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Biological activities and chemical profile of *Hericium erinaceus* mycelium cultivated on mixed red and white jasmine rice

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Abstract

Functional foods have received considerable attention due to their numerous health benefits. Lion's mane mushroom (*Hericium erinaceus*) is a functional food source that contains bioactive compounds of medicinal importance. The main aim of this study was to explore antimicrobial and anticancer activities and identify bioactive compounds of *H. erinaceus* mycelia cultivated on mixed red and white jasmine rice (HMR) with the aid of solid-state fermentation (SSF). The HMR extract and its fractions were analyzed for antibacterial activity via disc diffusion and minimum inhibitory concentration (MIC) assays and anticancer activity against cervical cancer (HeLa) examined with the [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] (MTS) assay. Chemical profiles were analyzed using gas chromatography-mass spectrometry (GC-MS). Our results showed that the lipophilic fraction (LF2) had antibacterial activity against *P. mirabilis* (MIC = 250 µg/mL). LF2 is primarily composed of fatty acid derivatives with antibacterial components, including nonanoic acid, 2-dodecen-1-yl(-) succinic anhydride, linoleic acid ethyl ester, 3,4-dimethylbenzoic acid, and lauryl acetate. However, all HMR fractions displayed relatively weak anticancer activity (IC_{50} > 100 µg/mL). Our collective findings support the potential utility of *H. erinaceus* mycelium cultivated on mixed red and white jasmine rice as an antibacterial agent.

Keywords: *Hericium erinaceus*; jasmine rice; SSF; lipophilic; bioactivities.

Practical Application: Our results provide a novel avenue for bioprospecting of *H. erinaceus* mycelium cultivated on mixed red and white jasmine rice and support its development as a nutraceutical with antibacterial activity. Further research, including standardization of crude extract materials and *in vivo* studies, should be undertaken to evaluate the effectiveness, safety, and side-effects of this medicinal mushroom.

1 Introduction

Functional foods include a wide range of compounds associated with disease prevention and health promotion. Mushrooms have been identified as an important functional food due to the presence of various mycochemicals. Lion's mane mushroom (*Hericium erinaceus*) is an edible and medicinal fungus known for its antimicrobial, antioxidant, anticancer, neuroprotective, and immunostimulatory properties (Friedman, 2015; Sokół et al., 2015). Some recognized bioactive constituents of *H. erinaceus* include polysaccharides, sterols, glycoproteins, and phenolic and volatile compounds (Thongbai et al., 2015).

Recently, the production of bioactive compounds from natural food sources has been a topic of considerable interest. Solid-state cultivation or solid-state fermentation (SSF) is a technique that involves the growth of microorganisms on moist solid substrates in the absence of free-flowing water (Wang et al., 2012). SSF is suitable for the daily formation of a number of compounds because it mimics the natural habitat of filamentous fungi (Costa et al., 2020). A well-known example is the application of SSF in production of "Anka" from Monascus-fermented rice with antihypertensive, antioxidant, antimicrobial, and anticancer properties (Wang et al., 2012).

The use of rice as a substrate in SSF of *H. erinaceus* is reported to facilitate identification of novel components with beneficial bioactivities and further improve bioactive compound production. For example, alkaloid erinacerins with potential antidiabetic properties and cytotoxicity against K562 cells (Wang et al., 2015a), isoindolin-1-one erinacerins with anticancer activity against A549 and HeLa cells (Wang et al., 2015b), and various medium polar bioactive compounds with antimicrobial activity against *Staphylococcus aureus* and *Cryptococcus neoformans* (MIC > 500 µg/mL) in *Hericium* sp. (Song et al., 2020). However, these bioactivities are reported to be relatively weak.

SSF of *H. erinaceus* using jasmine rice as a substrate may aid in improving its therapeutic biological activities. Jasmine rice is a type of rice with nutritional, softness, adhesive, and unique fragrance properties that underlie its high economic importance in global trade (Attaviroj & Noomhorm, 2014). Some of the most popular varieties are non-pigmented white jasmine rice and pigmented red jasmine rice (Charoenthaikij et al., 2021). Compared to white jasmine rice, the red variety has higher phenolic and antioxidant content but lower moisture (Charoenthaikij et al., 2021; Phanurak, 2021; Vichapong et al., 2010). Therefore, mixed

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varieties of jasmine rice could be effectively used as cultivation substrate with additional health benefits.

To our knowledge, information on the biological activities and chemical profile of *H. erinaceus* mycelia cultivated on jasmine rice is lacking in the literature. SSF of *H. erinaceus* using mixed red and white jasmine rice is expected to improve its biological activities. To validate this premise, we investigated the antimicrobial and anticancer activities as well as bioactive compounds contributing to these properties of *H. erinaceus* mycelia cultivated on a mixture of red and white jasmine rice in this study.

2 Materials and methods

2.1 Chemicals and media

The chemicals used for extraction and GC-MS analysis were purchased from Sigma-Aldrich (Saint Louis, USA). Potato Dextrose Agar (PDA) was obtained from HiMedia (Mumbai, India). Nutrient Agar, Mueller–Hinton Agar (MHA), and Mueller–Hinton Broth (MHB) were purchased from Oxoid (Basingstoke, UK). DMEM and FBS media were obtained from Gibco (Paisley, Scotland, UK) and doxorubicin from Fresenius Kabi (Pune, India). All other chemicals were acquired from Merck (Darmstadt, Germany) and SRL Chem (Hyderabad, India). White jasmine rice (*Oryza sativa* L. cv. KDML105) and red jasmine rice (*Oryza sativa* L.cv.HMD) were obtained from a local market (Buriram, Thailand).

2.2 Microorganisms and cell lines

H. erinaceus MW 672510.1 spawn originating from the Thailand Mushroom Collection Center was purchased from Marayat Farm (Pathumthani, Thailand). Bacterial strains (*Staphylococcus epidermidis, Salmonella* Typhimurium, and *Proteus mirabilis*) and the HeLa (cervical cancer) cell line were obtained from the Department of Microbiology (Kasetsart University, Thailand Culture Collection).

2.3 Solid-state fermentation

Mushroom spawns were transported to the laboratory, developed for 14 days at room temperature, and subsequently transferred to PDA slants. As a pre-culture, five discs (5 cm^2 in diameter) of 14 day-old mushroom mycelia grown on PDA slants were transferred to 50 mL sterile yeast peptone dextrose (YPD) broth in 250 mL flasks. Pre-culture flasks were incubated on a rotary shaker (160 rpm) at 30 °C for 14 days. The rice medium for mushroom SSF experiments was prepared using 20 g of a mixture of white and red jasmine rice per jar (1:1) with the addition of 40 mL liquid nutrient (15 g/L glucose, 15 g/L sucrose, 51 g/L potato, two eggs, and 1 L distilled water). The mixture of rice and liquid nutrient was poured into 16-oz Mason Jars with air-filtered plastic caps and autoclaved at 121 °C for 20 minutes. After cooling, the medium was inoculated with 5 mL of pre-culture. SSF of *H. erinaceus* mycelia on jasmine rice was conducted in an air-conditioned laboratory at 22 ± 2 °C for approximately 6 weeks (Darmasiwi et al., 2022).

2.4 Extraction and fractionation

Briefly, 200 g unfermented jasmine rice medium (UR) and *H. erinaceus* mycelia cultvivated on mixed red and white jasmine rice (HMR) samples were soaked in 800 mL of 95% ethanol (1:4, *w/v*) for 7 days at 25 °C. Samples were filtered, concentrated via rotary evaporation (Heidolph, Schwabach, Germany), freeze-dried (ScanVac, Allerød, Denmark), and kept at -20 °C before the assay. The ethanol extract of *H. erinaceus* mycelia (13.78 g) was fractionated following the method of Kawagishi (2005) with modifications, using a chloroform:water (1:1) mixture. The chloroform fraction was further subjected to column chromatography using silica gel (60–120 mesh size, SRL Chem, Hyderabad, India) and equilibrated with chloroform:ethyl acetate (10:1, 8:2, 7:3, 5:5, 3:7, 1:10). The fractions were run on a TLC silica gel plate using chloroform:ethyl acetate (3:7) as the mobile phase. Eluted fractions with the same TLC profiles were combined, yielding two distinct lipophilic fractions, specifically, LF1 (0.98 g) and LF2 (0.15 g). The aqueous fraction was equilibrated with ethanol as the mobile phase (20:80), yielding a hydrophilic fraction HF (0.49 g). UR, HMR, LF1, LF2, and HF were further subjected to GC-MS and analyzed for bioactivities. A graphical workflow of these experiments is shown in (Figure 1).

2.5 Disc diffusion assay

An aliquot (100 µL) of overnight bacterial culture was spread on sterile MHA plates. Next, 6 mm filter paper discs were soaked with 10 µL sample (1000 µg/mL in 5% DMSO) and placed on the agar surface. Plates were incubated for 24 h at 37 °C and examined for clear inhibition zones around the discs. Ampicillin was used as the positive control (Liu et al., 2013).

2.6 Minimum Inhibitory Concentration (MIC) test

MIC tests were performed following the method described by Bach et al. (2019). Briefly, 50 µL of two-fold serial dilutions of samples (concentrations of 125–1000 µg/mL) were placed in a 96-well microplate containing Mueller–Hinton Broth (MHB), followed by the addition of 50 µL microbial suspension (10^8 CFU/mL) to each well. Ampicillin was used as the positive control. After 24 h of incubation at 37 °C, absorbance was measured at 600 nm with a Multiskan GO microplate reader (Thermo Fisher Scientific, MA, USA).

2.7 Analysis of anticancer activity

Cervical cancer (HeLa) cells were maintained in DMEM containing 10% FBS and antibiotics (50 U/mL penicillin and 50 µg/mL streptomycin). A cytotoxicity test was performed using the MTS assay (Promega, Madison, USA) following the manufacturer's protocol. Aliquots (100 µL) of cell cultures ($3x10^4$ cells/mL) were seeded in 96-well plates and incubated at 37 °C for 24 h under 5% CO_2 (Shel Lab, Orlando, USA). Following the addition of samples (125–1000 µg/mL), cultures were incubated at 37 °C for 72 h. Next, 20 µL MTS reagent was added and incubated at 37 °C for 2 h. Cell viability was measured at 490 nm with a Multiskan GO microplate spectrophotometer (Thermo Fisher Scientific, MA, USA). Medium was used as a negative control and doxorubicin as a positive control (Ghosh et al., 2020).

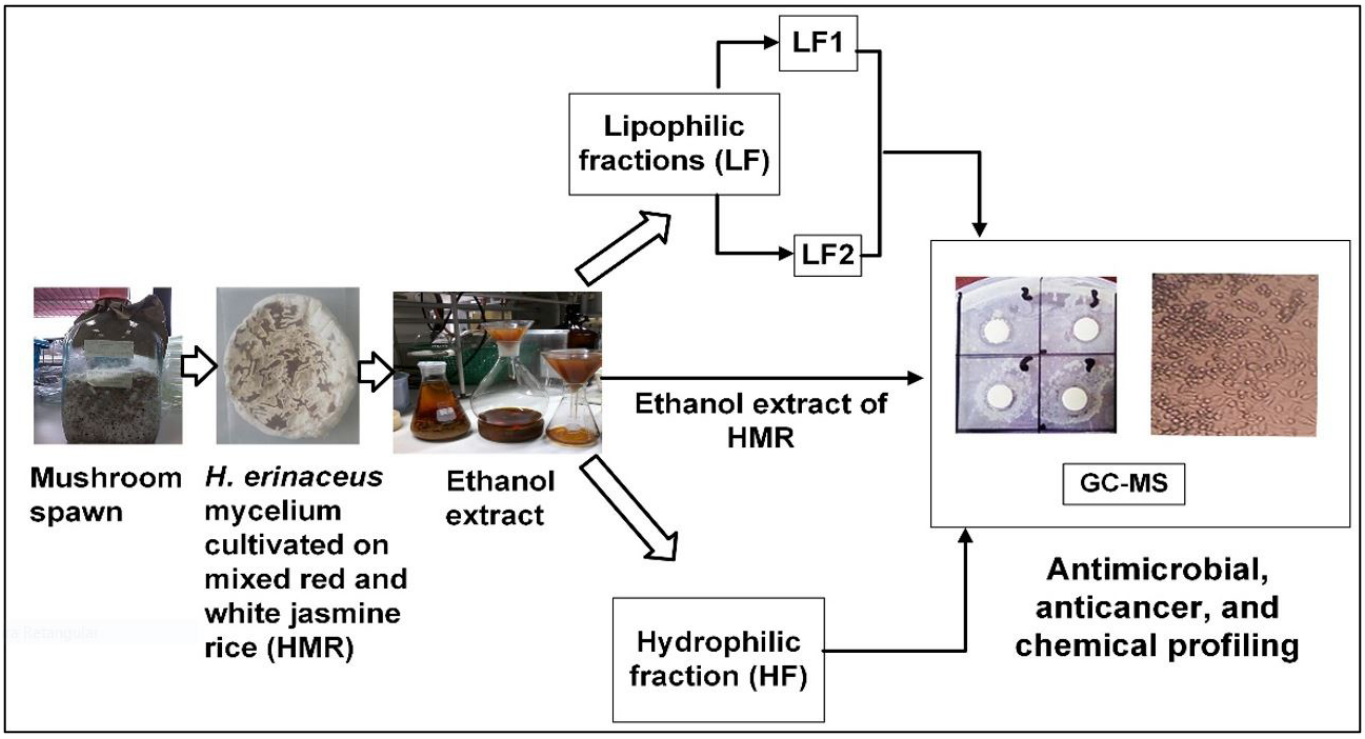

Figure 1. Graphical workflow of the experiments.

2.8 GC-MS analysis

Prior to GC-MS analysis, samples (0.1 g/mL) were dissolved in ethanol at a 20:80 ratio. GC-MS analysis was performed using a Shimadzu QP2020 system (Shimadzu, Tokyo, Japan) on a fused silica capillary column filled with 5% phenyl methylpolysiloxane. The column temperature was set to 50 °C (2 min), followed by 300 °C (5 °C/min for 10 min), with a running time of 64 min, vaporization temperature of 250 °C, and He (99.99%) as the gas carrier. The gas flow rate was 1.0 mL/min with column pressure of 7.65 psi, ion source temperature of 230 °C, quadrupole temperature of 150 °C, electron energy of 70 eV, emission current of 34.6 A, multiplier voltage of 1294 V, interface temperature of 280 °C, and mass range of 29–500 amu. The m/z spectra were identified using the NIST Mass Spectral database (Song et al., 2019).

2.9 Statistical analysis

All experiments were performed in triplicate and results expressed as mean ± standard deviation. Statistical evaluation of one-way ANOVA followed by Duncan's multiple range test (DMRT) was conducted in SPSS version 22 (IBM, NY, USA), and data considered significant at $P < 0.05$.

3 Results

3.1 In vitro antimicrobial assay

The antimicrobial activities of unfermented jasmine rice (UR), extracts of *H. erinaceus* mycelia cultivated on mixed red and white jasmine rice (HMR), and active fractions were evaluated by measurement of zone of inhibition (ZOI) from the disc diffusion assay and minimum inhibitory concentration (MIC), as shown in Table 1. Data from the disc diffusion assay showed that LF2 had the highest antimicrobial activity with ZOI values of 7.67, 8.3 and 7.3 mm against *S. epidermidis, S.* Typhimurium and *P. mirabilis*, respectively.

MIC values of the extracts and active fractions against *S. epidermidis, S.* Typhimurium, and *P. mirabilis* are presented in Table 2. All extracts and fractions showed low antibacterial activity against *S. epidermidis* and *S.* Typhimurium at the concentrations examined. HMR, LF1 and HF displayed low to moderate antibacterial activity (MIC = 500 µg/mL) whereas LF2 had moderate to high antibacterial activity (MIC = 250 µg/mL) against *P. mirabilis*.

3.2 Cytotoxicity against HeLa cells

Cytotoxic effects were evaluated based on IC_{50} values, signifying the ability of compounds to inhibit 50% of cancer cell growth. UR, HMR, and all active fractions displayed weak activity in inhibiting HeLa cell proliferation, as determined based on an IC_{50} range of 100–1000 µg/mL. LF1 and HF exerted higher cytotoxicity than other samples, with IC_{50} values of 142.09 µg/mL and 118.63 µg/mL, respectively. However, these values were not comparable to the high cytotoxicity of doxorubicin (IC_{50} = 7.72 µg/mL) against HeLa cells (Figure 2).

3.3 Chemical profiles of HMR and active fractions

The bioactive compounds of HMR displayed different chemical profiles to unfermented jasmine rice, as shown in Table 3. The HMR was composed of sulfoxide (45.89%), fatty

Table 1. ZOI values of unfermented jasmine rice, *H. erinaceus* mycelia cultivated on mixed red and white jasmine rice, and active fractions (mm).

Bacteria	UR	HMR	LF1	LF2	HF	PC
S. epidermidis	8 ± 0^{a}	7.3 ± 0.06^{a}	7.3 ± 0.06^{a}	7.67 ± 0.06^{a}	7.3 ± 0.06^{a}	9 ± 0^{b}
S. Typhimurium	6.3 ± 0.06^{b}	8.3 ± 0.06^{c}	6 ± 0.06^{ab}	8.3 ± 0.12^{c}	5 ± 0^{a}	16.7 ± 0^{d}
P. mirabilis	5 ± 0^{a}	6 ± 0^{b}	7 ± 0^{c}	7.3 ± 0.06^{cd}	7.7 ± 0.06^{d}	21 ± 0^{e}

UR = unfermented jasmine rice; HMR = *H. erinaceus* mycelia cultivated on mixed red and white jasmine rice; LF = lipophilic fractions; HF = hydrophilic fraction; PC = positive control. Mean values with different letters in the same column are significantly different according to Duncan's multiple range test at the level of $p < 0.05$.

Table 2. MIC values of unfermented jasmine rice, *H. erinaceus* mycelia cultivated on mixed red and white jasmine rice, and active fractions (µg/mL).

Bacteria	UR	HMR	LF1	LF2	HF	PC
S. epidermidis	1000	1000	> 1000	1000	> 1000	< 125
S. Typhimurium	1000	> 1000	1000	1000	> 1000	< 125
P. mirabilis	1000	500	500	250	500	< 125

UR = unfermented jasmine rice; HMR = *H. erinaceus* mycelia cultivated on mixed red and white jasmine rice; LF = lipophilic fractions; HF = hydrophilic fraction; PC = positive control.

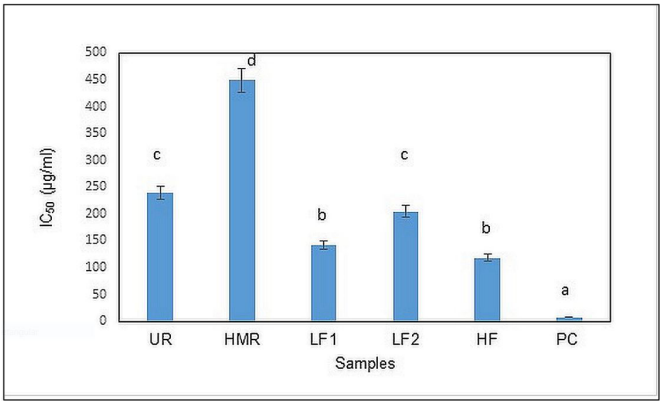

Figure 2. Cytotoxic activity (IC_{50} value) of unfermented jasmine rice, *H. erinaceus* mycelia cultivated on mixed red and white jasmine rice, and active fractions against the HeLa cell line. UR = unfermented jasmine rice; HMR = *H. erinaceus* mycelia cultivated on mixed red and white jasmine rice; LF = lipophilic fractions; HF = hydrophilic fraction; PC = positive control (doxorubicin). Mean values with different letters were significantly different at the level of $p < 0.05$.

acid derivatives (4.5%), and alcohols (0.35%) while unfermented jasmine rice mostly contained aldehydes (5.61%) and fatty acid derivatives (3.36%).

Despite these differences, a number of general similarities were detected among UR, HMR, and active fractions. The common compounds identified in all the samples were 3,4-dimethylbenzoic acid (Retention Time/RT = 20.55) and lauryl acetate (RT = 25.64) classified as fatty acid derivatives and caprolactam (RT = 16.68) of the lactam group (others). All active fractions comprised fatty acid derivatives, with concentrations of 4.5%, 12.5%, and 4.06% in LF1, LF2, and HF, respectively.

The major fatty acid derivative in LF1 and HF was 3,4-dimethylbenzoic acid (concentrations of 3.25% and 3.13%, respectively) while those in LF2 were nonanoic acid (RT = 17.17), 2-dodecen-1-yl (-) succinic anhydride (RT = 41.21) and linoleic acid ethyl ester (RT = 36.83) (concentrations of 3.19% and 2.53%, respectively). Terpenoids, such as ocimene quintoxide (RT = 27.35), epicedrol (RT = 42.68), and ledol (RT = 50.23), were identified in LF1, LF2, and HF (0.76%, 0.28%, and 0.97%, respectively). Additionally, ketones, such as 2-pentanone, 4-hydroxy-4-methyl- (RT = 12.42), were identified in LF1 and HF and acetophenone, 2', 4'-dimethoxy-3'-methyl- (RT = 28.84) in LF2.

4 Discussion

Several bacterial pathogens, such as *Staphylococcus epidermidis, Proteus mirabilis*, and *Salmonella* Typhimurium, are linked to gastrointestinal and urinary tract infections. While antibiotics are commonly used to treat particular pathogenic infections, novel antimicrobial agents from natural sources remain an urgent medical requirement. Nutraceutical sources, such as mushrooms, are a recent ongoing focus of research for production of efficient antimicrobial compounds. *H. erinaceus* is reported to inhibit a wide range of bacteria including *S. aureus*, *H. pylori*, *B. subtilis*, *E. coli*, *E. faecalis*, *P. aeruginosa*, and *S.* Typhimurium (Liu et al., 2016; Wong et al., 2009; Kim et al., 2012).

Results of the antimicrobial assay in our study showed that HMR extracts and fractions had poor inhibitory activity against *S. epidermidis* and *S.* Typhimurium but higher activity against *P. mirabilis*. An earlier study by Hood et al. (2003) suggests that the use of MIC in antimicrobial assays could generate more consistent results compared to agar diffusion due to difficulties in stable dispersion of compounds through the agar medium. HMR, LF1, and HF exerted low to moderate antibacterial effects (MIC = 500 g/mL) whereas LF2 showed potent antibacterial activity (MIC = 250 g/mL) against *P. mirabilis*.

The LF2 fraction had the highest fatty acid composition among all the samples (12.5%), which was potentially associated with its high antimicrobial activity. The highest contents of fatty acid derivative compounds in LF2 of 3.6%, 3.19% and 2.53% were obtained for nonanoic acid, 2-dodecen-1-yl(-) succinic anhydride, and linoleic acid ethyl ester, respectively. Nonanoic acid has been found in *Laetiporus sulphureus* mushroom as antimicrobial against *Micrococcus flavus* and *P. aeruginosa* (Petrović et al., 2013). Bactericidal 2-dodecen-1-yl(-) succinic anhydride has been identified in *Agaricus bisporus* mushroom and endophytic fungi *Fusarium fujikuroi* and *Aspergillus tubingensis* (Nisa et al., 2020; Mohamed, 2012) while linoleic acid ethyl ester has also been reported in *Ganoderma lipsiense*

Table 3. Main components of unfermented jasmine rice, *H. erinaceus* mycelia cultivated on mixed red and white jasmine rice, and active fractions.

RT (min)	Compound name	Peak area (%)				
		UR	HMR	LF1	LF2	HF
	Acids and derivatives					
17.17	Nonanoic acid			0.16	3.6	
20.55	3,4-Dimethylbenzoic acid	1.71	1.4	3.25	0.6	3.13
20.63	2-Pentenoic acid		0.1	0.47		
25.64	Lauryl acetate	0.26	0.18	0.62	0.16	0.75
36.83	Linoleic acid ethyl ester		1.28		2.53	
41.21	2-Dodecen-1-yl(-)succinic anhydride				3.19	
41.67	1-Adamantanecarboxylic acid, 2-tridecyl ester	0.14				
41.75	Fumaric acid, 2-dimethylaminoethyl nonyl ester	1.25				
46.84	Octacosyl acetate					1.68
	Alcohols					
33.37	1-Cyclohexene-1-ethanol, 2,6,6-trimethyl		0.35	0.4		
46.28	7,8-Epoxylanostan-11-ol, 3-acetoxy-					0.26
	Aldehydes					
4.19	Hexanal	1.11			0.58	
7.95	2-Heptenal, (Z)-	0.26				
12.27	Nonanal	0.28			0.13	
16.44	2-Decenal, (E)-	2.12				
18.39	2,4-Decadienal, (E,E)-	1.22				
19.62	2-Undecenal	0.62			0.2	
50.88	Benzaldehyde, 3-bromo-4-hydroxy-				0.17	
	Hydrocarbons					
4.132	2,4-Dimethylheptane	0.8				
19.31	Octadecane, 6-methyl-	0.13				
37.08	1-Chloromethyl-1-heptyloxy-1-silacyclohexane	0.16	0.06			
	Ketones					
10.29	3-Octen-2-one	0.19				
12.42	2-Pentanone, 4-hydroxy-4-methyl-			0.17		0.18
28.84	Acetophenone, 2',4'-dimethoxy-3'-methyl-				0.27	
	Oleamides					
46.59	9-Octadecenamide, (Z)-	0.15	0.26	0.31		
	Sterols					
51.15	Cholesterol	7.68				
51.75	Ethyl iso-allocholate		0.24			
52.21	Ergosterol		1.18			
52.53	Ergost-5-en-3-ol, (3.beta.)-	0.29				
52.88	Stigmasta-4,22-dien-3.beta.-ol	0.16				
53.76	.gamma.-Sitosterol	0.95				
	Sulfoxides					
4.81	Dimethyl Sulfoxide		45.89			0.47
	Terpenoids					
27.35	Ocimene quintoxide		0.81	0.76		
42.68	Epicedrol				0.28	
50.23	Ledol					0.97
	Others					
16.68	Caprolactam	0.64	0.56	1.65	0.14	0.2
18.22	Furan, tetrahydro-2,2,4,4-tetramethyl-		0.05	0.67		
25.91	Ethyl.alpha.-d-glucopyranoside		0.93			
26.06	1H-Indole-3-ethanamine, N-methyl-		0.14			
26.68	(3-Glycidoxypropyl)dimethylethoxysilane		0.28	0.34		
30.20	Methoxsalen		0.49	0.66		
44.76	1-Cyclohexyldimethylsilyloxybutane	0.14				

UR = unfermented jasmine rice; HMR = *H. erinaceus* mycelia cultivated on mixed red and white jasmine rice; LF = lipophilic fractions; HF = hydrophilic fraction.

mushroom grown on red rice as effective antimicrobial agents against *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Costa et al., 2020).

In general, all extracts and fractions contained 3,4-dimethylbenzoic acid (RT = 20.55) and lauryl acetate (RT = 25.64), as reported previously. Antimicrobial activities of fatty acid derivatives, e.g., 3,4-dimethylbenzoic acid, fumaric acid, 2-dimethylaminoethyl nonyl ester, and lauryl acetate, against *Salmonella* sp., *B. subtilis, E. coli, S. aureus, Sarcina* sp., *P. aeruginosa, L. monocytogenes*, and *Vibrio* species have been demonstrated by several research groups (Peixoto et al., 2017; Jatin & Priya, 2016; Marzlan et al., 2020), which may contribute to the antimicrobial effects of extracts.

The antimicrobial mechanisms of fatty acids and derivatives are not well understood due to their non-specific modes of action. The cell membrane is proposed as the primary site of antimicrobial activity where fatty acids disrupt the electron transport chain and oxidative phosphorylation to interfere with the cellular energy production of pathogens. In addition, fatty acids may inhibit enzyme activity, impair nutrient uptake, generate peroxidation and auto-oxidation degradation products, and cause direct bacterial cell lysis. Owing to their broad antimicrobial spectrum, fatty acids are suitable candidates as antibacterial agents for a variety of applications in medicine, agriculture, and food preservation, particularly in situations where the use of conventional antibiotics is undesirable or prohibited (Desbois & Smith, 2010).

Aside from fatty acid derivatives, other bioactive compounds, such as terpenoids, aldehydes, ketones, sterols and hydrocarbons, exert beneficial effects. LF2 additionally contained epicedrol (RT = 42.68) at a concentration of 0.28%. Antimicrobial activities of epicedrol against *E. coli, B. subtilis, B. cereus, M. flavus*, and *S. epidermidis* have been reported previously (Cavalli et al., 2003). Liposoluble compounds can alter the fluidity and decrease the barrier effect of bacterial cell membranes, allowing the passage of several compounds (Nunes et al., 2021), while volatile compounds, such as terpenoids, act by causing partitioning of the lipid layer of the bacterial cell membrane, thus enhancing its permeability (Schmidt et al., 2015). Accordingly, these compounds may synergistically exert inhibitory or killing effects against pathogenic bacteria.

In addition to the biological properties of HMR, anticancer activities were investigated in this study. UR, HMR, and all the fractions examined showed weak anticancer activity against HeLa cells (IC_{50} > 100 μg/mL). The low cytotoxic activity of the jasmine rice extract alone and *H. erinaceus* mycelia against HeLa cells has also been described in an earlier report (Uttama & Ittharat, 2010; Wang et al., 2015b). Therefore, improvement in the anticancer properties of mushroom extract could not be achieved with the SSF method.

In our experiments, LF1 and HF exerted the highest cytotoxic effects. The chemical profiles of both active fractions were nearly identical. LF1 and HF contained 3,4-dimethylbenzoic acid (RT = 20.55) at concentrations of 3.25% and 3.13%, lauryl acetate (RT = 25.64) at concentrations of 0.62% and 0.75%, and 2-pentanone at concentrations of 0.17% and 0.18%, respectively. Terpenoids, including ocimene quintoxide and ledol, were present in both LF1 and HF at concentrations of 0.76% and 0.93%, respectively.

Limited studies to date have focused on the utility of 3,4-dimethylbenzoic acid compounds as anticancer agents. Lauryl acetate was recently reported as an anticancer compound against MCF-7 breast cancer cells in *Calocybe indica* mushroom (Mohanasundaram et al., 2021). The anticancer mechanisms of action of fatty acids have been discussed by Jóźwiak et al. (2020), who suggest that fatty acids work by attenuating cellular proliferation and differentiation, inducing apoptosis, modifying membrane permeability by incorporating phospholipids into the cell membrane, and increasing substance transport throughout the cell membrane.

A previous study by Pettersson et al. (2008) showed that 2-pentanone contained in banana fruit (*Musa* sp.) inhibits prostaglandin (PGE2) production and cyclooxygenase-2 (COX-2) protein expression in tumor necrosis factor (TNF)-stimulated colon cancer cells (HT29). Low molecular weight compounds such as terpenoids exert anticancer effects by modulating several cellular signal transduction pathways (nuclear factor-B [NF-B] and mitogen activated protein kinase [MAPK]) and inhibiting a number of carcinogenic processes including differentiation, angiogenesis, carcinogenesis, and metastasis (Silva et al., 2012).

Overall, our results showed for the first time that bioactive compounds of HMR possess antibacterial activity against *P. mirabilis*. However, *H. erinaceus* mycelium exhibits low anticancer activity against HeLa cells. The potential presence of beneficial components of *H. erinaceus* mycelium with unknown bioactivities should be investigated in further studies.

5 Conclusion

The current study provides evidence of antimicrobial and anticancer activities along with bioactive components of *H. erinaceus* cultivated on mixed red and white jasmine rice. Our experiments revealed that *H. erinaceus* mycelia extracts have the ability to inhibit bacteria, particularly *P. mirabilis,* with moderate activity. Additionally, the fatty acid components of *H. erinaceus* mycelia and their derivatives may effectively serve as antibacterial compounds. In summary, bioactive compounds of *H. erinaceus* mycelia cultivated on mixed red and white jasmine rice display weak activity against HeLa cells and are therefore not suitable as anticancer agents but have potential as antibacterial candidates.

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