ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF MUSHROOMS FROM A COMMUNITY ENTERPRISE IN LAMPANG PROVINCE, THAILAND

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Pornanan Boonkorn[1], Sastra Ladpala[1], Angkhana Chuajedton[1], Metarin Somboon[1], Anukool Sroikum[1], Kanjanavadee Khumsem[1], Pimkamol Kunakham[1], Jumnian Meesumlee[2], Sarayut Malai[3] and Haruthai Thaisuchat[1*]

[1]Faculty of Science, Lampang Rajabhat University, Lampang, Thailand
[2]Faculty of Agricultural Technology, Lampang Rajabhat University, Lampang, Thailand
[3]Faculty of Industrial Technology, Lampang Rajabhat University, Lampang, Thailand
*corresponding author e-mail: haruth@g.lpru.ac.th

Abstract

The objectives of this research were to evaluate the antibacterial and antioxidant activities of ethanolic and aqueous extracts of five mushrooms cultivated by a community enterprise in Lampang Province, Thailand. Five mushrooms including *Lentinus edodes*, *Lentinus polychrous*, *Lentinus squarrosulus*, *Pleurotus ostreatus*, and *Dictyophora indusiata* were identified through ITS sequencing analysis. The ethanolic and aqueous extracts of these mushrooms were analyzed using the agar disc diffusion method and the DPPH radical scavenging activity assay, followed by statistical analysis using a one-way analysis of variance (ANOVA). Results showed that the ethanolic extract of *L. squarrosulus* exhibited the antibacterial activity against *Proteus mirabilis* DMST8212, *Enterobacter aerogenes* DMST8841, and *Salmonella typhimurium* DMST562, with clear zone diameters of 12.83 mm, 6.83 mm, and 6.00 mm, respectively. The growth of *Staphylococcus aureus* DMST8840 was inhibited by the ethanolic extracts of *P. ostreatus* and *L. edodes* with clear zone diameters of 6.67 mm and 6.00 mm, respectively. The ethanolic extract of *L. polychrous* inhibited *P. mirabilis* growth, resulting in a clear zone diameter of 11.33 mm. Antioxidant activity varied among species. The highest DPPH radical-scavenging activity was observed in ethanolic *L. edodes* and aqueous *L. polychrous* extracts at 10 mg/ml, with %DPPH reduction values of 92.23% and 96.69% respectively. The IC_{50} values ranged from 4.951 to 7.952 mg/ml for ethanolic extracts, showing significant differences, and from 0.003 to 0.014 mg/ml for aqueous extracts, showing no significant differences. These findings highlight the potential of these mushrooms as natural antibacterial agents and antioxidants, offering opportunities for community enterprises to enhance income and sustainability through value-added products like dietary supplements, medicines, and cosmetics.

Keywords: Edible mushroom, Antibacterial activity, Antioxidant activity, Pathogenic bacteria, DPPH assay

Introduction

Mushrooms are valuable organisms belongs to the kingdom of Fungi. They play a crucial role in ecosystems by acting as natural decomposers and facilitating the cycling of carbon and nutrients. In addition to their environmental importance, mushrooms are an excellent source of food and offer health benefits for humans. Mushroom basidiocarps are typically low in calories but rich in protein and fiber. They contain a wide range of beneficial bioactive substances, providing health-promoting and medicinal properties, including antioxidant, antimicrobial, anticancer, anti-inflammatory, cholesterol lowering and immunostimulatory effects (Kosanić et al., 2012, p.1095; Valverde et al., 2015; Gupta et al., 2019, p.1815; Niazi & Ghafoor, 2021, pp.450-452).

Macromycetes are interestingly an alternative resource of antibiotics to microorganisms. Various both cultured and wild mushrooms have been estimated for their capability to inhibit human pathogenic bacteria especially food-borne bacteria including *Escherichia coli*, *Bacillus cereus*, *Enterobacter aerogenes*, *Micrococcus luteus*, *Proteus vulgaris*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. The ethanolic and water-ethanolic extracts of *P. ostreatus* exhibited high levels of antibacterial activity against *S. aureus*, *Listeria innocua*, *E. coli*, and *S. typhimurium* (Torres-Martínez et al., 2021, p.5). The hydroethanolic extracts of *L. edodes* had a bactericidal effect on *S. aureus*, *Acinetobacter baumannii*, and *E. coli*, with the minimum bactericidal concentration (MBC) equal to the minimum inhibitory concentration (MIC) (20 mg/ml), while *P. ostreatus* only affected *E. coli* at the same level (Machado-Carvalho et al., 2023). The *L. edodes* extract efficiently inhibited all four tested bacteria (*B. cereus*, *S. aureus*, *S. enteritidis*, and *E. coli*) with MIC values of 12.50 mg/ml, 1.56 mg/ml, 100 mg/ml, and 100 mg/ml, respectively (Bach et al., 2019, p.219).

One extensively studied property of mushrooms is their antioxidant activity, which is considered for the utilization of mushrooms in medicinal and cosmetic applications. Three extraction solvents, including aqueous, ethanol, and aqueous-ethanol, had no differing effects on the antioxidant activity of *P. ostreatus* extracts, with DPPH reduction percentages of 89.1%, 91.4%, and 91.1%, respectively (Torres-Martínez et al., 2021, p.6). The ethanolic extract of *P. ostreatus* exhibited antioxidant activity by scavenging free radical DPPH by 21.43% at a concentration of 100 ppm (Egra et al., 2019, p.17). The extract of *L. edodes* presented the strongest DPPH radical scavenging activity (53.90%, and 64.34% at 500 ug/ml for aqueous and 50% ethanol, respectively) compared to three other mushrooms (Boonsong et al., 2016, p.93). The crude polysaccharides extracted from *L. edodes* using hot water exhibited radical scavenging ability with an EC50 value of 0.51 ± 0.05 mg/ml, which was lower compared to a previous report (Muñoz-Castiblanco et al., 2022).

The increasing demand for mushrooms as a health food has led to expanded mushroom production. Among the most widely cultivated mushroom species are *Agaricus bisporus* (J.E. Lange) Imbach, *Lentinus edodes* (Berk.) Pegler, and *Pleurotus* spp. (Rathore et al., 2017, p.36). In Lampang Province, Thailand, there are over 30 community enterprises involved in mushroom production. The commonly cultivated mushrooms in these enterprises include *L. edodes* (Berk.) Pegler, *Lentinus polychrous* Lév, *Lentinus squarrosulus* Mont., *Pleurotus ostreatus* (Jacq.) P. and the newly

popular *Dictyophora indusiata* (Vent. Ex Pers.) Fish Phallaceae. Bioactive compounds in mushrooms and their properties are not only dependent on the mushroom species but may also be influenced by environmental factors and other variables such as the substrate, growing conditions, maintenance and harvesting methods (Asri et al., 2019, p.516; Mkhize et al., 2022, pp.1-2). Research from various countries has shown differences in the properties of the same mushrooms. Therefore, mushrooms that are popularly cultivated and consumed in each region should be subjected to bioactivity analysis. The results of this study offer fundamental data regarding future investigations and product development involving mushrooms for the purpose of promoting local economic growth.

Research objective

To evaluate the antibacterial and antioxidant activities of ethanolic and aqueous extracts of five mushrooms cultivated by a community enterprise in Lampang Province, Thailand.

Research methodology

Mushroom samples

The mushroom samples used in the study were obtained from a community enterprise in Lampang Province, where they are a popular and regularly produced mushroom variety. Samples were collected between June and November 2022, targeting both immature and mature fruiting bodies, which were at a generally suitable growth stage for consumption. The sources of the mushrooms are shown in Table 1.

Table 1 Sources of mushrooms

Mushroom Species	Common name / Local name	Source	Latitude Longitude
Lentinus edodes (Berk.) Pegler	Shiitake / Hed Hom	Ban Pang Ma-O Shiitake mushroom group community enterprise, Mae Ta district	18°06'14.9"N 99°38'12.6"E
Lentinus polychrous Lév	- / Hed Lom	Ban Wiang Hong greenhouse mushroom cultivation community enterprise, Mae Moh district	18°24'51.5"N 99°51'34.7"E
Lentinus squarrosulus Mont.	- / Hed Khon Khao	Ban Wiang Hong greenhouse mushroom cultivation community enterprise, Mae Moh district	18°24'51.5"N 99°51'34.7"E
Pleurotus ostreatus (Jacq.) P.	oyster mushroom / Hed Nang Rom	Ban Rong mushroom farming group community enterprise, Ngao district	19°00'08.5"N 99°47'43.5"E

Mushroom Species	Common name / Local name	Source	Latitude Longitude
Dictyophora indusiata (Vent ex. Pers.) Fischer	bamboo mushroom / Hed Yua Phai	Ban Kor Ruak bamboo mushroom group community enterprises, Mae Moh district	18°24'51.5"N 99°51'34.7"E

Internal transcribed spacer (ITS) sequencing for mushroom identification

Molecular identification was conducted using the internal transcribed spacer (ITS) genetic region. Fungal DNA was extracted from approximately 0.1 g of the fruiting body stem using the RBC genomic DNA extraction kit (RBC Bioscience Corp., New Taipei City, Taiwan). The extracted DNA served as a template for PCR amplification, employing Excel Taq 5X PCR Master Dye Mix (SMOBIO Technology, Inc., Hsinchu City, Taiwan) and two universal primers, ITS3 (5'-gCATCgATgAAgAACgCAgC-3') and ITS4 (5'-TCCTCCgCTTATTgATATgC-3') (Thaisuchat et al., 2023, p.541). The PCR reaction was carried out in 25 μl containing 5 μl 5X master mix, 1-3 μl template DNA (100 ng), 1.25 μl of each 10 μM forward (ITS3) and reverse (ITS4) primer and top up with distilled water to reach a total volume of 25 μl. The thermocycling parameters for amplification were 2 minutes at 94 °C for the initial denaturation of template DNA, 30 amplification cycles (30 seconds at 94 °C, 30 seconds at 50-55 °C, and 20 seconds at 72 °C), and 1 minute at 72 °C for the final extension, following the guidelines provided by the manufacturer. The amplified ITS DNA fragments, approximately 400 bp in size, were determined using 1.2% agarose gel electrophoresis. Subsequently, they were purified and sequenced bidirectionally by Macrogen Inc. (Seoul, Korea). The obtained ITS sequences were aligned with known nucleotide sequences in the National Center for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov/) using the BLASTN search option.

Crude extract preparation

Ethanol (80%) and sterilized distilled water were used as solvents for mushroom extraction. The fruiting bodies were dried in an incubator at 45 °C for 12 hours before being sliced into small pieces. Five hundred grams of sliced mushrooms were immersed in a solvent at a ratio of 1:2 (w/v) at 4 °C for 7 days. Subsequently, the solution was filtered with Whatman No. 1 filter paper, and the filtrate was collected for further evaporation. The mushroom extract soaked in ethanol was evaporated using a rotary evaporator (Rotavapor R-100, BÜCHI Labortechnik AG, Flawil, Switzerland) at 40-50 °C until a dry crude extract was obtained. The mushroom soaked in distilled water was evaporated using an incubator at 50-60 °C until a dry crude extract was achieved. Ethanolic and aqueous extracts of 5 mushrooms were diluted to a concentration of 250 mg/ml with 80% ethanol and distilled water, respectively. They were stored at 4 °C before being used directly in the agar disc diffusion method or as a stock for dilution in the free radical scavenging assay.

Agar disc diffusion method for antibacterial activity assessment

The antibacterial activity of mushroom extracts was assessed against six pathogenic bacteria using the agar disc diffusion method described by Avcı et al. (2014, p. 431) with some modifications. *Escherichia coli* O157: H7 (DMST12743), *Staphylococcus aureus* (DMST8840), *Enterobacter aerogenes* (DMST8841), *Shigella flexneri* (DMST4423), *Proteus mirabilis* (DMST8212) and *Salmonella typhimurium* (DMST562) were cultivated in tryptic soy broth at 37 °C overnight. They were subsequently subcultured into Mueller-Hinton broth and incubated at 37 °C for 2-4 hours to achieve a concentration of $1x10^7$ CFU/ml, determined by measuring the optical density at 600 nm (OD_{600}). A sterile cotton swab was aseptically used to transfer the bacteria onto Mueller-Hinton agar plate. Sterile paper discs (5 mm in diameter) were then saturated with either 10 µl of mushroom extracts or a control solution and placed on the inoculated media. Chloramphenicol (CHL; 10 µg/µl) and the solvent used for diluting mushroom crude extract (80% ethanol and distilled water) were used as positive and negative control, respectively. The Petri dishes were incubated at 37 °C for 24 hours. The experiment was carried out in triplicate, and the radius of the clear inhibition zone was measured and recorded in millimeters.

DPPH free radical scavenging (DPPH) assay for antioxidant activity assessment

The DPPH radical scavenging assay, as described by Avcı et al. (2014, p. 430) with some modifications, was carried out to assess the antioxidant activity of mushrooms. All mushroom extracts were prepared using a 5-fold serial dilution, starting from 5 mg/ml and reaching a final concentration of 0.04 mg/ml in separate 10 ml tubes. For each extract, including the 10 mg/ml stock solution, 1.5 ml was mixed with an equal volume of a 0.1 mM DPPH (1,1-diphenyl-2-picrylhydrazyl) solution in ethanol. The mixture was vigorously shaken and allowed to stand in the dark for 30 minutes. The absorbance of the mixture was measured at 517 nm using a UV-visible spectrophotometer (Specord 50 Plus, Analytik Jena AG, Jena, Germany). Ethanol served as the blank, while ascorbic acid was used as the standard. The ability to scavenge the DPPH radical was calculated using the following formula:

$$\%\text{DPPH reduction} = (Ac - As/Ac) \times 100$$

Where Ac and As represent the absorbance of the control reaction (containing all reagents except the extract sample) and the absorbance of the extract sample, respectively. The IC_{50} value (mg/ml) of DPPH scavenging activity was determined at the concentration where the extract showed 50% scavenging activity against DPPH, derived from the standard curve plotted between %DPPH reduction and the concentration. Ascorbic acid was used as a standard to compare the free radical scavenging capability of the sample.

Statistical analysis

The experimental configuration followed a completely randomized design (CRD) methodology. A one-way analysis of variance (ANOVA) was performed on the data using the SPSS software (version 29). With a confidence interval of 95%, significant differences between treatments were ascertained utilizing Duncan's multiple range tests.

Results

Mushroom identification

PCR yielded the ITS sequences of five mushrooms with a size range of 300-450 bp. With percentage similarity of 100%, 100%, 98%, 100%, and 95% match on GenBank, they were identified as *Lentinus edodes* (MN622792.1), *Lentinus polychrous* (KP283487.1), *Lentinus squarrosulus* (OR826100.1), *Pleurotus ostreatus* (OQ947058.1), and *Dictyophora indusiata* (HQ414538.1), respectively.

Mushroom extract yield (%)

The highest extract yield was found in *L. edodes* at percentages of 2.50 and 2.68 from ethanol and aqueous extraction, respectively. The ethanolic extract yields of the other four species ranged from 0.48% to 0.59%, while their aqueous extract yields ranged from 0.95% to 1.34%. Aqueous extraction methods for all mushrooms yielded higher dry extracts than ethanol extraction.

Antibacterial activity of mushroom extract

The ethanolic extract of *L. squarrosulus* exhibited the best antibacterial activity against *E. aerogenes* (6.83 mm), *P. mirabilis* (12.83 mm) and *S. typhimurium* (6.00 mm) (Table 2). The *L. edodes* and *P. ostreatus* extracts only inhibited one bacterial species, *S. aureus*, with clear zone of 6.00 and 6.67 mm, respectively. *L. polychrous* demonstrated a slightly lower zone of inhibition against *P. mirabilis* (11.33 mm) compared to *L. squarrosulus*. *E. coli* O157:H7 and *S. flexneri* showed no sensitivity to any of the tested extracts. None of the aqueous extracts from the mushrooms showed antibacterial activity against the tested bacterial species.

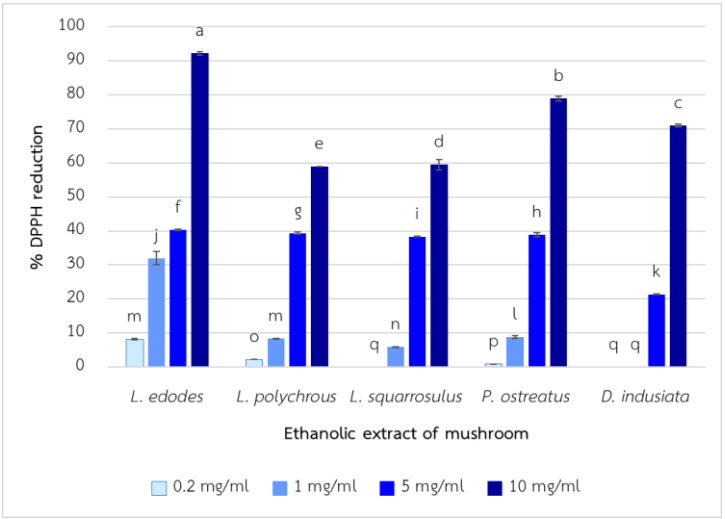
Table 2 Inhibition zone (mm) of ethanolic extracts of mushrooms against pathogenic bacteria

Mushroom /Control	*E. coli* O157:H7	*S. aureus*	*E. aerogenes*	*S. flexneri*	*P. mirabilis*	*S. typhimurium*
L. edodes	ni	6.00±0.41^{f}	ni	ni	ni	ni
L. polychrous	ni	ni	ni	ni	11.33±1.25^{c}	ni
L. squarrosulus	ni	ni	6.83±0.47^{e}	ni	12.83±0.47^{b}	6.00±0.00^{f}
P. ostreatus	ni	6.67±0.24^{ef}	ni	ni	ni	ni
D. indusiata	ni	ni	ni	ni	ni	ni
Solvent (C-)	ni	ni	ni	ni	ni	ni
CHL (C+)	12.67±0.85^{b}	12.33±0.62^{b}	10.17±0.47^{d}	7.17±0.24^{e}	6.83±0.62^{e}	17.00±0.82^{a}

ni = no inhibition found; Values with the same letters are not statistically different (P>0.05) according to Duncan's multiple range test.

Antioxidant activity of mushroom extract

All mushroom extracts exhibited antioxidant activity, and the DPPH free radicals were more effectively scavenged at higher extract concentrations. At the highest concentration tested (10 mg/ml), the ethanolic and aqueous extracts of mushrooms showed %DPPH reduction within the range of 59.01-92.23% and 72.02-96.69%, respectively (Fig 1). The most potential antioxidant activity was observed in the ethanolic extract of *L. edodes* following by *P. ostreatus*, *D. indusiata*, *L. squarrosulus* and *L. polychrous*. The aqueous extract of *L. polychrous* exhibited the highest antioxidant activity, followed by *D. indusiata*, *L. edodes*, *P. ostreatus* and *L. squarrosulus*. The IC_{50} values varied from 4.951-7.952 mg/ml and from 0.003-0.014 mg/ml for ethanolic and aqueous extracts, respectively (Table 3). Among the mushroom samples tested, significant different in IC_{50} values were found in the ethanolic extract but not in the aqueous extract.



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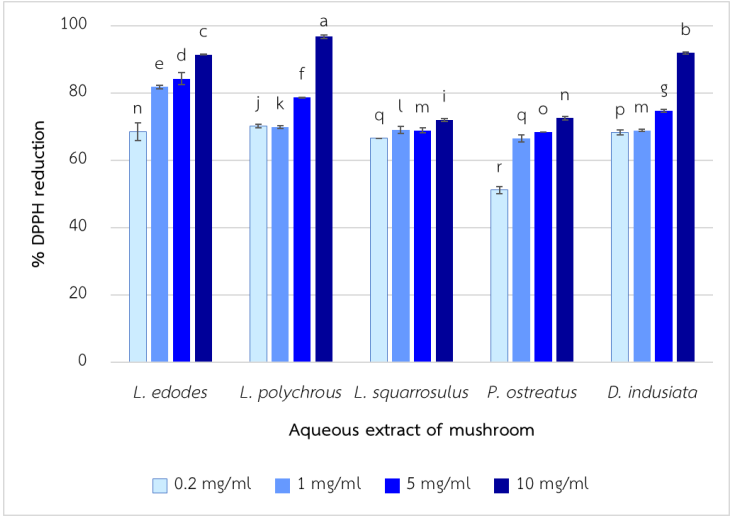

B

Fig 1 The DPPH free radical scavenging activity of ethanolic (A) and aqueous (B) extracts of mushrooms Error bars present means and SD, respectively, from triplicate samples. Values with the same letters are not significantly different (P>0.05) according to Duncan's multiple range test.

Table 3 The IC_{50} values (mg/ml) against DPPH of ethanolic and aqueous extract of mushrooms

Mushroom /Standard	Ethanolic extract	Aqueous extract
L. edodes	4.951 ± 0.01^{e}	0.004 ± 0.00^{h}
L. polychrous	7.932 ± 0.00^{b}	0.003 ± 0.00^{h}
L. squarrosulus	7.952 ± 0.01^{a}	0.012 ± 0.00^{h}
P. ostreatus	6.318 ± 0.01^{d}	0.014 ± 0.00^{h}
D. indusiata	7.745 ± 0.01^{c}	0.004 ± 0.00^{h}
Ascorbic acid	3.350 ± 0.01^{f}	0.057 ± 0.00^{g}

Values with the same letters are not statistically different (P>0.05) according to Duncan's multiple range test.

Discussion

Aqueous extracts of all mushrooms consistently yielded higher results compared to ethanol solvents. This may be attributed to the higher content of polar components such as proteins, as well as essential amino acids, in the mushrooms (Al Azad and Ai Ping, 2021, p.291).

Lentinus mushroom have been shown to possess antibacterial properties. Extracts from two species of *Lentinus* mushroom exhibited promising antibacterial activities against four pathogenic bacteria: *B. subtilis*, *E. coli*, *Pseudomonas aeruginosa* and *S. aureus*. The methanolic extract of the mycelium showed a higher inhibition zone at the highest concentration (50 mg/ml) against *B. subtilis* (20.33 mm) and *S. aureus* (14.16 mm) (Dutta et al., 2023). Sutthisa and Chaiyacham (2022, p.444) reported that ethanolic extract of *L. squarrosulus* mycelia was effectively inhibited *B.*

cereus at the highest concentration. However, the basidiocarp extract, at all concentrations, was unable to inhibit the growth of *E. coli*, *S. aureus*, *Bacilllus cereus* and *P. aeruginosa*. *L. edodes* extract demonstrated greater efficiency against all tested bacteria, particularly gram-positive bacteria (Bach et al., 2019, pp. 218-219). This finding aligns with a review by Alves et al. (2012, p.1707), which reported *L. edodes* broad antimicrobial activity against both gram-positive and gram-negative bacteria. However, it contrasts with the results of this study where only *S. aureus* was susceptible to *L. edodes*. *L. edodes* extract has been reported to exhibit excellent antibacterial activity against Gram-positive bacteria such as *S. aureus* and *B. subtilis*, but not against *E. coli*. Inhibiting Gram-negative bacteria poses a challenge for different extracts because they contain lipopolysaccharide components on the outer peptidoglycan layer, making the cell walls water-resistant to lipophilic solutes (Dutta et al., 2023).

In the current study, *P. ostreatus* exhibited weak inhibition, primarily against *S. aureus*. This contrasts with the findings of Chaiharn et al. (2018, p.1720), who reported that the ethanolic extract of *P. ostreatus* displayed antibacterial activity against several strains, including *B. cereus*, *E. aerogenes*, *P. vulgaris* and *S. typhimurium*, but not *S. aureus* and *E. coli* using the disc diffusion method. The growth of *S. typhimurium*, *S. aureus*, *P. aeruginosa*, *E. coli*, and *B. cereus* was inhibited by the methanolic extract of *P. ostreatus* (Fogarasi et al., 2020, p.6). Brazkova et al. (2022) revealed that pleuran, the most studied polysaccharides, specifically the water-insoluble (1–3)-β-d-glucan obtained from *P. ostreatus*, exhibit numerous medicinal activities, including antibacterial properties.

D. indusiata has been previously reported to possess numerous biological activities and has found extensive use in traditional medicine, functional foods, and skincare products. The hot aqueous extract from *D. indusiata* exhibited the ability to inhibit the growth of both bacteria and fungi used as indicator organisms, demonstrating a broad-spectrum antimicrobial activity at a concentration of 200 mg/ml (Oyetayo et al., 2009, p.25). However, in the present study, *D. indusiata* did not show any antibacterial activity.

Mushrooms contain numerous natural active agents against gram-positive and gram-negative bacteria. Antimicrobial compounds from mushrooms have been reported such as proteins and peptides (plectasin and peptaibol), terpenes and sesquiterpenes, steroids, organic acid (oxalic acid and gallic acid) benzoic acid derivatives, ribonuclease, fraction B (Alves et al., 2012, p. 1712; Bach et al., 2019, p.219; Huguet et al., 2022). While mushrooms contain antimicrobial compounds, the efficacy of mushroom extracts in inhibiting microorganisms may depend on various factors, such as mushroom species, mushroom parts used, mushroom cultivation, extraction methods, testing methods, and the evaluation of test results (Asri et al., 2019, p. 516; Mkhize et al., 2022, pp.1-2).

No antibacterial activity was detected in the aqueous extracts of all mushrooms. Similarly, previous research has shown that aqueous extracts of both wild edible and non-edible mushrooms

exhibited the lowest level of antibacterial activity, whereas ethanolic extracts demonstrated the strongest activity. It has been suggested that the active compounds within mushrooms may dissolve more effectively in organic solvents than in aqueous solvents (Reid et al., 2016, p.983). In contrast, the hot water extraction of wild mushrooms displayed better antimicrobial activity against all tested organisms when compared to 70% ethanol and chloroform extractions (Gebreyohannes et al., 2019). The aqueous extract of *P. ostreatus* inhibited the growth of *B. cereus* with an MIC value of less than 170 mg/ml (Aramsirirujiwet et al., 2016, p.595). The aqueous extract of *P. squarrosolus* showed the highest antimicrobial activity against *S. aureus* when compared with ethanol (Nwachukwu and Uzoeto, 2010, p. 2463). Although there are variations in the antibacterial activity of mushrooms extracted using aqueous methods, water is the preferred solvent since it is readily available, inexpensive, and non-toxic.

The antioxidant activity of four *Lentinus* mushrooms in Thailand was investigated by Rungprom (2018, pp. 75-80). The study revealed that the ethanolic extracts of *L. edodes*, *L. polychrous* and *L. squarrosulus* (at a concentration of 10 mg/ml) exhibited % DPPH inhibition levels of approximately 35%, 65% and 81%, respectively. Furthermore, the metnanolic extracts of *L. edodes* and *L. squarrosulus* mycelium, at a concentration of 0.5 mg/ml, displayed the highest % DPPH reduction at 53.37 and 70.37 %, respectively (Jiamworanunkul, 2019, p.216). These findings did not align with the results of this study, where *L. edodes* showed higher activity compared to the other *Lentinus* mushroom. However, *L. edodes* demonstrated high levels of antioxidant components, and *L. edodes* crude polysaccharides was confirmed as a source of natural antioxidants, as reported by Boonsong et al., (2016, p. 89) and Muñoz-Castiblanco et al. (2022), respectively.

L. edodes displayed a higher antioxidant capacity when compared to *P. ostreatus*. In another study, the ethanolic extract of *P. ostreatus* exhibited the lowest antioxidant activity among the four tested mushrooms, even lower than that of *L. edodes* (Machado-Carvalho et al., 2023).

The aqueous extract of *D. indusiata*, at concentrations ranging from 0.2-10 mg/ml exhibited DPPH reduction ability of 68.54% to 91.39%. These findings align with a previous study that reported that a hot water extract of *D. indusiata*, at concentration from 0.0625 to 2 mg/ml, exhibited scavenging abilities ranging from 18.12% to 97.58% (Oyetayo et al., 2009, p.22). For the extraction of *D. indusiata* polysaccharides, ultrasonic-microwave-assisted extracted was suggested due to its superior comprehensive antioxidant capacity when compared to hot water, microwave, or ultrasonic extraction methods (Zhang et al., 2023).

Antioxidant activity varies among mushrooms, likely influenced by multiple factors that affect the production of active compounds and their potency. The species of mushroom and the type of liquid medium demonstrated dominant effects in the antioxidant activity of the extracts (Jiamworanunkul, 2019, p.217). The type and composition of growing substrates may also influence

the bioactive compounds found within mushrooms, thus affecting their medicinal properties (Mkhize et al., 2022). Boonsong et al. (2016, pp.89-96) reported that the choice of extractants and the types of mushroom samples clearly influenced their antioxidant constitution, resulting in divergent antioxidant effects. They found that 50 % (v/v) ethanol was the most suitable for antioxidant extraction from the mushroom samples. However, the results of antioxidant activities, as assessed by the DPPH assay, ABTS assay, and ferricyanide/prussian blue method, showed no significant differences between extraction solvents (aqueous, ethanol, and aqueous-ethanol) analyzed in *P. ostreatus* extracts (Torres-Martínez et al., 2021, p.6). Elbatrawy et al. (2015, p.471) revealed that the aqueous extracts of mushrooms exhibited the highest percentage of DPPH scavenging activity compared to all other solvent extracts (ethanol, ethyl acetate, acetone, chloroform, hexane, and petroleum ether). Additionally, *L. edodes* exhibited strong DPPH radical-scavenging activity in aqueous extraction, confirming that a water-based extraction protocol was sufficient to assess the inhibitory effects (Finimundy et al., 2013, p.76). In our study, the aqueous extracts of all mushrooms showed higher antioxidant activity than the ethanolic extracts, especially at lower concentrations.

Mushroom components renowned for their potent antioxidant properties include phenolics, flavonoids, glycosides, polysaccharides, tocopherols, ergothioneine, carotenoids, and ascorbic acid (Chun et al., 2021). Bioactive compounds such as gallic acid, protocatechuic acid, catechin, and tocopherols were found in *L. edodes*, while ß-carotene, lycopene, and flavonoids were found in *L. squarrolosus*. Various antioxidant compounds, including ß-glucans, gallic acid, homogentisic acid, naringin, myricetin, tocopherols, glycoproteins, ß-D-glucan (pleuran), and lectin, were reported from *P. ostreatus* (Sánchez, 2017, pp.18-19). Most of the bioactive compounds in mushrooms can be extracted using polar solvents, primarily aqueous and ethanol. Therefore, the use of both types of solvents is commonly reported in a significant number of research studies.

Conclusion and suggestions

The ethanolic extracts of *L. edodes*, *L. polychrous*, *L. squarrosulus* and *P. ostreatus* displayed antibacterial activity against at least one of the tested pathogenic bacteria. However, both ethanolic and aqueous extract of *D. indusiata*, along with aqueous extracts from the other mushrooms, did not inhibit any of the tested bacteria. Furthermore, all mushroom extracts exhibited potential DPPH free radical scavenging activity at varying level, ranging from 59.01% to 96.69%. Notably, *L. edodes* demonstrated superior antioxidant activity in comparison to the other mushrooms tested, considering ethanol extraction.

Recommendation for using to benefit

The bioactive properties of each analyzed mushroom species can be used by community enterprises as supporting data for marketing. This information can be used to develop other products such as dietary supplements, medicines, and cosmetics to create added value.

Recommendation for future research

Alternative methods for confirming the antioxidant activity of mushroom substances should be considered. Solvents and extraction procedures that facilitate easy extraction by community members should be investigated, including methods that are simple to prepare and safe for both extraction and subsequent use in product development. Additionally, the effectiveness of the active compounds in mushrooms is influenced by various factors. Therefore, further testing should be conducted, including experiments with mushrooms grown using different substrates, various mushroom parts or developmental stages, and employing different extraction and evaluation methods, to maximize the potential benefits of mushrooms.

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