

# Antibacterial, Antioxidant and Anti-Inflammatory Effect with GC-MS Profile of Edible Mushroom Fermented by LAB

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## ABSTRACT

**Background:** Nutritional modifications arise from an enhanced understanding of the importance of food and its impact on human well-being. **Objectives:** This study investigates the fermentation-based preservation of edible mushrooms using *Lactobacillus* sp. **Methods:** The research examines the effects of LAB-mediated fermentation on edible mushrooms and evaluates their bioactive potential. **Results:** The findings indicate that the extract of *Agaricus bisporus* exhibits superior properties compared to *Coriolus versicolor*. Nevertheless, both fermented mushrooms displayed significantly lower metal chelating capacity than DPPH radical inhibition efficiency. *Agaricus bisporus* showed greater antioxidant activity than *Calocybe indica*, but exhibited lower anti-inflammatory properties. The analyzed LAB effectively fermented *Agaricus bisporus*, preserving the mushroom and sustaining its biological activity, as evidenced by the identification of more than 30 different volatile compounds through gas chromatography. **Conclusion:** The results suggest that LAB-dependent fermentation of *Agaricus bisporus* holds promising medicinal value, exhibiting higher antioxidants, antibacterial, and anti-inflammatory properties compared to non-fermented mushrooms. This study highlights the potential of edible mushrooms as sources of antibacterial, antioxidant, and anti-inflammatory compounds, indicating the necessity for further assays to evaluate fermented fungal compounds as safe alternatives to combat antibiotic resistance.

**Keywords:** Fungi, antioxidant, *Lactobacillus*, Mushroom, Phenol, DPPH.

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## INTRODUCTION

Around the world, people create and eat fermented food, with probiotic bacteria probably the most prevalent type of fermentation. Because of their distinct flavor and its universal recognition as a cuisine marvel, mushrooms are used all over the world (Bakratsas *et al.*, 2021). In addition to their organoleptic quality, therapeutic qualities, and socioeconomic relevance, mushrooms are regarded as a delicacy with high nutritional and functional value and are also recognized as nutraceutical foods. In recognition of its anticancer properties, lactic fermentation has long been utilized in many parts of the world for maintaining the fruiting bodies of edible mushrooms (Atila *et al.*, 2021). The *Agaricus bisporus* mushroom is among the most widely grown in the globe (Ramos *et al.*, 2019). Diverse antimicrobial substances, primarily secondary metabolites along with certain primary metabolites as oxalic acid, peptides, and proteins, may be found in mushrooms. The most extensively researched species, *Lentinus edodes*, appears to exhibit antibacterial properties

against both bacteria and fungi (Ergonul *et al.*, 2013). They also guard towards inflammation and the growth of tumors. The most significant polysaccharide in contemporary medicine is glucan, a highly well-known and adaptable metabolite with a broad range of biological activities (Alves *et al.*, 2012). In addition to a variety of amino acids, dietary mushrooms are a good source of riboflavin (vitamin B2), niacin, folates and trace amounts of vitamin C, B1, B12, D, and E. (Pereira *et al.*, 2012) Strong immune boosters have been discovered in a protein from *A. polytricha* and a lectin from *A. bisporus*. It has been demonstrated that *A. bisporus* extract stops breast cancer cells from proliferating (Ferreira *et al.*, 2010; Jabłońska *et al.*, 2019). Although lactic acid fermentation of mushrooms is common in Southeast Asia and Eastern Europe, it is not commonly used in industry. Therefore, the purpose of this work is to investigate the *in vitro* antioxidant and antibacterial activity of the acetone and methanol extract of the *Russula cyanoxantha*, *Lactarius piperatus*, *Amanita rubescens*, and *Cantharellus cibarius* mushrooms (Kosanić *et al.*, 2016).

## MATERIALS AND METHODS

### Raw material processing

Experimental study on preservation of produced edible mushrooms has focused on improving the procedure thoroughly



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using curd samples. Commercially procured *Agaricus bisporus* and *Calocybe indica* were the two types of mushrooms used in these investigations. Typically, only the mushroom caps are utilized for lactic fermentation; the stems are thrown away. The edible parts of the mushrooms used in this study were chopped into short pieces, measuring between 3.5 and 4.5 cm and then blanched in boiling water for 60 sec. For fermentation, just about 25 g of stems and fruiting bodies were used. When a washing procedure is done correctly, dirt can be removed during dry cleaning, and surface microorganisms can be partially reduced (Liu et al., 2016).

### Starter cultures

The majority of investigations on fermented mushrooms used starter cultures of Lactic Acid Bacteria (LAB), which are obtained from curd samples on MRS agar, to carry out lactic fermentation. The curd sample was serially diluted and plated on MRS agar and subsequently subcultured on MRS broth. The cell pellet was collected by centrifugation and the OD at 600 nm was adjusted to 1 using phosphate buffer.

### Fermentation

In a sterile vial, 50 g of edible mushroom parts are combined with 5 mL of LAB at  $1 \times 10^{-8}$  CFU sample and thoroughly mixed. It is advised to use the raw material in a 1:2 ratio to the brine that contains 2% salt and 1% sucrose in this about 25 mL sterilized brine solution. The samples, which were stored at 30°C for 25 days (Bernas and Jaworska, 2012).

### Extraction

10 mL of the sample were obtained, and it went through a centrifuge for 15 min at 10,000 rpm. After 3 hr, the clear aqueous phase was gathered and extracted using 10 mL of ethanol. Following a 10-min centrifugation at 5000 rpm, the supernatant was filtered through Whatman filter paper No. 2, dried in a Rotary Evaporator, and then reconstituted in 1 mg/mL of double-distilled water.

### Total Antioxidant activity

The antioxidant capability of the isolated biological compound assessed using 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) at a concentration of 1 mg/mL. A 100 µg quantity of ascorbic acid served as the evaluation's standard. 0.2 mL of DPPH reagent was incorporated to 2 mL of ethanol solution in a test tube, and everything was thoroughly mixed. The reaction tube filled with 0.1 mL of the fermented aqueous sample. After that, the tube was covered with aluminum foil and allowed to sit at room temperature for 20 min and OD recorded at 517 nm, absorbance was measured against ethanol blank. The percentage was calculated by equation 1

$$\text{Eq1. DPPH scavenging} = \frac{(\text{OD of control} - \text{OD of test})}{\text{OD of control}} \times 100$$

### Metal Chelating Activity

Metal chelating activity carried out on the extract's by mixing 1 mL of ethanol with 100 µL of the recovered fermented mushroom with 50 µL of 2 mM ferrous chloride, 0.2 mL of 5 mM ferrozine was added to initiate the reaction. After 10 min in a dark environment, absorbance at 562 nm was then measured. EDTA is used as standard. For the control, distilled water was utilized. The equation that follows was used to determine the metal chelating efficiency.

$$\text{Eq2. Inhibition (\%)} = (1 - \frac{A_{562}(\text{extract})}{A_{562}(\text{control})}) \times 100$$

### The anti-inflammatory protein denaturation

The extract's anti-inflammatory properties were investigated using the protein denaturation prevention technique at a concentration of 1 mg/mL. 0.1 mL of egg albumin, 2.8 mL of phosphate buffered saline (pH: 6.4), and 0.1 mL of fermented extracts at different concentration was prepared. PBS used as a negative control and dichlofenac used as standard drug. After 15 min of incubation at  $37 \pm 2^\circ\text{C}$ , the mixtures were heated to  $70^\circ\text{C}$  for 5 min. After cooling, the vehicle was used as a blank to measure their absorbance at 660 nm. The following formula was used to determine the percentage inhibition of protein denaturation.

$$\text{Eq3. Percentage of anti-inflammatory} = \frac{(\text{OD of Control} - \text{OD of sample})}{\text{OD of Control}} \times 100$$

### Antibacterial activity

100 µg of both fermented and crude mushroom extracts were placed onto a sterile disk after being dissolved in the vehicle for 30 min at room temperature. The disc had been placed on over the MH agar plates that had been inoculated with the test pathogen for 24 hr. The bacterial lawn agar Petri plates were coated with 30 µg of ofloxacin. The regions of inhibition surrounding each disc were measured in millimeters to assess sensitivity to either antibiotics or mushroom extracts.

### GC-MS analysis

The GC Clarus 500 Perkin-Elmer apparatus with an autosampler (AOC-20i) and Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) were the instruments used to perform the GC-MS analysis of these extracts. The temperature was set at  $250^\circ\text{C}$  for the injector and  $280^\circ\text{C}$  for the transfer line. The oven temperature was programmed to rise by  $10^\circ\text{C}$  every min from  $60^\circ\text{C}$  to  $280^\circ\text{C}$ , with a 9 min isothermal at  $280^\circ\text{C}$ . Software for handling mass spectra and chromatograms is called Turbo Mass Ver. 5.0. The HP 5973 mass detector's settings included scan mode, ion ass/charge ratio, and 30-550 m/z. Every mass spectrum was compared to the NIST library.

## RESULTS

### Antioxidant DPPH activity of fermented mushroom

The LAB is used for fermentation of two different edible mushroom kept for 21 days changed into dark brown colour during fermentation and the pH is sustained 6 to 7. Acidification reached and pH is gradually decreased to 6.6, 6.4 and 6.2 during 7<sup>th</sup>, 14<sup>th</sup> and 21<sup>st</sup> day of fermentation. During fermentation the LAB grown well and produced large filamentous like vegetative cells (Figure 1). Both the metal chelating assay and the DPPH radical inhibition assay were used to measure antioxidant effects. Comparison of the rate of probiotic fermented mushroom and raw mushroom on DPPH was given in Table 1. The DPPH antioxidant activity of raw mushroom and fermented mushroom was given in Figure 2. The percentage of free radical was  $51.46 \pm 0.503\%$  on *Agaricus bisporus* and  $55.6 \pm 0.529\%$  in *Calocybe indica*. Compare to LAB fermented *Agaricus bisporus* have  $69.866 \pm 0.503\%$  and significant  $p$  value was 0.000177. Likewise, *Calocybe indica* LAB showed  $62.266 \pm 0.305\%$  significant with  $p$  value 0.001394. The percentage of DPPH antioxidant activity of ascorbic acid was  $78.46 \pm 0.416$ . The independent DPPH of sample and standard was significant  $p < 0.05$  except fermented sample ( $p > 0.05$ ).

### Metal chelating activity

The Metal chelating antioxidant activity of non-fermented mushroom is and fermented mushroom activity given in Figure 3 as percentage of inhibition. The data represent that both the fermented mushroom have greater activity than raw mushroom. The maximum activity of  $72.33 \pm 0.305$  was recorded in fermented *Agaricus bisporus* whereas non fermented extract have  $57.86 \pm 0.41\%$ . The activity of fermented *Calocybe indica* was  $68.4 \pm 0.2\%$  and non-fermented sample have  $54.3 \pm 0.1\%$ . The dependent  $t$ -test among *Agaricus bisporus* ( $p = 0.0002$ ) was significant at  $p < 0.05$  and *Calocybe indica* was not significant ( $p = 2.5$ ). The independent  $t$  test among sample and standard shows except fermented *Agaricus bisporus* ( $p = 0.027$ ) all other activities were not significant  $p > 0.05$  (Table 2).

### Anti-inflammatory activity

Figure 4 represent the results indeed verified the *in vitro* anti-inflammatory activities of LAB<sup>+</sup> *Agaricus bisporus* stem reveals greatest anti-inflammatory (74%) Similarly in the albumin denaturation inhibition assay (plate 4), LAB<sup>+</sup> *Calocybe indica* stem extract had shown the good inhibition capacity with 70% inhibition. LAB<sup>+</sup> *Agaricus bisporus* FB have less significant activity compare to stem and recorded as 15-20% Similarly in the albumin denaturation inhibition assay, of non-fermented *Calocybe indica* extract had shown the less significant inhibition capacity with 30% inhibition and 56% by non-fermented *Agaricus bisporus* at 21<sup>st</sup> day (Table 3).

### Antibacterial activity of fermented mushroom

The antibacterial activity of ethanol extract of LAB fermented fruiting body and stem of basidiomycete *Coriolus versicolor* and *Agaricus bisporus* were examined by well diffusion method against test pathogens. The zone of inhibition against tested pathogens were given in Table 4 data reveals fermented Button mushroom-stem showed significantly greater antibacterial effect against all tested pathogens and the zone of inhibition  $15.66 \pm 0.57, 15.66 \pm 0.57, 15 \pm 0, 17.33 \pm 0.57$  mm against *Escherichia coli*, *P. aeruginosa*, *K. pneumoniae*, *E. faecalis* were recorded on *Agaricus bisporus* fermented by LAB<sup>+</sup>. The non-fermented crude extract showed  $< 10$  mm to be less significant. The antibacterial activity of fermented *Calocybe indica* were found to be moderately effective and less significant zone of inhibition on *E. coli* ( $12.33 \pm 0.57$ ), *P. aeruginosa* ( $14 \pm 0$ ) and *E. faecalis* ( $12.33 \pm 0.57$ ) whereas not active on *K. pneumoniae* ( $4.6 \pm 0.57$ ). Similarly, the raw extract of *Calocybe indica* failed to exhibit antibacterial activity. The  $p$  value of dependent  $t$  test of *Agaricus* sp. was 0.000467007 and *Calocybe indica* was 0.010124591 found to be significant. In case of the independent  $t$  test of sample of fermented *Agaricus* sp. and standard 0.02 ( $p < 0.05$ ) and non-fermented  $p$  value 0.20 ( $p > 0.05$ ). The independent  $p$  value of fermented *Calocybe indica*

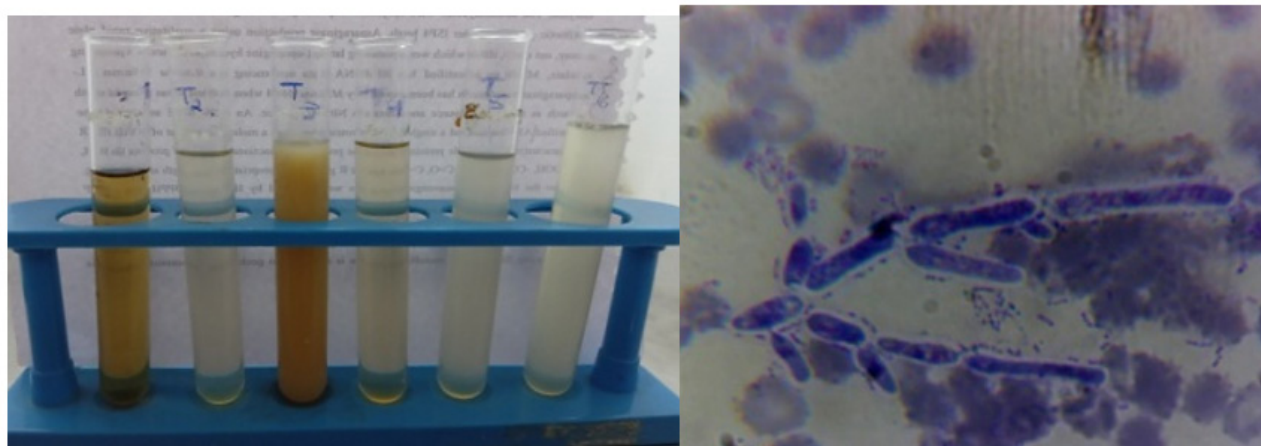


Figure 1: LAB mediated Fermentation.

**Table 1: Antioxidant Activity of Lab Fermented Mushroom.**

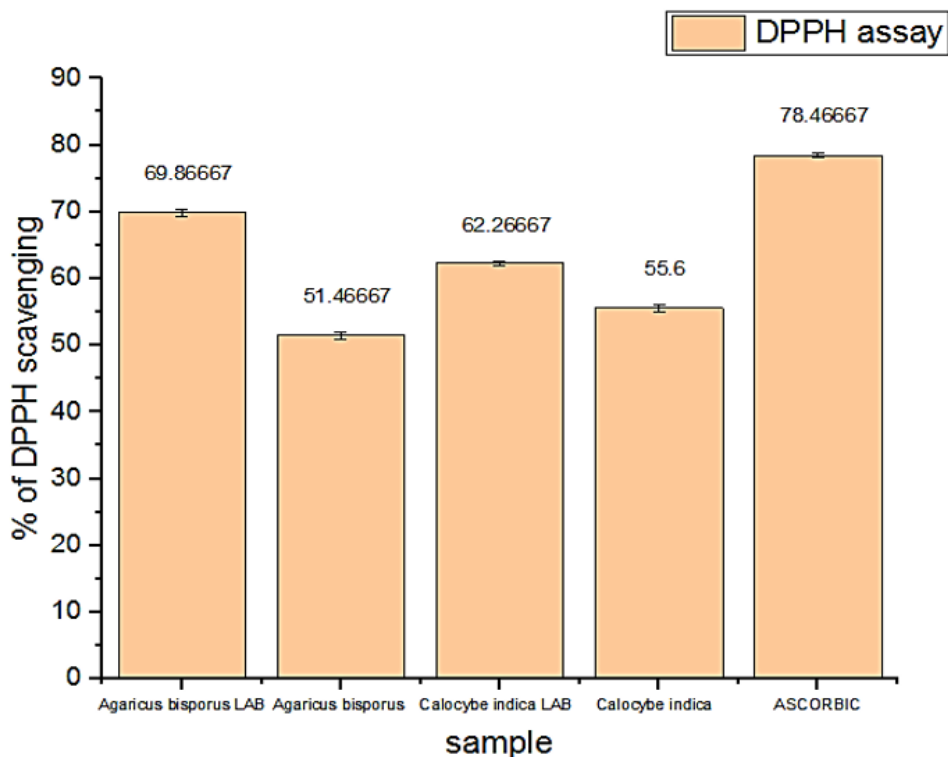
Sample	T1	T2	T3	Mean	SD	Dependent	Independent
<i>Agaricus bisporus</i> LAB	69.8	69.4	70.4	69.866	0.503	0.000177	0.0010781
<i>Agaricus bisporus</i>	52	51	51.4	51.466	0.503		0.0001919
<i>Calocybe indica</i> LAB	62	62.2	62.6	62.266	0.305	0.001394	7.61905
<i>Calocybe indica</i>	56	55	55.8	55.6	0.529		0.00025
Ascorbic	78	78.8	78.6	78.46	0.416		

**Table 2: Metal Chelating Activity of Lab Fermented Mushroom.**

Sample	T1	T2	T3	Mean	SD	Dependent	Independent
<i>Agaricus bisporus</i> LAB	72	72.4	72.6	72.33	0.305	0.0002	0.027
<i>Agaricus bisporus</i>	58	57.4	58.2	57.86	0.41		6.51
<i>Calocybe indica</i> LAB	68.4	68.6	68.2	68.4	0.2	2.51	5.06
<i>Calocybe indica</i>	54.4	54.3	54.2	54.3	0.1		3.909
EDTA	72	71.4	71.6	71.66	0.30		

**Table 3: Anti-inflammatory Activity of Lab Fermented Mushroom.**

Sample	T1	T2	T3	Mean	SD	Dependent	Independent
<i>Agaricus bisporus</i> LAB	51	52	51	51.33	0.577	0.00016	0.00030
<i>Agaricus bisporus</i>	24	23.6	24.2	23.93	0.3055		1.858
<i>Calocybe indica</i> LAB	62	62.6	63.4	62.66	0.702	1.9652	0.006536
<i>Calocybe indica</i>	20	19.8	20.6	20.13	0.416		3.0486
Dichlofenac	68	67.6	67.4	67.66	0.305		



**Figure 2:** Percentage of DPPH antioxidant activity of mushroom Samples.

and standard was 0.00025 and crude was 0.001049 indicates both are significant ( $p < 0.05$ ).

### Mass spectrum analysis of LAB fermented *Agaricus bisporus*

GC MS analysis of fermented Button mushroom reveals the presence of 35 peaks (Figure 5) and 32 different compounds. All the detected compounds are enlisted on Table 5. The maximum detected compounds are n-Hexadecanoic acid (14.33%), 4.87% of 2-Piperidinone (RT 11.288). Dibutyl phthalate (9.7%), 3.93% of 1,2-Benzenediol (RT 11.432), 4.24% of 2-Methoxy-4-Vinylphenol and 6.32% Hydrocinnamic acid. In addition to fatty acid methyl esters, spectrum shows presence of Phenylpropanoic acid or hydrocinnamic acid is a carboxylic acid and 1-Nonadecene alkenes were identified from the fermented sample. Piperidin-2-one is a delta-lactam that is piperidine with an oxo group at position 2, according to a Pub chem investigation. It has a role as L-glutamate gamma-semialdehyde dehydrogenase inhibitor. Presence of 11-octadecenoic acid, phenolic acids. *A. bisporus* extracts suggest that there may be several bioactive compounds that inhibit the growth of both Gram-positive and Gram negative pathogens.

## DISCUSSION

The sensory results of fermented edible mushroom such as odour, consistency was acceptable, except for the texture and colour found to be dark and juicy. All the parameters were triplicated and the bioassay pH was maintained at 6.5. Bello and Akinyele proposed an identical pattern in the use of fermenting mushroom

medicinal value using fruiting bodies (Bello and Akinyele, 2007). Free radical scavenging of crude edible mushroom was lower than fermented mushroom. Both metal chelation and DPPH activity was concentration dependent. Significant protein denaturation inhibition was recorded and found to be close to standard. Bai *et al.*, (2013) have reported that presence of phenolics aid in scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydroxyl (OH) radicals. The application of the LAB starter as fermenter also act as preservative in the food industry and enhance the use of the industrial fermentation and production of more health-oriented foods (Beganovic *et al.*, 2014). Ascorbic acid equivalent antioxidant activities of extracts of five edible mushrooms was previously reported by (Boonsong *et al.*, 2016). Metal chelation potential of mushroom *Pleurotus ostreatus* was previously reported by (Oluwamodupe *et al.*, 2013). Mushroom components like phenolics and flavonoids and ascorbic acid promotes the antioxidant potential (Nakamura *et al.*, 2019). The data is correlates the study of (Nataraj *et al.*, 2022) who reported metal chelating capacity of mushrooms lower than EDTA. The  $\beta$ -glucan-rich preparation from mushrooms has cytotoxic in nature antioxidant effects that are controlled by glucan. Its anti-inflammatory effects are achieved by inhibiting Cyclooxygenase (COX) and Nitric Oxide Synthase (NOS) inhibition. According literature survey it was reported that *C. indica* significantly showed anti-inflammatory, antioxidant and antimicrobial activity (Meghna *et al.*, 2022). A number of studies showed that mushrooms extracts express a higher antimicrobial activity against pathogenic bacteria. Recently (Jankov *et al.*, 2024) have reported the antibacterial and Synergistic activities of *Agaricus bisporus* against *S. aureus*, *E. coli*, *B. subtilis*. According to

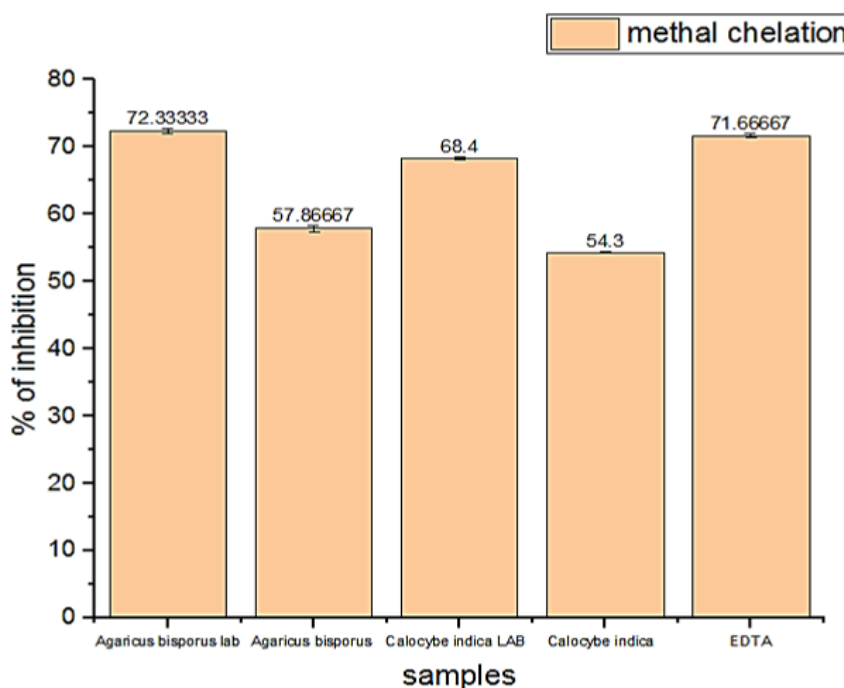


Figure 3: Percentage of Metal Chelating Activity.

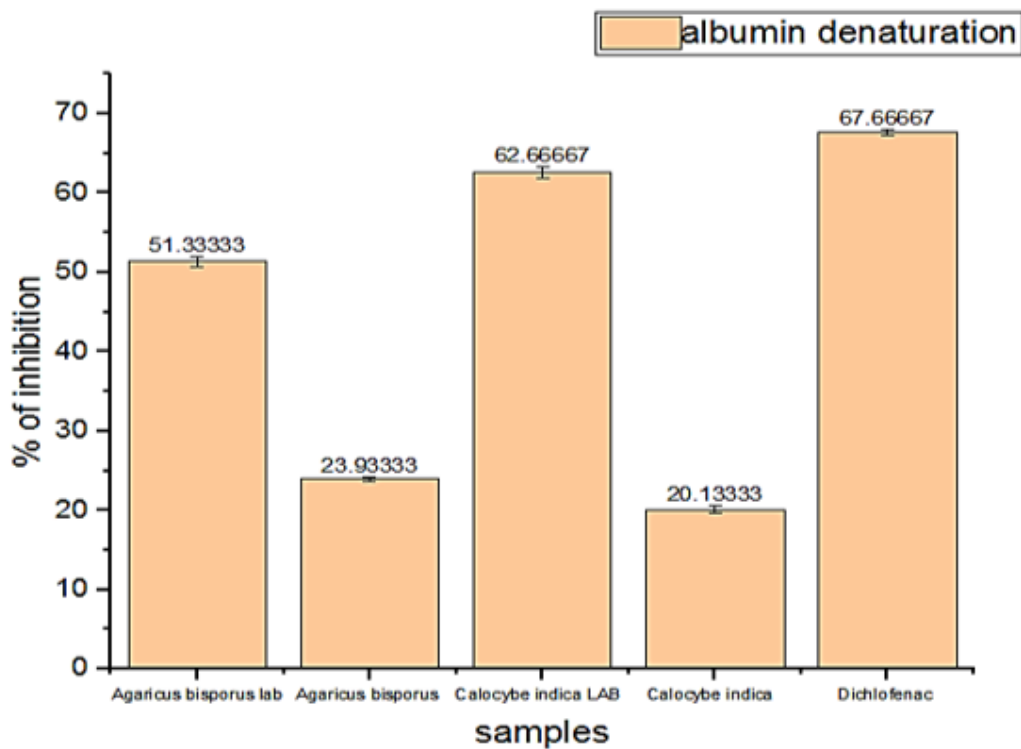


Figure 4: Percentage of anti-inflammatory activity.

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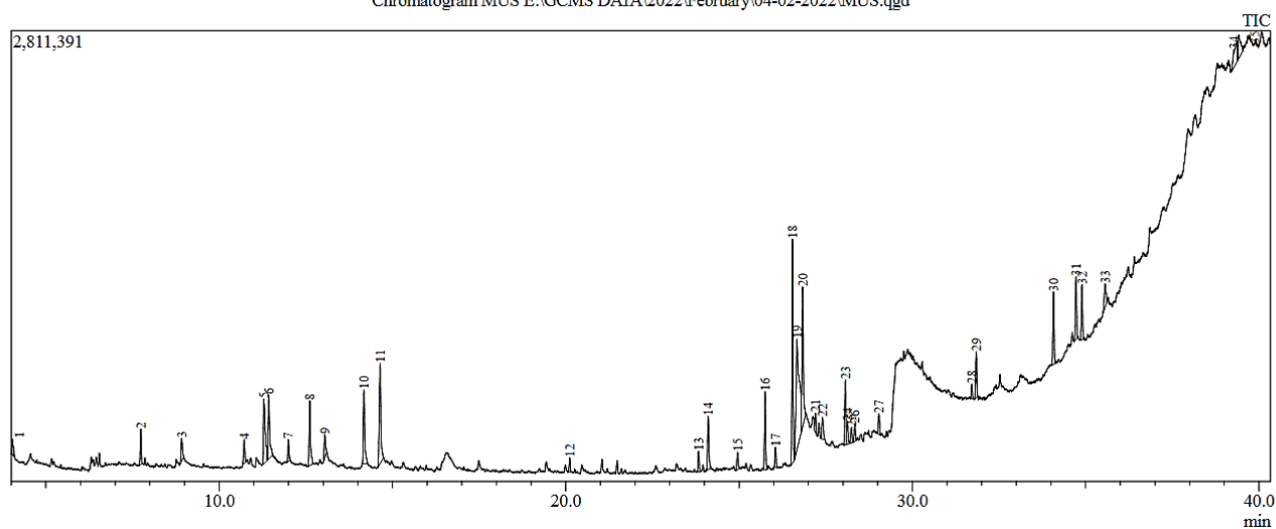


Figure 5: GCMS spectrum of fermented *Agaricus bisporus* (Button mushroom).

Table 4: Antibacterial activity of fermented and raw edible mushroom.

Sample 1 mg/mL	EC		PA		KP		EF	
	Mm	SD	Mm	SD	Mm	SD	Mm	SD
LAB <i>Agaricus bisporus</i>	15.66	0.57	15.66	0.57	15	0	17.33	0.57
LAB <i>Calocybe indica</i>	12.33	0.57	14	0	4.6	0.57	12.33	0.57
<i>Agaricus bisporus</i>	5.66	0.57	6.66	1.15	7.33	1.15	6.33	0.57
<i>Calocybe indica</i>	4.66	1.15	0	0	0	0	0	0
Standard	13.33	1.154	12.66	0.57	14.33	0.57	12.33	0.57

EC-E. coli, PA-P. aeruginosa, KP-K. pneumoniae, EF-E. faecalis.

**Table 5: NIST library matched GCMS eluted active compounds.**

Peak	Retention time	Area %	Name
1	4.035	0.76	Butanoic acid, 2-methyl-
2	7.737	1.57	Benzyl alcohol
3	8.919	1.29	Phenol, 2-methoxy-
4	10.725	1.12	Benzenepropanal
5	11.288	4.87	2-Piperidinone
6	11.432	3.93	1,2-benzenediol
7	11.996	1.01	2,3-dihydro-benzofuran
8	12.613	3.54	4-phenyl-2-butanone
9	13.045	1.25	Phenol, 4-amino-
10	14.176	4.24	2-Methoxy-4-vinylphenol
11	14.644	6.32	Hydrocinnamic acid
12	20.111	0.6	1-Nonadecene
13	23.833	0.92	1-Nonadecene
14	24.108	2.91	3-Buten-2-one, 4-(4-hydroxy-3-methoxyphenyl)-
15	24.956	0.85	1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester
16	25.747	3.56	1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester
17	26.048	1.01	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester
18	26.541	9.79	Dibutyl phthalate
19	26.67	14.33	n-Hexadecanoic acid
20	26.837	7.95	Phthalic acid, butyl 2-pentyl ester
21	27.204	0.86	n-Nonadecanol-1
22	27.405	1.33	1,2-benzenedicarboxylic acid, butyl octyl ESTER
23	28.069	3.01	Dibutyl phthalate
24	28.127	1.22	9,12-Octadecadien-1-ol, (Z,Z)-
25	28.241	0.82	1,2-Benzenedicarboxylic acid, butyl octyl ester
26	28.339	0.76	Diamyl phthalate
27	29.035	1.04	1,2-benzenedicarboxylic acid, dibutyl ester
28	31.712	0.59	Glycidyl palmitate
29	31.841	2.18	1H-Imidazole, 4,5-dihydro-2-(phenylmethyl)-
30	34.075	3.36	Bicyclo[10.1.0]tridec-1-ene
31	34.722	3	6h-benzofuro[3,2-c][1]benzopyran, 6a,11a-dihydro-3,9-dimethoxy-, (6ar-cis)-
32	34.895	2.59	Bis(2-ethylhexyl) phthalate
33	35.56	1.66	Hexacontane
34	39.275	3.15	Phytyl dodecanoate
35	39.426	2.6	1,1,3,3-Tetraallyl-1,3-disilacyclobutane

(Bartkiene *et al.*, 2023) during fermentation *A. bisporus* showed decreased 1-Octen-3-ol content while benzyl alcohol, acetoin, and 2,3-butanediol increased in most fermented sample. Another study shows the FA profile of *A. bisporus* comprises stearic acid, palmitic, linoleic, caprylic, oleic, erucic, and eicosanoic acid (Saiqa *et al.*, 2008). It was also reported that Linoleic acid is converted to 13-hydroxy-9-octadecenoic acid or the antifungal 10-hydroxy-12-octadecenoic acid (Ogawa *et al.*, 2001).

## CONCLUSION

This study yielded useful data that can be used to produce fermented mushrooms on a large-scale. Additionally, a broader range of LAB for *A. bisporus* fermentation can be investigated in the future to suggest the most suitable LAB strains for the mushroom fermentation sector as well as the healthiest and most palatable outcome for the end user.

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## CONFLICT OF INTEREST

The study was carried out without any financial or commercial ties that might be interpreted as a conflict of interest, agreed to the authors.

## ABBREVIATIONS

**FA:** Fatty acid; **FB:** Fruiting bodies; **OD:** Optical density; **LAB:** Lactic acid bacillus; **Mm:** millimole.

## SUMMARY

In addition to increasing phytochemical during fermentation with LAB strain altered the physical characteristics of *A. bisporus* and *Calocybe indica*. *A. bisporus* showed the highest degree of bioactivity. The primary volatile substances found in the samples of mushrooms were hydrocinnamic acid, nonadecanol-2-peridinone, hexadecanoic acid, and 2-methyl-4-vinylphenol. The general acceptance of fermented and non-fermented mushrooms was comparable. The *A. bisporus* variety and the LAB strain employed for fermentation were important contributors to the majority of biomedical application.

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