC is expected to be lower than the TPC. A higher TPC and TFC was obtained from the acetone fraction due to the higher polarity of acetone compared to ethyl acetate.

Table 1 List of LC-MS-QT of-identified compounds in the acetone fraction of *Bunapi shimeji* caps

S/N	Component name	Formula	Identification status	Observed neutral mass (Da)	Observed m/z	Mass error (mDa)	Mass error (ppm)	Observed RT (min)	Response	Adducts	Observed CCS (\AA^2)	Total fragments found
1	Sodium ferulate	$\text{C}_{10}\text{H}_9\text{NaO}_4$	Identified	216.0399	261.0381	0.1	0.2	0.35	670	+HCOO	142.27	0
2	Tribulusamide B	$\text{C}_{36}\text{H}_{34}\text{N}_2\text{O}_9$	Identified	638.2256	683.2238	-0.8	-1.2	0.40	196	+HCOO	229.03	3
3	Mulberrofuran P	$\text{C}_{34}\text{H}_{22}\text{O}_9$	Identified	574.1259	573.1186	-0.5	-0.9	0.43	884	-H	209.89	3
4	Mulberrofuran P	$\text{C}_{34}\text{H}_{22}\text{O}_9$	Identified	574.1258	573.1186	-0.6	-1.0	0.44	851	-H	217.41	2
5	3,7-Dihydroxy-2,4-dimethoxyphenanthrene-3- <i>O</i> -glucoside	$\text{C}_{22}\text{H}_{24}\text{O}_9$	Identified	432.1404	477.1386	-1.7	-3.5	0.44	425	+HCOO	195.32	16
6	1- <i>O</i> -Methyl-3,5- <i>O</i> -dicaffeoylquinic acid	$\text{C}_{27}\text{H}_{28}\text{O}_{12}$	Identified	544.1568	543.1495	-1.3	-2.4	0.44	415	-H	210.00	10
7	3,4-Dihydroxybenzamide	$\text{C}_7\text{H}_7\text{NO}_3$	Identified	153.0431	198.0413	0.5	2.6	0.44	215	+HCOO	166.31	0
8	2,7-Dihydroxy-4-methoxyphenanthrene-2- <i>O</i> -glucoside	$\text{C}_{21}\text{H}_{22}\text{O}_8$	Identified	402.1295	447.1277	-1.9	-4.3	0.44	610	+HCOO	187.24	20
9	1,5-Bis(4-hydroxy-3-methoxyphenyl)-1,4-pentadien-3-one	$\text{C}_{19}\text{H}_{18}\text{O}_5$	Identified	326.1141	325.1068	-1.4	-4.2	0.44	1714	-H	165.86	6
10	Norbergenin	$\text{C}_{13}\text{H}_{14}\text{O}_9$	Identified	314.0629	359.0611	-0.9	-2.4	0.44	548	+HCOO	165.49	4
11	Blestrianol C	$\text{C}_{37}\text{H}_{30}\text{O}_7$	Identified	586.2006	585.1933	1.4	2.5	0.44	469	-H	220.84	1
12	Norbergenin	$\text{C}_{13}\text{H}_{14}\text{O}_9$	Identified	314.0624	359.0606	-1.4	-3.9	0.45	736	+HCOO	165.10	6
13	Ehretioside B	$\text{C}_{14}\text{H}_{17}\text{NO}_7$	Identified	311.1004	356.0986	-0.1	-0.2	0.46	1170	+HCOO	172.63	21
14	L-Noradrenaline	$\text{C}_8\text{H}_{11}\text{NO}_3$	Identified	169.0737	214.0719	-0.2	-1.1	0.47	632	+HCOO	164.65	0
15	Ehretioside B	$\text{C}_{14}\text{H}_{17}\text{NO}_7$	Identified	311.1014	310.0942	0.9	3.0	0.48	172	-H	165.48	4
16	Blestrianol C	$\text{C}_{37}\text{H}_{30}\text{O}_7$	Identified	586.1982	631.1964	-1.0	-1.6	0.49	174	+HCOO	226.10	1
17	Polygoaceto-phenoside	$\text{C}_{14}\text{H}_{18}\text{O}_{10}$	Identified	346.0888	391.0870	-1.2	-3.2	0.67	346	+HCOO	173.26	0
18	Dendrocandin C	$\text{C}_{16}\text{H}_{18}\text{O}_5$	Identified	290.1154	289.1081	-0.1	-0.2	0.73	143	-H	159.05	0
19	(+)-Threo- <i>N</i> -Methyltyramine	$\text{C}_{10}\text{H}_{14}\text{O}_5$	Identified	214.0845	259.0827	0.3	1.3	1.02	925	+HCOO	207.20	0
20	<i>N</i> -Methyltyramine	$\text{C}_9\text{H}_{13}\text{NO}$	Identified	151.0991	196.0973	-0.7	-3.3	1.10	150	+HCOO	144.26	0
21	Smilaxin	$\text{C}_{17}\text{H}_{16}\text{O}_6$	Identified	316.0966	361.0948	1.9	5.4	1.15	881	+HCOO	171.13	1
22	Synephrine	$\text{C}_9\text{H}_{13}\text{NO}_2$	Identified	167.0942	166.0869	-0.5	-2.8	1.22	165	-H	163.92	0
23	Yakuchinone B	$\text{C}_{20}\text{H}_{22}\text{O}_3$	Identified	310.1560	355.1542	-0.9	-2.7	1.25	280	+HCOO	178.17	0
24	Cimidarhine	$\text{C}_{14}\text{H}_{20}\text{O}_8$	Identified	316.1151	361.1133	-0.7	-2.0	1.28	667	+HCOO	175.46	0
25	2,4,6-Trihydroxyacetophenone-2,4-di- <i>O</i> - β -D-glucopyranoside	$\text{C}_{20}\text{H}_{28}\text{O}_{14}$	Identified	492.1464	491.1391	-1.6	-3.2	1.31	4680	-H	199.32	0
26	Koaburaside	$\text{C}_{14}\text{H}_{20}\text{O}_9$	Identified	332.1101	377.1083	-0.6	-1.7	1.54	425	+HCOO	171.52	0

Table 1 (continued)

S/N	Component name	Formula	Identification status	Observed neutral mass (Da)	Observed m/z	Mass error (mDa)	Mass error (ppm)	Observed RT (min)	Response	Adducts	Observed CCS (Å ²)	Total fragments found
27	Kuzubutenolide A	C ₂₃ H ₂₄ O ₁₀	Identified	460.1380	505.1362	1.0	2.0	1.79	852	+HCOO	201.98	1
28	Synephrine	C ₉ H ₁₃ NO ₂	Identified	167.0945	212.0927	-0.1	-0.7	1.84	586	+HCOO	142.99	0
29	Isotachioside	C ₁₃ H ₁₈ O ₈	Identified	302.0990	301.0917	-1.2	-4.0	2.39	317	-H	229.14	0
30	<i>p</i> -Cresol	C ₇ H ₈ O	Identified	108.0577	153.0559	0.1	0.9	2.48	131	+HCOO	126.57	0
31	Synephrine	C ₉ H ₁₃ NO ₂	Identified	167.0949	212.0931	0.3	1.2	2.50	649	+HCOO	150.96	0
32	Homoarbutin	C ₁₃ H ₁₈ O ₇	Identified	286.1033	285.0960	-2.0	-6.9	2.62	182	-H	147.54	0
33	Ehretioside B	C ₁₄ H ₁₇ NO ₇	Identified	311.1002	356.0984	-0.3	-0.7	2.68	229	+HCOO	175.63	0
34	1-Galloyl-β-D-glucose	C ₁₃ H ₁₆ O ₁₀	Identified	332.0732	331.0659	-1.1	-3.4	2.72	544	-H	155.57	0
35	<i>p</i> -Hydroxy-acetamide	C ₈ H ₉ NO ₂	Identified	151.0635	196.0617	0.2	1.1	2.75	273	+HCOO	134.75	0
36	3,7-Dihydroxy-2,4-dimethoxyphenanthrene-3- <i>O</i> -glucoside	C ₂₂ H ₂₄ O ₉	Identified	432.1435	431.1363	1.5	3.5	2.84	529	-H	188.32	2
37	2,3',4,4',6'-Pentrahydroxybenzophenone	C ₁₃ H ₁₀ O ₆	Identified	262.0468	307.0450	-0.9	-3.0	3.60	125	+HCOO	166.54	1
38	Homoarbutin	C ₁₃ H ₁₈ O ₇	Identified	286.1045	285.0972	-0.8	-2.7	4.17	657	-H	151.26	0
39	Norbergenin	C ₁₃ H ₁₄ O ₉	Identified	314.0635	313.0563	-0.2	-0.8	4.22	379	-H	162.59	0
41	4-Ethylphenol	C ₈ H ₁₀ O	Identified	122.0729	167.0711	-0.3	-1.7	4.25	207	+HCOO	130.12	0
41	Moracin E	C ₁₉ H ₁₆ O ₄	Identified	308.1063	353.1045	1.5	4.2	4.25	250	+HCOO	173.18	0
42	Salidroside	C ₁₄ H ₂₀ O ₇	Identified	300.1195	345.1177	-1.4	-4.0	4.32	278	+HCOO	173.60	0
43	Isoarundinin II	C ₂₂ H ₂₂ O ₄	Identified	350.1505	349.1433	-1.3	-3.7	4.36	340	-H	177.68	0
44	Synephrine	C ₉ H ₁₃ NO ₂	Identified	167.0951	212.0933	0.5	2.3	4.41	147	+HCOO	147.49	0
45	1-Galloyl-β-D-glucose	C ₁₃ H ₁₆ O ₁₀	Identified	332.0733	331.0661	-1.0	-3.0	4.43	789	-H	244.90	0
46	Sinapaldehyde	C ₁₁ H ₁₂ O ₄	Identified	208.0738	253.0720	0.3	1.1	4.43	269	+HCOO	173.15	0
47	Salidroside	C ₁₄ H ₂₀ O ₇	Identified	300.1196	345.1178	-1.3	-3.8	4.71	213	+HCOO	246.70	0
38	1-(4-Hydroxy-benzyl)-4-methoxy-2,7-dihydroxy phenanthrene	C ₂₂ H ₁₈ O ₄	Identified	346.1220	391.1202	1.5	3.8	4.74	218	+HCOO	177.89	0
49	Cassialactone	C ₁₆ H ₁₆ O ₆	Identified	304.0942	349.0924	-0.5	-1.3	5.12	254	+HCOO	205.58	0
50	Lavandulifoside	C ₃₄ H ₄₄ O ₁₉	Identified	756.2466	755.2394	-1.0	-1.4	5.22	179	-H	246.01	0
51	Forsythoside D	C ₂₀ H ₃₀ O ₁₃	Identified	478.1684	477.1612	-0.2	-0.4	5.36	3619	-H	198.94	2
52	Digupigan A	C ₁₈ H ₂₆ O ₁₂	Identified	434.1424	433.1351	0.0	0.0	6.06	323	-H	186.12	8
53	Forsythoside D	C ₂₀ H ₃₀ O ₁₃	Identified	478.1679	523.1661	-0.8	-1.5	6.54	123	+HCOO	269.36	0
54	Forsythoside D	C ₂₀ H ₃₀ O ₁₃	Identified	478.1676	523.1658	-1.1	-2.0	6.55	454	+HCOO	207.74	3
55	Dendrocandian I	C ₃₂ H ₃₂ O ₈	Identified	544.2079	589.2061	-1.8	-3.1	7.67	219	+HCOO	224.25	0

Table 1 (continued)

S/N	Component name	Formula	Identification status	Observed neutral mass (Da)	Observed m/z	Mass error (mDa)	Mass error (ppm)	Observed RT (min)	Response	Adducts	Observed CCS (\AA^2)	Total fragments found
56	Blettiol B	$\text{C}_{27}\text{H}_{26}\text{O}_7$	Identified	462.1694	461.1622	1.6	3.4	8.36	911	-H	199.76	1
57	Feralolide	$\text{C}_{18}\text{H}_{16}\text{O}_7$	Identified	344.0904	389.0886	0.8	1.9	8.37	3257	+HCOO	183.57	0
58	Xanthoxylin	$\text{C}_{10}\text{H}_{12}\text{O}_4$	Identified	196.0751	241.0733	1.5	6.3	9.25	1368	+HCOO	241.81	0
59	Nobilin C	$\text{C}_{18}\text{H}_{22}\text{O}_6$	Identified	334.1419	379.1401	0.3	0.8	10.83	321	+HCOO	192.72	0
60	Dendrocandian I	$\text{C}_{32}\text{H}_{32}\text{O}_8$	Identified	544.2085	589.2067	-1.2	-2.1	10.88	512	+HCOO	231.94	0
61	Decaffeoylacteoside	$\text{C}_{20}\text{H}_{30}\text{O}_{12}$	Identified	462.1750	461.1677	1.3	2.8	11.33	632	-H	200.60	2
62	N-Dihydrocaffeoyl tyramine	$\text{C}_{17}\text{H}_{19}\text{NO}_4$	Identified	301.1304	346.1286	-1.0	-2.9	11.35	204	+HCOO	180.77	0
63	6-Gingerdione	$\text{C}_{17}\text{H}_{24}\text{O}_4$	Identified	292.1679	337.1661	0.5	1.4	12.20	2787	+HCOO	177.97	0
64	4'-O-Methylbrazilin	$\text{C}_{17}\text{H}_{18}\text{O}_5$	Identified	302.1143	347.1125	-1.1	-3.1	12.25	195	+HCOO	183.22	0
65	2'-Acetylacteoside	$\text{C}_{31}\text{H}_{38}\text{O}_{16}$	Identified	666.2151	711.2133	-0.9	-1.2	12.39	124	+HCOO	248.60	0
66	Osmanthuside H	$\text{C}_{19}\text{H}_{28}\text{O}_{11}$	Identified	432.1637	431.1565	0.6	1.3	12.48	454	-H	197.95	2
67	N-Dihydrocaffeoyl tyramine	$\text{C}_{17}\text{H}_{19}\text{NO}_4$	Identified	301.1319	346.1301	0.5	1.4	12.48	280	+HCOO	249.19	0
68	N-Dihydrocaffeoyl tyramine	$\text{C}_{17}\text{H}_{19}\text{NO}_4$	Identified	301.1318	346.1300	0.4	1.2	12.49	3678	+HCOO	172.00	0

Table 2 List of LC-MS-QToF-identified compounds in the ethyl acetate fraction of *Bunapi shimeji* caps

S/N	Component name	Formula	Identification status	Observed neutral mass (Da)	Observed m/z	Mass error (mDa)	Mass error (ppm)	Observed RT (min)	Response	Adducts	Observed CCS (Å ²)	Total fragments found
1	6-Gingerol	C ₁₇ H ₂₆ O ₄	Identified	294.1844	293.1771	1.3	4.4	0.18	524	-H	185.51	10
2	Dendrocinanin I	C ₃₂ H ₅₂ O ₈	Identified	544.2078	543.2005	-1.9	-3.5	0.24	240	-H	237.28	0
3	Asebotin	C ₂₂ H ₂₆ O ₁₀	Identified	450.1514	495.1496	-1.2	-2.5	0.28	532	+HCOO	216.31	8
4	Ciwujiatone	C ₂₂ H ₂₆ O ₉	Identified	434.1586	479.1568	0.9	1.9	0.32	510	+HCOO	216.74	0
5	Asebotin	C ₂₂ H ₂₆ O ₁₀	Identified	450.1542	495.1524	1.6	3.3	0.37	584	+HCOO	211.44	12
6	Asebotin	C ₂₂ H ₂₆ O ₁₀	Identified	450.1542	495.1524	1.6	3.3	0.38	616	+HCOO	220.39	0
7	2'-Hydroxy-3',4'-dimethoxy-isoflavan-7-O-β-D-glucoside	C ₂₃ H ₂₈ O ₁₀	Identified	464.1678	509.1660	-0.4	-0.8	0.46	349	+HCOO	224.79	0
8	Asebotin	C ₂₂ H ₂₆ O ₁₀	Identified	450.1525	495.1507	-0.1	-0.2	0.48	572	+HCOO	221.00	2
9	Kukoamine A	C ₂₈ H ₄₂ N ₄ O ₆	Identified	530.3124	529.3051	2.0	3.7	0.48	305	-H	242.46	18
10	(3R,4R)-3,4-Trans-7,2'-dihydroxy-4'-methoxy-4-[(3R)-2',7'-dihydroxy-4'-methoxy-isoflavan-5'-yl]-isoflavan	C ₃₂ H ₃₀ O ₈	Identified	542.1956	541.1883	1.5	2.9	0.56	541	-H	239.55	1
11	Ciwujiatone	C ₂₂ H ₂₆ O ₉	Identified	434.1592	479.1574	1.6	3.3	0.59	459	+HCOO	215.74	0
12	Bletilol A	C ₂₈ H ₂₈ O ₈	Identified	492.1800	537.1782	1.6	3.0	0.59	307	+HCOO	228.94	0
13	Forsythoside D	C ₂₀ H ₃₀ O ₁₃	Identified	478.1667	477.1594	-2.0	-4.1	0.61	711	-H	220.91	1
14	2'-Hydroxy-3',4'-dimethoxy-isoflavan-7-O-β-D-glucoside	C ₂₃ H ₂₈ O ₁₀	Identified	464.1701	509.1683	1.9	3.6	0.64	920	+HCOO	229.36	1
15	Asebotin	C ₂₂ H ₂₆ O ₁₀	Identified	450.1535	495.1517	0.9	1.8	1.33	481	+HCOO	219.12	0
16	Mulberrofuran C	C ₃₄ H ₂₈ O ₉	Identified	580.1723	579.1651	-1.0	-1.7	1.38	232	-H	253.73	0
17	6-Gingerol	C ₁₇ H ₂₆ O ₄	Identified	294.1836	293.1764	0.5	1.8	1.38	679	-H	183.22	0
18	Bletilol A	C ₂₈ H ₂₈ O ₈	Identified	492.1779	537.1761	-0.5	-0.9	1.65	339	+HCOO	244.26	1
19	Ciwujiatone	C ₂₂ H ₂₆ O ₉	Identified	434.1595	479.1577	1.8	3.7	1.84	224	+HCOO	217.43	3
20	Mulberrofuran Q	C ₃₄ H ₂₄ O ₁₀	Identified	592.1370	637.1352	0.0	0.1	2.15	281	+HCOO	249.25	0
21	6-Gingerol	C ₁₇ H ₂₆ O ₄	Identified	294.1846	293.1774	1.5	5.2	2.16	579	-H	186.95	0
22	Asebotin	C ₂₂ H ₂₆ O ₁₀	Identified	450.1543	495.1525	1.7	3.4	2.45	798	+HCOO	221.31	0
23	2'-Hydroxy-3',4'-dimethoxy-isoflavan-7-O-β-D-glucoside	C ₂₃ H ₂₈ O ₁₀	Identified	464.1699	509.1681	1.6	3.2	3.30	993	+HCOO	227.34	1
24	Asebotin	C ₂₂ H ₂₆ O ₁₀	Identified	450.1537	495.1519	1.1	2.3	4.15	432	+HCOO	215.00	0
25	Mulberrofuran Q	C ₃₄ H ₂₄ O ₁₀	Identified	592.1352	591.1279	-1.8	-3.0	4.16	293	-H	238.17	1

Table 2 (continued)

S/N	Component name	Formula	Identification status	Observed neutral mass (Da)	Observed m/z	Mass error (mDa)	Mass error (ppm)	Observed RT (min)	Response	Adducts	Observed CCS (\AA^2)	Total fragments found
26	Yakuchinone B	$\text{C}_{20}\text{H}_{22}\text{O}_3$	Identified	310.1576	355.1558	0.7	2.0	4.34	366	+HCOO	195.72	0
27	Yakuchinone B	$\text{C}_{20}\text{H}_{22}\text{O}_3$	Identified	310.1584	355.1566	1.5	4.3	4.39	314	+HCOO	198.99	0
28	Mulberrofuran G	$\text{C}_{34}\text{H}_{26}\text{O}_8$	Identified	562.1612	607.1594	-1.6	-2.6	4.49	240	+HCOO	249.55	0
29	Dendrocandian I	$\text{C}_{32}\text{H}_{32}\text{O}_8$	Identified	544.2098	543.2025	0.1	0.1	4.50	267	-H	239.50	0
30	Yakuchinone B	$\text{C}_{20}\text{H}_{22}\text{O}_3$	Identified	310.1589	355.1571	2.0	5.6	4.63	603	+HCOO	198.91	5
31	Kukoamine A	$\text{C}_{28}\text{H}_{42}\text{N}_4\text{O}_6$	Identified	530.3112	529.3039	0.7	1.4	4.90	269	-H	241.60	0
32	Erigeroster A	$\text{C}_{27}\text{H}_{26}\text{O}_{13}$	Identified	558.1382	603.1364	0.8	1.4	5.08	272	+HCOO	251.42	0
33	Oxyphyllacicol	$\text{C}_{20}\text{H}_{26}\text{O}_3$	Identified	314.1894	313.1822	1.2	4.0	5.10	255	-H	194.80	7
34	Erigeroster A	$\text{C}_{27}\text{H}_{26}\text{O}_{13}$	Identified	558.1379	603.1361	0.6	1.0	5.16	224	+HCOO	250.06	0
35	Tubuloside C	$\text{C}_{43}\text{H}_{54}\text{O}_{24}$	Identified	954.2992	953.2919	-1.3	-1.4	5.18	248	-H	193.06	20
36	6-Gingerol	$\text{C}_{17}\text{H}_{26}\text{O}_4$	Identified	294.1848	293.1776	1.7	5.9	5.20	592	-H	197.31	0
37	Kukoamine A	$\text{C}_{28}\text{H}_{42}\text{N}_4\text{O}_6$	Identified	530.3103	529.3030	-0.1	-0.3	6.48	4.6	-H	240.39	0
38	(3R,4R)-3,4-Trans-7,2'-dihydroxy-4'-methoxy-4-[(3R)-2',7'-dihydroxy-4'-methoxy-isoflavan-5'-yl]-isoflavan	$\text{C}_{32}\text{H}_{30}\text{O}_8$	Identified	542.1952	541.1880	1.2	2.2	8.90	236	-H	244.66	0
39	6-Gingerol	$\text{C}_{17}\text{H}_{26}\text{O}_4$	Identified	294.1843	293.1770	1.2	4.1	10.37	333	-H	187.69	0
40	Tubuloside C	$\text{C}_{43}\text{H}_{54}\text{O}_{24}$	Identified	954.2994	999.2976	-1.1	-1.1	16.64	232	+HCOO	294.96	10
41	Kukoamine A	$\text{C}_{28}\text{H}_{42}\text{N}_4\text{O}_6$	Identified	530.3122	529.3050	1.8	3.4	16.65	405	-H	240.18	3
42	2,7-Dihydroxy-1-(4'-hydroxybenzyl)-4-methoxy-9,10-dihydrophenanthrene-4'-O-glucoside	$\text{C}_{28}\text{H}_{30}\text{O}_9$	Identified	510.1871	555.1853	-1.9	-3.5	16.70	295	+HCOO	235.68	0
43	Dryocrassin	$\text{C}_{43}\text{H}_{48}\text{O}_{16}$	Identified	820.2929	865.2911	-1.4	1.6	18.62	239	+HCOO	308.09	2

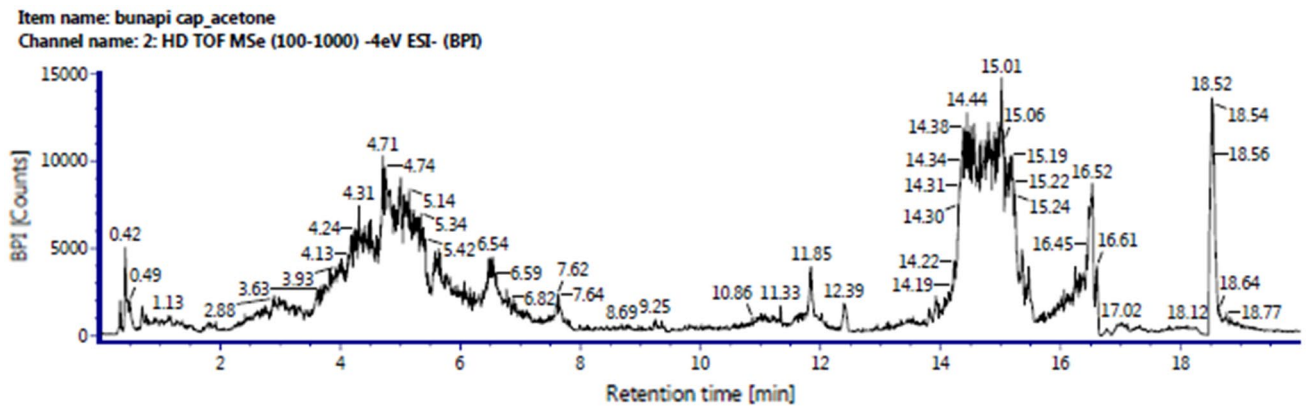


Fig. 1 Base peak intensity plot of the LCMS-QToF-identified compounds in the acetone fraction of Bunapi shimeji caps

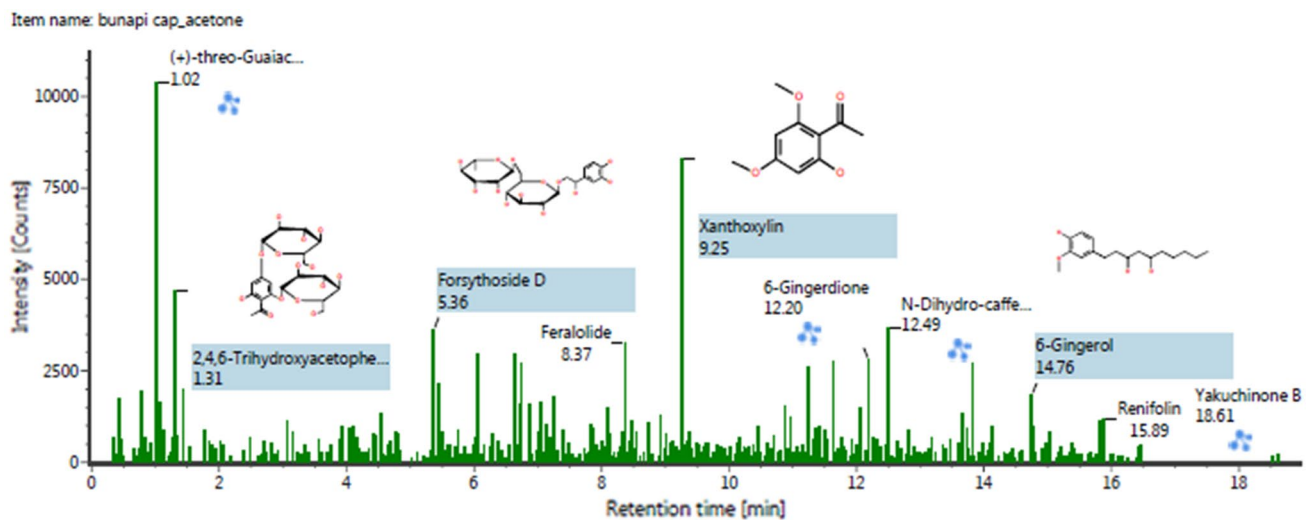


Fig. 2 Some of the LCMS-QToF-identified phytochemicals in the acetone fraction of Bunapi shimeji caps

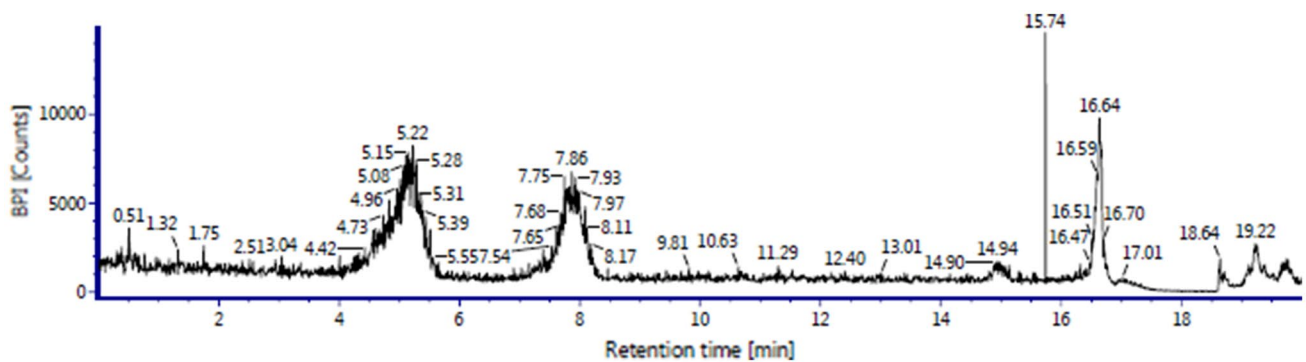


Fig. 3 Base peak intensity plot of the LCMS-QToF-identified compounds in the ethyl acetate fraction of Bunapi shimeji caps

Several other factors such as the presence of phytochemicals with different side chains (including methoxy, hydroxyl, and methyl groups) in the extracts, the formation of glycosidic

bond between molecules, and the degree of aromatic rings conjugation within the phytochemicals can interfere with the solubility of several phytochemicals in different solvents [11,

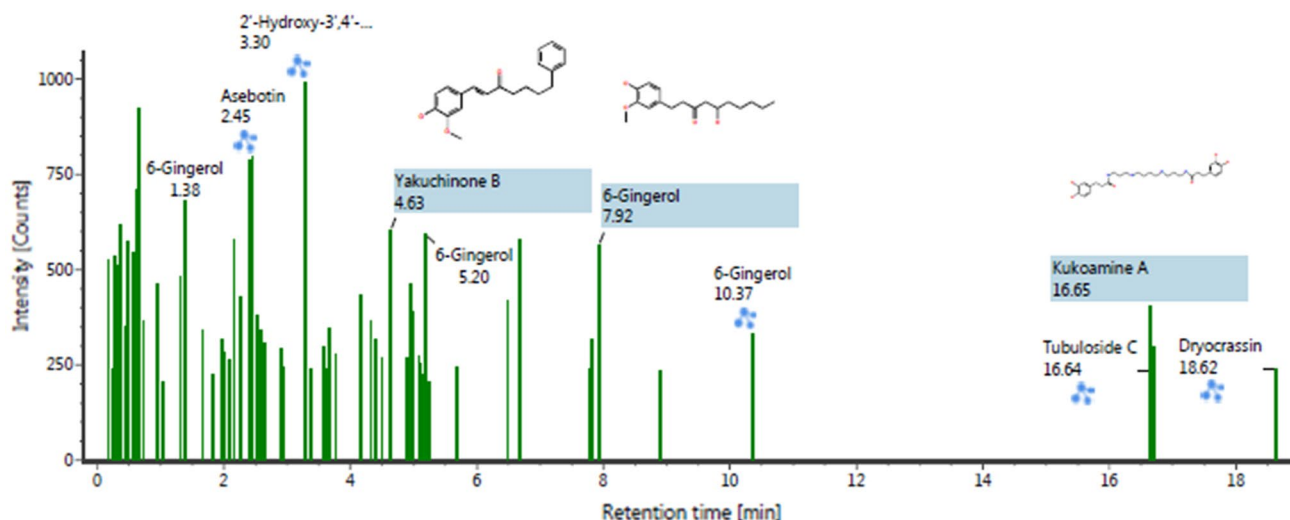


Fig. 4 Some of the LCMS-QToF-identified phytochemicals in the ethyl acetate fraction of Bunapi shimeji caps

20]. Phenolic compounds are classified as either phenolic acids, simple phenolics, flavonoids, or hydroxycinnamic acid derivatives [11], these classes have different levels of biological activities.

Previously, Addai et al. [24] and Dar et al. [25] reported solvent polarity-dependent differences in the TPC and TFC of some mushroom species. The solubility of phenolic compounds in different solvents is influenced by two factors: first is the level of the polarity of the solvent, and second is the number of OH^- groups contained in the compound [26]. Compounds with a higher number of OH^- groups are more soluble in polar solvents than those with less number of OH^- groups [11]. The positive effect of dipole formation in the form of hydrogen bond formation in polar solvents on the solubility of solvating molecules has been reported by Visht and Chaturvedi [27]. Furthermore, different species of the same mushroom can contain different phytochemicals, however, their biological activities mainly depend on the type of phytochemicals they contain. Similarly, the TPC and TFC of extracts can vary due to the influence of

environmental factors and genetic variations among. Hence, these results showed that Bunapi shimeji cap is a potential good natural source of phenolic compounds which has been shown to impact positively on human health owing to their antioxidant, anti-inflammatory, anticancer, and hypoglycemic properties [28].

In vitro antioxidant activity

A positive correlation has earlier been found between the antioxidant activity of extracts and their TPC. Phenolic compounds act as hydrogen donors, singlet oxygen quenchers, reducing agents, or as radical scavengers to bring about redox stability [5]. This study deployed three chemical-based assays (DPPH and H_2O_2 radicals scavenging and FRAP) to determine the antioxidant activities of the studied extracts [29]. The free radical-based assays (such as DPPH and H_2O_2 assays) manifest the presence of proton donors in a color formation or fade in original color [30], however, the metal-reducing-based methods (such as FRAP) manifest the ability of the extract to bring about a shift in the oxidation state of metals, such as the reduction of ferric ions to the ferrous state (Fe^{3+} to Fe^{2+}).

Scavenging activity of DPPH and H_2O_2 radicals

One mechanism of lipid oxidation inhibition through antioxidants is its free radical scavenging as it can project the antioxidant activity of an extract within a short period [6]. Figures 5 and 6 illustrate the observed free DPPH and H_2O_2 radicals scavenging activities respectively, of acetone and ethyl acetate extracts of Bunapi shimeji compared to that of ascorbic acid. From the figures, the antioxidant

Table 3 Total phenolic content (TPC) and total flavonoid content (TFC) of acetone and ethyl acetate fractions of Bunapi shimeji caps

Acetone fraction	Ethyl acetate fraction
TPC ($\mu\text{gGAE}/\text{mg}$)	
53.22 ± 0.11^a	49.38 ± 0.06^a
TFC ($\mu\text{gQE}/\text{mg}$)	
48.24 ± 0.15^b	43.53 ± 0.15^b

Different superscripts in the same column indicate statistically significant difference at $p < 0.05$

GAE gallic acid equivalent, QE quercetin equivalent; $n = 3$

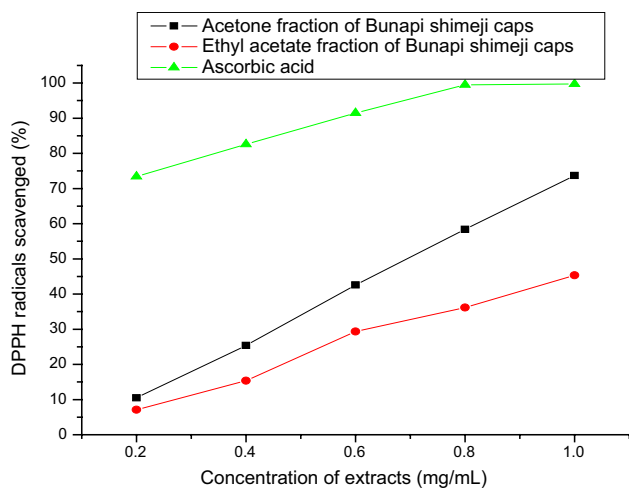


Fig. 5 DPPH radical scavenging activities of acetone and ethyl acetate fractions of Bunapi shimeji caps compared to standard ascorbic acid

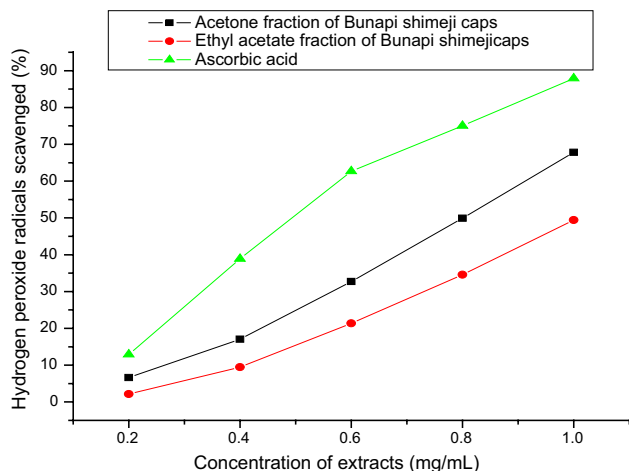


Fig. 6 H₂O₂ radical scavenging activities of acetone and ethyl acetate fractions of Bunapi shimeji caps compared to standard ascorbic acid

activity of the acetone fraction was significantly higher against DPPH radicals (IC₅₀ = 0.76 mg/mL) and H₂O₂ radicals (IC₅₀ = 0.84 mg/mL) compared to that of the ethyl acetate fraction against DPPH radicals (IC₅₀ = 1.10 mg/

mL) and H₂O₂ radicals (IC₅₀ = 1.26 mg/mL) (p < 0.05). A reduced scavenging activity was observed in ethyl acetate fraction probably due to the type of flavonoids extracted by ethyl acetate (lower polarity) compared to that of acetone. It should be noted that the abundance of flavonoids in extracts have a correlation with their antioxidant activities [31]. Another contributor to the antioxidant activity of extracts is the degree of hydrogen atom substitution by sugar on the C-3 position. This substitution has been reported to result in a slightly better antioxidant activity especially in compounds with one hydroxyl group [32]. The activities of extracts were also concentration dependent [12]. These findings agreed with previous studies where biological extracts have been reported to show a high antioxidant activity against DPPH and H₂O₂ [9, 32]. Siddhuraju and Becker reported the negative influence of glycosides presence in flavonoids on their antioxidant activity [33]. The scavenging activity of phenolics is reliant on the number of phenolic and hydroxyl groups they contain as these groups facilitate the transfer of protons to radicals, leading to the formation of phenoxide radicals and a consequent radical stabilization [34]. Cyclocurcumin, one of the identified phytochemicals in the extracts have been reported to have antioxidant activities [24].

Ferric reducing antioxidant power (FRAP)

The ferric reducing power (FRAP) of the extracts is shown in Table 4. The FRAP of the extracts showed a similar concentration-dependent activity as observed with the radical scavenging assays. The table showed the acetone fraction to have more ferric reducing power compared to the ethyl acetate fractions. The reasons for the notable differences in the FRAP of the fractions are not far from the reasons for the differences in the radical scavenging activities that were earlier discussed. Hodzic et al. [35] and Schafer and Buettner [36] suggested FRAP as a good method for assessing the antioxidant activity of extracts. Although other factors such as solvent polarity can influence the biological activity of extracts, Moure et al. [37] further suggested that the FRAP of extracts can be due to their hydrogen ion donating ability.

Table 4 Ferric reducing antioxidant power (FRAP) of Bunapi shimeji caps extracted with acetone and ethyl acetate (absorbance@700 nm)

	0.2 mg/mL	0.4 mg/mL	0.6 mg/mL	0.8 mg/mL	1.0 mg/mL
Acetone extract	0.036 ± 0.01 ^{a,*}	0.075 ± 0.01 ^a	0.150 ± 0.03 ^a	0.251 ± 0.01 ^a	0.322 ± 0.01 ^a
Ethyl acetate extract	0.033 ± 0.01 ^{a,*}	0.072 ± 0.02 ^a	0.132 ± 0.02 ^b	0.211 ± 0.01 ^b	0.285 ± 0.01 ^b
Ascorbic acid	0.202 ± 0.02 ^b	0.796 ± 0.05 ^b	1.262 ± 0.01 ^b	1.824 ± 0.02 ^b	2.130 ± 0.02 ^b

Similar superscripts in the same column indicate statistically significant difference at p < 0.05, while different superscripts in the same column indicate statistically significant difference at p < 0.005; *non-statistically significant difference in the same column. Values are mean ± SD of triplicate measurements

Cytotoxicity assay

The concentrations of the extracts that inhibited the proliferation of the studied cell lines by 50% (IC_{50} values) are presented in Table 5. From the table, the acetone fraction was seen to be more cytotoxic against MCF-7 (IC_{50} range = 0.051–0.055 mg/mL) and MDA-MB-231 (IC_{50} range = 0.122–0.131 mg/mL) compared to MCF-10a ($IC_{50} \geq 250$ mg/mL). Similarly, ethyl acetate fraction was more potent against MCF-7 (IC_{50} range = 0.075–0.096 mg/mL) and MDA-MB-231 (IC_{50} range = 0.161–0.164 mg/mL). The normal cells (MCF-10a) were significantly less sensitive to the extracts compared to cancerous cells ($p < 0.05$) likely due to the absence of specific proteins which were expressed by the cancerous cells but lacking in the normal cells. These proteins may contribute to the affinity of the active phytochemicals in the extracts towards binding on the cell surfaces to induce apoptosis [12, 38]. Similarly, the type of phenolic compound present in extracts contributes to their anticancer activities. Doxorubicin, a standard chemotherapeutic agent showed more antiproliferative activity on both cells ($IC_{50} = 0.009$ – 0.010 mg/mL against MDA-MB-231, 0.007 – 0.008 mg/mL against MCF-7, and 0.012 – 0.016 mg/mL against MCF-10a) compared to the extracts ($p < 0.05$).

In this study, a selective cytotoxicity was observed against the studied cell lines likely due to the type of protein markers expressed by the different cell lines [12]. The simple mechanism for the observed anti-inflammatory activities could be

Table 5 MTT-based anticancer activity of Bunapi shimeji caps extracted with acetone and ethyl acetate against MDA-MB-231, MCF-7, and MCF-10a

Duration of treatment	24 h	48 h	72 h
Acetone fraction (IC_{50} mg/mL)			
MDA-MB-231	0.131 ^{a,b}	0.126 ^{a,b}	0.122 ^{a,b}
MCF-7	0.096 ^{a,b}	0.094 ^{a,b}	0.075 ^{a,b}
MCF-10a	> 0.200	> 0.200	> 0.200
Ethyl acetate fraction (IC_{50} mg/mL)			
MDA-MB-231	0.164 ^{a,b}	0.161 ^{a,b}	0.162 ^{a,b}
MCF-7	0.051 ^{a,b}	0.055 ^{a,b}	0.053 ^{a,b}
MCF-10a	> 0.200	> 0.200	> 0.200
Doxorubicin* (IC_{50} mg/mL)			
MDA-MB-231	0.010	0.009	0.009
MCF-7	0.007	0.007	0.008
MCF-10a	0.016	0.014	0.012

Values are presented as the concentration of extracts (mg/mL) required to inhibit 50% of the viable cell population (IC_{50})

*Standard chemotherapeutic agent

^aSignificantly different compared to MCF-10a treated with the extracts ($p < 0.05$)

^bSignificantly different compared to MCF-10a treated with doxorubicin ($p < 0.05$), $n = 3$

attributed to the induction of apoptosis by the polyphenol content of the extracts. The observed activity is mediated by the interaction of the hydroxyl groups contained in the phytochemicals with the polar receptor site of the mitochondrial cytochrome P450 enzyme. The attachment of these hydroxyl groups to the P450 enzyme could manifest in the intrinsic apoptotic pathway which is characterized by increased membrane permeability and its associated mitochondrial swelling, outer mitochondrial membrane rupture, and the discharge of proapoptotic factors from the intermembrane space [39].

Different classes of polyphenols have different mechanisms of inducing apoptosis and exerting antiproliferative effects. Flavonols inhibit cell proliferation by increasing the population of apoptotic cells and downregulating nuclear factor κB (NF- κB). They also induce apoptosis by increasing the expression of pro-apoptotic Bax proteins, Apaf-1, p53, cytosolic Cyt *c*, caspases-9 & -3, and cleavage of poly (ADP-ribose) polymerase (PARP). This cleavage of PARP results in the reduced expression of AKT, Bcl-2, Bcl-xL, and Mcl-1 which are anti-apoptotic proteins in different cancer cell lines [40]. Flavonols also induce the depolarization of the mitochondrial membrane potential [41] and generally mediate apoptosis through a dose-dependent up-regulation of Bax protein expression, activation of caspase-3 and caspase-9, the release of Cyt *c* to the cytosol, and the increase of the mRNA levels of p53 as reported in mouse neuroblastoma cells [42]. Isoflavones induce apoptosis through the regulation of the protein kinase B survival signaling pathway as reported in breast and cancer cells [43]. They also regulate cell proliferation through the regulation of Bcl-2/Bax proteins expression and caspase-3 activation [44]. Anthocyanins regulates cell proliferation by blocking the cell cycle at the G0/G1 phase. They also reduce the expression of anti-apoptotic proteins (Bcl-2, xIAP, cIAP-1, and cIAP-2) [45]. Apoptosis due to anthocyanins manifests in the formation of apoptotic bodies, poly (ADP-ribose) polymerase proteolysis, and caspase-3 activation. Phenolic acids induce apoptosis through the inhibition of Bcl-2 activity, leading to the activation of caspase-3 and a subsequent release of Cyt *c*. These could represent are some of the possible mechanisms of the antiproliferative activities exerted by the extracts reported in this study [46].

Over the years, several reports on the bioactivity of several mushrooms have been documented, however, few have been taken to the next level of drug development likely due to the limited availability of the source mushrooms or due to the potentially high level of toxicity of such sources to human [11, 47]. Hence, this study was carried out on Bunapi shimeji, a widely available and non-toxic mushroom in Malaysia and South-East Asia which can be produced in great quantities with minimal labor and cost inputs. The antiproliferative activities of different mushroom extracts have

been reported against different cancer cells [20, 23]. For instance, Kim et al. [48] reported the in vivo antiproliferative activity of *L. deliciosus*-derived hetero-polysaccharides, while Xu et al. [49] reported the ability of water extract of *M. procera* to inhibit the metastasis of 26-M3.1 cells. Previously, Kusavadee et al. [1] reported the antiproliferative activity of *Sanghuangporus* sp.1 extracts through a caspase-dependent pathway.

Conclusion

In this study, the in vitro anticancer activity of Bunapi shimeji caps extracted with acetone and ethyl acetate were studied against MCF-10a, MCF-7, and MDA-MB-231 cell lines. Both fractions of the extracts were biologically characterized before studying their anticancer activities. From the study, acetone extract showed more biological activities (antioxidant and anticancer) compared to ethyl acetate fraction ($p < 0.05$). Furthermore, MCF-7 cell line was more sensitive to both extracts based on the presented IC_{50} values compared to MDA-MB-231, while MCF-10a was generally less sensitive to both extracts, this might be due to the absence of some protein markers on the normal cells compared to the cancerous cells. Thus, the caps of Bunapi shimeji have the potential of being used as the good source of antioxidant and anticancer agents.

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