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Termitomyces mushroom extracts and its biological activities

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Abstract

Termitomyces mushrooms are affluent in bioactive components contributing to their therapeutic properties. This study aimed to investigate the antioxidant, antibacterial, antifungal activities and the toxicity of *Termitomyces* extracts. *Termitomyces* mushrooms were collected from the South of Vietnam, separated into different parts, air-dried, ground into powder, and extracted with methanol and ethanol at a divergent temperature of evaporation. *Termitomyces* mycelial biomass extract was discovered to be efficient in scavenging the free radicals through DPPH assay. The extract exhibited potent efficacy against Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella typhimurium*) and Gram-positive (*Bacillus cereus*, *Staphylococcus aureus*) bacteria along with *Candida albicans* fungus applying the disc-diffusion method. *Termitomyces* extract underwent *in vitro* and *in vivo* experiments revealed no toxicity. The *Termitomyces* mycelial biomass extract is a potential source in developing novel antioxidant, antibacterial and antifungal agents.

Keywords: antibacterial; antifungal; antioxidant; *Termitomyces* mushroom.

Practical Application: Mushroom extract-based functional food.

1 Introduction

Mushrooms play a significant role in curing various degenerative diseases and food processing by their chemical composition, nutritional value, and therapeutic properties. Mushrooms were determined to be affluent in unsaturated fatty acids, fat-soluble vitamins, ergosterol, vitamin D, along with bioactive molecules consisting of β-glucans, triterpenoids, antioxidants (Rathore et al., 2017).

Termitomyces mushrooms were naturally endowed with high nutritional values, containing a full range of non-replaceable amino acids and minerals at high concentrations (Nakalembe et al., 2015). *Termitomyces heimii,* isolated from Negeri Sembilan, Malaysia, was cultivated in liquid fermentation, producing endopolysaccharide and exopolysaccharide (β-glucan) exhibited antibacterial property (Ahmad et al., 2021). Both exopolysaccharides (EPS) and depolymerized-exopolysaccharides (DEPS) from *Termitomyces albuminosus* exerted pharmacological *in vitro* antioxidant, hepatoprotective, and hypolipidaemic effects on mice with high-fat emulsion-induced hyperlipidemia (Zhao et al., 2017). In addition, *Termitomyces clypeatus* contained AkP, an effective biomolecule for killing cancer cells (Majumder et al., 2016). In India, *Termitomyces heimii* and *Termitomyces microcarpus* were used to alleviate fever, colds, and fungal infections (Venkatachalapathi & Paulsamy, 2016). In Cameroon, *Termitomyces titanicusis* was used to treat gastric complications (Rosemary et al., 2017).

According to the research results of Ulziijargal & Mau (2011), the difference in the content of the essential components involving fruiting bodies and mycelia among fungal species of genera named *Agaricus*, *Auricularia*, *Cordyceps*, *Flammulina*, *Ganoderma*, *Lentinus*, *Pleurotus*, and so on showed close resemblance. Therefore, the study on the production process of termite mycelium implemented a liquid culture method guiding a premise for future studies on culturing other species.

2 Materials and methods

2.1 Termitomyces mushroom extracts

100 g of dry biomass powder and fruiting body of 5 samples were extracted by methanol as a solvent with a sample ratio 5:1 at 40 °C, shaken for 24 h at 150 rpm, and filtered. The residual material underwent a double extraction with 300 mL of methanol afterward. The total extracts were evaporated at 50 °C to release the solvent, as reported by Giri et al. (2012).

2.2 Evaluation of DPPH radical scavenging activity assay

The mixture consisted of 0.5 mL of DPPH (2, 2-diphenyl-1-picrylhydrazyl) solution 2 mL of the extract with different concentrations. After incubation for 30 min, the absorbance of the solution was measured in a spectrophotometer at 517 nm and compared with the methanol control sample.

2.3 Evaluation of antibacterial activity

The antibacterial capacity was assessed using the agar well diffusion method (Palaksha et al., 2010). Microbial strains, namely *Escherichia coli* ATCC 8739, *Bacillus cereus* ATCC 11778, *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa*

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ATCC 27853, *Salmonella typhimurium* ATCC 14028, *Candida albicans* ATCC 10231 were activated in meat peptone broth for 24 h. The bacterial density was determined by optical density at 660 nm, adjusted in the range of 106-107 CFU/mL, and inoculated in solid mediumfor validation afterward.

A volume of 40 µL of the extract was diluted into different concentration ranges and added to the wells on the agar plate covered with bacteria. Chloramphenicol (10 mg/mL) was utilized as a positive control. The antibacterial potency was the distinction between the diameter of the inhibition zone and that of the well after 24 h. The increase the difference, the greater the antibacterial capacity.

The MIC (Minimum Inhibitory Concentration) on a 96-well plate method was employed to quantify the antibacterial effect of the extracts (Clinical & Laboratory Standards Institute, 2010). The solution involving 100 µL of liquid-form bacteria and 100 µL of different concentration ranges extract was incubated at 37 °C for 16-24h. Then, 20 µL of 0.01% resazurin reagent was made up serially to each well. The conversion of the resazurin solution from blue to pink indicated bacterial presence. MIC was defined as the lowest concentration of extracts inhibiting bacterial growth (without discoloring resazurin).

2.4 Toxicity testing

In-vitro MTT assay

The cytotoxic activity of the extract was investigated by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] method as described by Mosmann (1983). LO-2 hepatocytes were seeded in 96 well microtiter plates at a density of 1×10^5 cells/mL at 37 °C and 5% CO_2. Cells were transferred to medium without bovine serum and treated with *Termitomyces* strains biomass extracts in various gradient concentrations. After 24 h incubation, LO-2 hepatocytes were rinsed, and MTT solution was applied simultaneously (0.5 mg/mL, final concentration) for 4 h. 100 µL dimethyl sulfoxide (DMSO) was placed in each well after eliminating the supernatant and resuspended until all formazan crystals were dissolved. The absorbance value was measured on a microplate reader using a filter at a wavelength of 540 nm. The percentage of cellular viability was quantified as a proportion of the absorbance between the test sample and negative controls.

In-vivo acute and semi-chromic toxicity

Evaluation of acute toxicity of the reagent in white mice orally was executed by the description of Litchfield & Wilcoxon (1949). White mice were plotted and numbered in order of offspring and drank water freely during the fasting time of 12 h. Then, rats had applied reagents with increasing doses, from the lowest dose of 0 mg/kg body weight to the highest dose of 10000 mg/kg body weight. The analogous volume of both distilled water and different dose levels of biomass extracts were inserted directly into the stomach of each rat in test plots by a blunt-tipped needle. Each rat drank 3 hours/time x 3 times/24 h. Mice should be monitored concerning general condition, dead mice, weight, and hematological index in 72 h, then all dead mice were dissected to evaluate the gross damage. White mice were further observed for up to 14 days.

According to Vietnam Health Ministry's regulation, the semi-chronic toxicity was evaluated on white mice. In total, 32 mice were used for this experiment, where 4 mice were stabilized for 24 h and taken blood to analyze physiological indicators. The remaining 28 rats were divided into 2 plots located in 4 cages. The same volume of distilled water and extracts were inserted directly into the stomach of each rat by a curved needle once a day in the mornings from 8-10 h and drank continuously for 30 days. Test rats drank 1000 mg/kg body weight of extracts while control mice consumed distilled water. Parameters including body weight, blood physiological, and biochemical blood index were verified before utilizing the extract within 30 days from the first dose.

3 Results and discussion

3.1 Extraction efficiency

The extract of *Termitomyces* biomass applied methanol solvent at 45 °C achieved the optimal efficiency, which accounted for 39.87%. In contrast, fruiting bodies extracts ranged from 29.74% to 32.21%, similar to the results of Puttaraju et al. (2006) reported *Termitomyces mummiformis* (32%), *Termitomyces heimii* (28%), *Termitomyces microcapus* (20%), gray oyster mushroom (*Pleurotus sajor-caju*) (32%), pink oyster mushroom (*Pleurotus djamor*) (21%).

3.2 Antioxidant activity

Termitomyces mycelial biomass extract was discovered to be efficient in scavenging the free radicals through the DPPH method, depending on the concentration. The biomass extract showed antioxidant potential at 3.0 mg/mL with 35.3% of inhibition and uplifted until reached the finest activity 77.5% at 5 mg/mL. The IC50 was 2.262 mg/mL indicating the significant role of the extract in the antioxidative activity, according to Mau et al. (2004). The antioxidant activity of both *Termitomyces* biomass and fruiting body extract was equivalent in comparison with the EC50 values (via DPPH free radical scavenging) of Agaricus Blazei Murill mushroom (*A. blazei*) 2.26 mg/mL (Huang et al., 1999), Lingzhi mushroom (*Ganoderma tsugae*) 4.28 mg/mL (Tsai, 2002), maitake mushroom (*Grifolia frondosa*) 2.00 mg/mL (Mau et al., 2002), shiitake mushroom (*Lentinus edodes*) 7.3 mg/mL (Heleno et al., 2015); turkey tail mushroom (*Trametes versicolor*) 1.3 mg/mL and penny bun mushroom (*Boletus edulis*) 4.67 mg/mL (Vamanu & Voica, 2017) and *Astraeus hygrometricus* (Biwas et al., 2010).

3.3 Antibacterial potential

Studies on the antibacterial ability of plant extracts prioritized methanol and ethanol solvents by their capability to dissolve natural compounds (El-Mahmood & Doughari, 2008); hence the extracts will be highly effective in both Gram-negative and Gram-positive bacteria (Turker et al., 2009).

The antibacterial activity of *Termitomyces* mushroom biomass and fruiting body extract against six strains of pathogenic

bacteria, including *Escherichia coli* ATCC 8739 (*EC*), *Bacillus cereus* ATCC 11778 (*BC*), *Staphylococcus aureus* ATCC 6538 (*SA*), *Pseudomonas aeruginosa* ATCC 27853 (*PA*), *Salmonella typhimurium* ATCC 14028 (*ST*), *Candida albicans* ATCC 10231(*CA*) was evaluated in Table 1. The biomass extract expressed potent efficacy against all strains of pathogenic bacteria with the inhibition zone diameters ranging from 12-14.5 mm, in which the ability against *EC* was weaker with the respective inhibition zone 8 mm. On the other hand, remaining extracts from the fruiting body were determined inactive against *SA* and *PA* and showed moderate resistance to other strains. In particular, extract M2 prevented the growth of *EC*, *BC*, *ST*, *CA* with the inhibition zone ranging from 6.5-10 mm. Extract M3 exposed a similar activity to extract M2 to EC, BC, and CA but strongly against ST with the inhibition zone diameter of 12 mm. Extract M4 exhibited weak activity inhibiting *BC* and *CA* but dynamically against *ST* (inhibition zones 12 mm). Extract M5 revealed no activity or weak efficacy resist the bacteria enumerated.

The tested bacterial strains were susceptible to 5 extracts from Termitomyces mushroom with low minimum inhibitory concentration (MIC) values ranging from 10.21 mg/mL to 46.2 mg/mL (Table 2). This result was in accordance with other studies on the antibacterial activity of mushrooms (Jiamworanunkul & Chomcheon, 2019). In particular, common puffball mushroom (*Lycoperdon perlatum*) extracted with ethanol methanol had the potential against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus cereus* with MICs ranging from 15.63 mg/mL to 125 mg/mL (Akpi et al., 2017); wood ear mushroom (*Auricularia auricula-judae*) resisted to *Staphylococcus aureus,* and *Escherichia coli* with MICs of 12.5 mg/mL and 6.25 mg/mL, respectively (Cai et al., 2015); white oyster mushroom (*Pleurotus Florida*) had the MIC value of 25 mg/mL for *Escherichia coli*, 50 mg/mL for *Streptococcus sp.*, and 75 mg/mL for *Proteus murabilus* (Thillaimaharani et al., 2013); *Caterpillar fungus* (*Cordyceps Sinensis*) inhibited the growth of *Bacillus subtilis* and *Streptococcus epidermidis* with MICs were 938 μg/mL and 469 μg/mL, respectively (Ren et al., 2014).

3.4 Toxicity

Before applying for large-scale production, termite mushroom biomass extract was tested for cytotoxic activity to ensure efficacy and safety. In the *in-vitro* model, the cytotoxicity of the mushroom was evaluated by the MTT method on LO-2 human hepatocytes. Cell survival was recorded above 90% at the concentration range from 100, 200, 300, 500, 1000, 1500, 2000 to the highest concentration of 2500 μg/mL, proving that all concentrations of termite mushroom extract had no toxicity on the hepatoma cell line LO-2. According to Youn et al. (2008) research, the extract of Chaga mushroom (Inonotus obliquus) at the concentration of 1000 μg/mL had no effect on the hepatocytes, and cell survival rate attained over 80% in 48 h. However, the termite mushroom biomass extract should be further inspected in multiple mouse models at different concentration ranges to have entire toxicity data.

The acute toxicity results monitored after 72 h indicated that at the maximum oral dose of 10,000 mg/kg body weight, 100% of mice were normal without extraordinary expressions or dead. The next 14 days showed no abnormalities. Thus, termite mushroom biomass extract did not cause oral toxicity in white mice with the consumed dose of D_{max} = 10,000 mg/kg body weight. There was no statistical difference in the weight of mice before and after 72 h of oral administration and physiological

Table 1. Antibacterial activity of biomass and fruiting body extracts of *Termitomyces* mushroom.

Samples	*EC*	*BC*	*SA*	*PA*	*ST*	*CA*
M1 (3.5 mg)	8.0 ± 0.2	13.0 ± 0.1	14.5 ± 0.2	12.5 ± 0.1	14.0 ± 0.2	12.0 ± 0.1
M2 (3.7 mg)	6.5 ± 0.2	10.0 ± 0.2	-	-	7.0 ± 0.2	7.0 ± 0.1
M3 (3.7 mg)	6.5 ± 0.2	7.0 ± 0.1	-	-	12.0 ± 0.2	7.0 ± 0.2
M4 (3.8 mg)	-	7.0 ± 0.1	-	-	12.0 ± 0.1	6.5 ± 0.1
M5 (3.3 mg)	-	10.0 ± 0.1	-	-	11.0 ± 0.2	-
Chloramphenicol (0.2 μg)	23 ± 0.3	24 ± 0.2	30 ± 0.2	34 ± 0.2	27 ± 0.2	33 ± 0.3

Values are given as mean ± SD of the triplicate experiment. *Escherichia coli* ATCC 8739 (EC), *Bacillus cereus* ATCC 11778 (BC), *Staphylococcus aureus* ATCC 6538 (SA), *Pseudomonas aeruginosa* ATCC 27853 (PA), *Salmonella typhimurium* ATCC 14028 (ST), *Candida albicans* ATCC 10231 (CA).

Table 2. Minimum Inhibitory Concentration (MIC) values of biomass and fruiting body extracts of *Termitomyces* mushroom.

Samples	MIC (mg/mL)					
	EC	BC	SA	PA	ST	CA
M1	10.98	10.98	10.98	10.98	10.98	10.98
M2	11.55	46.20	11.55	11.55	11.55	11.55
M3	11.42	11.42	11.42	45.67	11.42	11.42
M4	23.50	23.50	23.50	23.50	23.50	23.50
M5	10.21	10.21	10.21	10.21	10.21	10.21
Chloramphenicol (ppm)	0.2	0.2	0.2	0.2	0.2	0.2

Escherichia coli ATCC 8739 (EC), *Bacillus cereus* ATCC 11778 (BC), *Staphylococcus aureus* ATCC 6538 (SA), *Pseudomonas aeruginosa* ATCC 27853 (PA), *Salmonella typhimurium* ATCC 14028 (ST), *Candida albicans* ATCC 10231 (CA).

blood indices between the experimental and control groups (Tables 3-4).

The semi-permanent toxicity observed after 30 days of applying extract at the concentration of 1000 mg/kg body weight revealed that the weight of mice in the treated group (29.787 ± 0.446) was inferior to that in the control group (30.563 ± 0.792). The difference between the 2 groups was not statistically significant but remarkably dissimilar to the initial mice weight (20.690 ± 0.264) (Table 5). The result proved that termite mushroom biomass extract did not affect mice's weight during 30 days.

A large amount of blood was required to check the biochemical indices in mice; hence, the blood of mice in the group was combined before centrifugation. The biochemical blood indices between the non-consumed extract and the consumed extract groups differed, but the difference and fluctuation were not too much. The fluctuation levels among biochemical blood indices of mice shown in Table 6 expressed the normal liver and kidney function. The liver and kidney specimens of the tested mice were not damaged compared to the control group after dissecting and observing under the microscope at the magnification of 400x.

Blood physiological indices between the control and test samples after 30 days utilizing pooled T-test and T-Satterthwaite exposed no statistical difference. On the other hand, MCV and RDW-SD indices in the experimental group were elevated in comparison with the control group ($p < 0.05$) (Table 7). However,

Table 3. Weight and lethal rate of mice after treated with mushroom extracts in acute toxicity experiment.

Dose (mg/kg body weight)	Number of mouse	Initial weight	Weight after 72 h	Number of deaths
0	6	20.000 ± 0.925	22.400 ± 1.115	0
2,000	6	20.437 ± 0.657	22.670 ± 1.078	0
4,000	6	20.733 ± 1.065	22.350 ± 1.405	0
6,000	6	20.367 ± 1.021	21.667 ± 1.013	0
8,000	6	20.633 ± 1.048	21.783 ± 1.520	0
10,000	6	20.883 ± 1.078	22.483 ± 1.042	0

Values are given as mean ± SD of triplicate experiment.

Table 4. Blood indices of tested mice acute toxicity experiment.

Blood indices	Unit	Dose 0 mg/kg	Dose 2.000 mg/kg	Dose 4.000 mg/kg	Dose 6.000 mg/kg	Dose 8.000 mg/kg	Dose 10.000 mg/kg
WBC	10*9/L	6.970 ± 2.470	5.800 ± 0.529	5.300 ± 0.600	4.000 ± 0.964	5.300 ± 1.510	6.93 ± 2.47
LYM - Lymphocyte **%**	%	76.17 ± 7.17	76.27 ± 3.55	74.70 ± 3.08	75.20 ± 0.866	77.00 ± 5.24	75.10 ± 3.72
MID%	%	11.77 ± 2.99	12.333 ± 1.115	13.47 ± 2.16	13.50 ± 1.73	10.77 ± 1.79	12.40 ± 3.40
NEUT- Neutrophil **%**	%	12.07 ± 4.56	11.40 ± 2.69	11.83 ± 2.40	11.30 ± 2.33	12.23 ± 3.50	12.50 ± 2.43
LYM- Lymphocyte **#**	10*9/L	5.40 ± 2.25	4.433 ± 0.473	7.30 ± 5.98	3.033 ± 0.723	3.900 ± 0.917	5.17 ± 1.86
MID#	10*9/L	0.800 ± 0.265	0.700 ± 0.100	0.733 ± 0.153	0.533 ± 0.208	0.567 ± 0.252	0.933 ± 0.462
NEUT- Neutrophil **#**	10*9/L	0.767 ± 0.058	0.667 ± 0.153	0.600 ± 0.200	0.433 ± 0.058	0.667 ± 0.351	0.833 ± 0.208
RBC- Red blood cell	10*12/L	7.17 ± 3.20	10.210 ± 0.466	10.237 ± 0.301	7.54 ± 3.82	6.91 ± 2.26	7.69 ± 1.92
HGB- Hemoglobin	g/dL	10.17 ± 4.57	14.500 ± 0.500	14.200 ± 0.700	10.43 ± 4.97	10.47 ± 3.30	10.97 ± 2.66
HCT- Hematocrit	%	36.10 ± 17.16	52.57 ± 3.35	52.17 ± 2.54	37.3 ± 18.6	35.70 ± 11.66	38.03 ± 9.31
MCV- Mean corpuscular volume	fL	49.73 ± 2.38	51.400 ± 0.800	51.000 ± 1.453	49.767 ± 0.737	51.667 ± 0.643	49.567 ± 1.201
MCH- Mean corpuscular hemoglobin	pg	14.100 ± 0.100	14.133 ± 0.153	13.833 ± 0.473	13.933 ± 0.924	15.133 ± 0.208	14.233 ± 0.651
MCHC- Mean corpuscular hemoglobin concentration	g/dL	28.500 ± 1.323	25.57 ± 4.15	27.167 ± 0.252	28.067 ± 1.422	29.333 ± 0.416	28.800 ± 0.700
RDW-Red distribution width -**SD**	fL	28.500 ± 1.039	31.600 ± 0.000	31.600 ± 0.000	30.37 ± 2.14	31.57 ± 1.85	30.33 ± 2.80
RDW- Red distribution width -**CV**	%	13.833 ± 0.569	14.767 ± 0.252	14.933 ± 0.451	14.733 ± 1.242	14.733 ± 0.874	14.767 ± 1.026
PLT- Platelet count	10*9/L	331.7 ± 93.0	436.7 ± 28.6	428.00 ± 14.18	383.0 ± 48.8	580.0 ± 188	345.7 ± 56.2
MPV- Mean platelet volume	fL	6.50 ± 1.85	7.233 ± 0.153	7.100 ± 0.400	7.033 ± 0.577	7.533 ± 0.289	8.267 ± 0.551
PDW- Platelet disrabution width	%	7.90 ± 3.30	8.333 ± 0.116	8.867 ± 0.569	9.200 ± 0.300	10.067 ± 0.404	11.067 ± 0.814
PCT- Plateletcrit	%	0.247 ± 0.071	0.33 ± 0.062	0.300 ± 0.030	0.263 ± 0.015	0.433 ± 0.153	0.277 ± 0.040
P-LCR- Platelet larger cell ratio	%	12.27 ± 1.91	10.833 ± 1.450	9.33 ± 4.46	7.37 ± 4.88	11.87 ± 2.22	19.70 ± 4.21

Values are given as mean ± SD of triplicate experiment.

physiological blood indices in animals possessed ranges of variation when analyzing. In particular, the MCV index of Syrian Hamsters ranged from 64.0-77.6 fL, European Hamsters approximated 58.7-71.4 fL, Djungarian Hamsters approached 53.6-65.2 fL (Heatley & Harris, 2009).

Termitomyces extracts were evaluated to possess valuable nutrition as well as pharmacological properties contributing to health benefits. Nakalembe et al. (2015) discovered high levels of thiamin, folic acid, vitamin C, and niacin within 3 species of edible *Termitomyces* mushrooms in Uganda namely *Termitomyces microcarpus* (Berk and Broom) R. Heim, *Termitomyces tyleranus* (Otieno), *Termitomyces clypeatus* (Heim). According to Zhao et al. (2017), depolymerized-exopolysaccharides and exopolysaccharides derived from *Termitomyces albuminosus* exposed scavenging

Table 5. Weight and lethal rate of mice after treated with mushroom extracts in semi-chronic toxicity experiment.

Treatment	Number of mouse	Weight of mice	Number of deaths
Initial mice	32	$20.690^{b} \pm 0.264$	0
Control mice	14	$30.563^{a} \pm 0.792$	0
Treated mice	14	$29.787^{a} \pm 0.446$	0

Values with different superscripts are significantly ($p < 0.05$) different. Values are given as mean ± SD of triplicate experiment.

Table 6. Blood indices of tested mice semi-chronic toxicity experiment.

Blood indices	Unit	Initial control samples	Control samples after 30 days	Treated samples after 30 days
WBC	10*9/L	5.275 ± 1.417	6.380 ± 2.54	5.650 ± 1.620
LYM%	%	63.200 ± 13.04	75.100 ± 11.000	71.850 ± 4.390
MID%	%	16.250 ± 1.923	11.550 ± 3.490	12.050 ± 1.507
NEUT%	%	20.550 ± 11.540	13.350 ± 7.840	16.100 ± 5.230
LYM#	10*9/L	3.225 ± 0.299	4.850 ± 2.210	4.025 ± 0.991
MID#	10*9/L	0.850 ± 0.332	0.750 ± 0.332	0.6750 ± 0.1708
NEUT#	10*9/L	1.200 ± 1.010	0.775 ± 0.479	0.950 ± 0.526
RBC	10*12/L	9.970 ± 1.731	8.590 ± 2.880	9.617 ± 1.131
HGB	g/dL	15.000 ± 2.720	11.850 ± 3.770	13.975 ± 1.328
HCT	%	53.130 ± 10.520	42.700 ± 14.380	49.650 ± 5.32
MCV	fL	53.175 ± 1.489	49.725 ± 1.231	51.750 ± 0.737
MCH	pg	15.000 ± 0.408	13.825 ± 0.330	14.525 ± 0.538
MCHC	g/dL	28.275 ± 0.650	27.875 ± 0.885	28.125 ± 0.634
RDW-SD	fL	29.250 ± 2.340	28.800 ± 1.039	31.575 ± 1.511
RDW-CV	%	13.250 ± 0.904	13.975 ± 0.650	14.275 ± 0.550
PLT	10*9/L	488.0 ± 41.1	389.3 ± 133.9	390.0 ± 80.8
MPV	fL	7.850 ± 0.238	7.275 ± 0.645	7.725 ± 0.403
PDW	%	9.625 ± 1.274	8.375 ± 0.991	9.575 ± 1.846
PCT	%	0.3775 ± 0.0359	0.2750 ± 0.0988	0.2975 ± 0.0618
P-LCR	%	15.250 ± 2.030	12.300 ± 3.380	15.270 ± 2.560

Values are given as mean ± SD of triplicate experiment.

Table 7. Blood indices of tested mice semi-chronic toxicity experiment compared to hamster Syria mice (Heatley & Harris, 2009).

Biochemical indices	Unit	Control batch	Treated batch	Mouse Hamster Syria
ALT (Alanine Aminotransferase)	U/L	59	50	22-128
AST (Aspartate aminotransferase)	U/L	31	29	28-122
BUN (Blood Urea Nitrogen)	mg/dL	17.9	14.3	12-26
Cholesterol	mg/dL	231.8	181	55-181
Creatinine	mg/dL	0.68	1.04	0.4-1
GGT (Gamma-glutamyl transferase)	U/L	50	77	-
HDL (high-density lipoprotein)	mg/dL	73.5	24.9	-
Triglycerides	mg/dL	161.6	103.5	72-227
LDL calculate	mg/dL	126	135	-

Values are given as mean ± SD of triplicate experiment.

free DPPH radicals activities at a concentration of 400 µg/mL. Besides, methanolic extract of *Termitomyces albuminosus* mycelia showed high antioxidant activity (Mau et al., 2004). However, varietal differences leaded to divergent biological activities, in particular, *Termitomyces heimii* and *Termitomyces mummiformis* displayed good antioxidant activity while *Termitomyces microcarpus* was contrary (Puttaraju et al., 2006). Aqueous extract of *Termitomyces striatus* were determined to inhibit several strains of bacteria and fungi due to the presence of phytochemicals namely alkaloids, flavonoids, sterols and steroids, saponins, phenols, carbohydrates, and proteins inside (Sitati et al., 2021). Both endopolysaccharide and exopolysaccharide derived from *Termitomyces heimii* RFES 230662 (THR2) were verified as β-glucan, which played a key role in the antibacterial activity of the mushroom extract (Ahmad et al., 2021). The research of Rathore et al. (2017) verified that *Termitomyces eurhizus* including significant homopolysaccharides supported anti-aging effects. Serine protease AkP isolated from *Termitomyces clypeatus* selectively prevented the growth of Hep-G2 cells by cleaving cell surface HSPG with the concomitant induction of apoptosis orchestrated by activation of caspase-3 (Majumder et al., 2016).

4 Conclusions

This study concluded that the methanol extract of *Termitomyces* mycelial biomass expressed antioxidant potential and potent efficacy against Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa,* and *Salmonella typhimurium*) and Gram-positive (*Bacillus cereus*, *Staphylococcus aureus*) bacteria. Besides, the extract showed an antifungal effect that inhibits *Candida Albicans* without causing toxicity. Therefore, the extract of Termitomyces mycelial biomass can be utilized as an antioxidant, antibacterial, and antifungal agent.

References

Ahmad, R., Sellathoroe, S., Ngadi, E., Saharuddin, T. S. T., Zakaria, I. I., Selvakumaran, S., & Wan-Mohtar, W. A. A. Q. I. (2021). Isolation, identification, cultivation and determination of antimicrobial β-glucan from a wild-termite mushroom *Termitomyces heimii* RFES 230662. *Biocatalysis and Agricultural Biotechnology*, 37(9), 102187.

Akpi, U. K., Odoh, C. K., Ideh, E. E., & Adobu, U. S. (2017). Antimicrobial activity of *Lycoperdon perlatum* whole fruit body on common pathogenic bacteria and fungi. *African Journal of Clinical and Experimental Microbiology*, 18(2), 79-85. http://dx.doi.org/10.4314/ajcem.v18i2.4.

Cai, M., Lin, Y., Luo, Y. L., Liang, H. H., & Sun, P. (2015). Extraction, antimicrobial, and antioxidant activities of crude polysaccharides from the wood ear medicinal mushroom *Auricularia auricula-judae* (higher basidiomycetes). *International Journal of Medicinal Mushrooms*, 17(6), 591-600. http://dx.doi.org/10.1615/IntJMedMushrooms.v17.i6.90.

Clinical & Laboratory Standards Institute – CLSI. (2010). *Performance standards for antimicrobial susceptibility testing Twentieth informational supplement M100-S20, 30(1)*. Wayne: CLSI.

El-Mahmood, A. M., & Doughari, J. H. (2008). Phytochemical screening and antibacterial evaluation of the leaf and root extracts of *Cassia alata* Linn. *African Journal of Pharmacy and Pharmacology*, 2(7), 124-129.

Giri, S., Biswas, G., Pradhan, P., Mandal, S. C., & Acharya, K. (2012). Antimicrobial activities of basidiocarps of wild edible mushrooms of West Bengal, India. *International Journal of PharmTech Research*, 4(4), 1554-1560.

Heatley, J. J., & Harris, M. C. (2009). Hamsters and gerbils. In M. A. Mitchell & T. N. Tully (Eds.), *Manual of exotic pet practice* (pp. 406-432). St. Louis: Elsevier. http://dx.doi.org/10.1016/B978-141600119-5.50018-4.

Heleno, S. A., Martins, A., Queiroz, M. J. R. P., & Ferreira, I.C.F.R. (2015). Bioactivity of phenolic acids: Metabolites versus parent compounds: a review. *Food Chemistry*, 173, 501-513.

Huang, L.-C., Huang, S.-J., Chen, C.-C., & Mau, J.-L. (1999, October). Antioxidant properties of *Antrodia camphorata*. In World Society for Mushroom Biology and Mushroom Products (Ed.), *Proceedings of the Third International Conference on Mushroom Biology and Mushroom Products* (pp. 12-16). Hong Kong: World Society for Mushroom Biology and Mushroom Products.

Jiamworanunkul, S., & Chomcheon, P. (2019). Screening of antimicrobial and antioxidant properties of ethyl acetate extracts from wild edible mushrooms. *Thai Journal of Pharmaceutical Sciences*, 43(3), 161-167.

Litchfield, J. T., & Wilcoxon, F. (1949). A simplified method of evaluating dose-effect experiments. *Journal of Pharmacology and Experimental Therapeutics*, 96(2), 99-113.

Majumder, R., Banik, S. P., & Khowala, S. (2016). AkP from mushroom *Termitomyces clypeatus* is a proteoglycan specific protease with apoptotic effect on HepG2. *International Journal of Biological Macromolecules*, 91, 198-207. http://dx.doi.org/10.1016/j.ijbiomac.2016.05.034. PMid:27180294.

Mau, J.-L., Chang, C.-N., Huang, S.-J., & Chen, C.-C. (2004). Antioxidant properties of methanolic extracts from *Grifola frondosa, Morchella esculenta* and *Termitomyces albuminosus* mycelia. *Food Chemistry*, 87(1), 111-118. http://dx.doi.org/10.1016/j.foodchem.2003.10.026.

Mau, J.-L., Lin, H.-C., & Song, S.-F. (2002). Antioxidant properties of several specialty mushrooms. *Food Research International*, 35(6), 519-526. http://dx.doi.org/10.1016/S0963-9969(01)00150-8.

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, 65(1-2), 55-63.

Nakalembe, I., Kabasa, J. D., & Olila, D. (2015). Comparative nutrient composition of selected wild edible mushrooms from two agro-ecological zones, Uganda. *SpringerPlus*, 4, 433. http://dx.doi.org/10.1186/s40064-015-1188-z. PMid:26306295.

Palaksha, M. N., Ahmed, M., & Das, S. (2010). Antibacterial activity of garlic extract on streptomycin-resistant Staphylococcus aureus and Escherichia coli solely and in synergism with streptomycin. *Journal of Natural Science, Biology and Medicine*, 1(1), 12.

Puttaraju, N. G., Venkateshaiah, S. U., Dharmesh, S. M., Urs, S. M. N., & Somasundaram, R. (2006). Antioxidant activity of indigenous edible mushrooms. *Journal of Agricultural and Food Chemistry*, 54(26), 9764-9772. http://dx.doi.org/10.1021/jf0615707. PMid:17177499.

Rathore, H., Prasad, S., & Sharma, S. (2017). Mushroom nutraceuticals for improved nutrition and better human health: a review. *PharmaNutrition*, 5(2), 35-46. http://dx.doi.org/10.1016/j.phanu.2017.02.001.

Ren, L., Hemar, Y., Perera, C. O., Lewis, G., Krissansen, G. W., & Buchanan, P. K. (2014). Antibacterial and antioxidant activities of aqueous extracts of eight edible mushrooms. *Bioactive Carbohydrates and Dietary Fibre*, 3(2), 41-51. http://dx.doi.org/10.1016/j.bcdf.2014.01.003.

Rosemary, F., Vitharana, U. W. A., Indraratne, S. P., Weerasooriya, R., & Mishra, U. (2017). Exploring the spatial variability of soil properties in an alfisol soil catena. *Catena*, 150, 53-61. http://dx.doi.org/10.1016/j.catena.2016.10.017.

Sitati, C. N. W., Ogila, K. O., Waihenya, R. W., & Ochola, L. A. (2021). Phytochemical profile and antimicrobial activities of edible mushroom

Termitomyces striatus. Evidence-Based Complementary and Alternative Medicine, 2021, 3025848. http://dx.doi.org/10.1155/2021/3025848. PMid:34712341.

Thillaimaharani, K. A., Sharmila, K., Thangaraju, P., Karthick, M., & Kalaiselvam, M. (2013). Studies on antimicrobial and antioxidant properties of oyster mushroom Pleurotus florida. *International Journal of Pharmaceutical Sciences and Research*, 4(4), 1540.

Tsai, S. Y. (2002). Antioxidant properties and their cytotoxic activities on tumor cells of Ganoderma tsugae and Agrocybe cylindracea and antimutagenic properties of Agrocybe cylindracea. Taichung, Taiwan: National Chung-Hsing University.

Turker, H., Yildirim, A. B., & Karakaş, F. P. (2009). Sensitivity of bacteria isolated from fish to some medicinal plants. *Turkish Journal of Fisheries and Aquatic Sciences*, 9(2), 45-50. http://dx.doi.org/10.4194/trjfas.2009.0209.

Ulziijargal, E., & Mau, J.-L. (2011). Nutrient compositions of culinary-medicinal mushroom fruiting bodies and mycelia. *International Journal of Medicinal Mushrooms*, 13(4), 343-349. http://dx.doi.org/10.1615/IntJMedMushr.v13.i4.40. PMid:22164764.

Vamanu, E., & Voica, A. (2017). Total phenolic analysis, antimicrobial and antioxidant activity of some mushroom tinctures from medicinal and edible species, by *in vitro* and *in vivo* tests. *Scientific Bulletin. Series F. Biotechnologies*, 21, 318-324.

Venkatachalapathi, A., & Paulsamy, S. (2016). Exploration of wild medicinal mushroom species in Walayar valley, the Southern Western Ghats of Coimbatore district Tamil Nadu. *Mycosphere*, 7(2), 118-130. http://dx.doi.org/10.5943/mycosphere/7/2/3.

Youn, M.-J., Kim, J.-K., Park, S.-Y., Kim, Y., Kim, S.-J., Lee, J.-S., Chai, K.-Y., Kim, H.-J., Cui, M.-X., So, H.-S., Kim, K.-Y., & Park, R. (2008). Chaga mushroom (*Inonotus obliquus*) induces G0/G1 arrest and apoptosis in human hepatoma HepG2 cells. *World Journal of Gastroenterology*, 14(4), 511-517. http://dx.doi.org/10.3748/wjg.14.511. PMid:18203281.

Zhao, H., Li, J., Zhang, J., Wang, X., Liu, M., Zhang, C., & Jia, L. (2017). Hepatoprotective and *in vitro* antioxidant effects of native depolymerised-exopolysaccharides derived from *Termitomyces albuminosus. Scientific Reports*, 7(1), 3910. PMid:28634377.