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GC-MS Profiling and in vitro Bioactivity Assessment of Ethanolic Extract of the Macrofungus *Phellinus rimosus* (Berk.) Pilát

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KEYWORDS

Phellinus rimosus, mushrooms, ethanolic extract, antioxidant, anti-inflammation, antidiabetic

ABSTRACT:

Introduction: Development of pharmaceutical agents from natural sources like mushrooms has been a hot spot of research due to their clinical efficacy and low toxicity. *Phellinus rimosus* (Berk) Pilat, a wood-rotting macrofungus found growing exclusively on jackfruit tree trunks in Kerala, India, is a less-explored medicinal mushroom and was reported to be traditionally used by local tribes to treat mumps.

Objectives: This investigation was carried out to characterise the active principles present in the ethanolic extract of *P. rimosus* and to evaluate its in vitro anti-inflammatory, antioxidant, and antidiabetic activities.

Methods: Ethanolic extract was prepared from sporocarps of *P.rimosus,* and analysed by Gas Chromatography-Mass Spectrometry (GC-MS) to characterise the active principles present in it. Anti-inflammatory activity was assessed through Cyclooxygenase (COX), Lipoxygenase (LOX), and Inducible Nitric Oxide Synthase (iNOS) inhibition assays. Antioxidant potential was evaluated using the Total Antioxidant Capacity Assay (TACA) and Total Reducing Power Assay (TRPA). The antidiabetic potential was studied via yeast glucose uptake and α-Amylase inhibitory assays.

Results: GC-MS analysis of the ethanolic extract from *P. rimosus* identified major constituents as Dotriacontane, Diethyl Phthalate, n-Hexadecanoic acid, Tetradecanoic acid, Hexadecanoic acid ethyl ester, and Azulene, along with other active compounds in moderate and lower concentrations. The extract showed significant inhibition for COX, LOX, and iNOS, at a rate of 62.02%, 62.47%, and 54.01%, respectively, at 100 µg/ml. TACA and TRPA showed dose-dependent antioxidant activity of *P.rimosus.* Although the extract did not enhance glucose uptake in vitro, it showed significant α-Amylase inhibition, suggesting its antidiabetic effect.

Conclusions: The ethanolic extract of *P. rimosus* showed profound pharmacological potential due to the presence of bioactive components profiled by GC-MS. The antioxidant and anti-inflammatory power may be responsible for its anti-diabetic activities. This investigation showed that *P. rimosus* could serve as a promising source for developing safe natural therapeutics.

1. Introduction

Natural sources, including fungi, plants, and microorganisms, have been continuously explored for the development and production of safe pharmacological agents.[1] Since ancient times, the profound pharmacological properties of medicinal mushrooms have been well known and are being continuously researched to identify their bioactive components.[2] Medicinal mushrooms are higher-class fungi with various nutraceutical properties such as low-fat content, high fiber content, and trans-isomers of unsaturated fatty acids, biologically active compounds like polysaccharides (especially glucans), alkaloids, steroids, polyphenols, terpenoids, etc.[3] Mushrooms have been reported to possess a wide range of medicinal properties, including anti-inflammatory, antimicrobial, antiviral, antiparasitic, antifungal, antioxidant, anti-HIV, hepatoprotective,

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antitumor, anticancer, cytotoxic, antidiabetic, hypocholesterolemic, anti-coagulant, and antiproliferative activities.[4]

Reactive oxygen species (ROS) play a crucial role in regulating inflammatory responses. Key ROS generated within cells include superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), peroxide anion (O_2^{2-}), hydroxyl ion (OH^-), and hydroxyl radical ($OH^{\cdot}$). Nitrosative stress is another detrimental condition damaging the cells when Reactive Nitrogen Species (RNS) react with ROS. Although nitric oxide (NO) is relatively less reactive, it can react with superoxide anion (O_2^-) to form peroxynitrite ($ONOO^-$).[5] $ONOO^-$ induces free radical pathways directly or indirectly, adversely affecting the DNA, proteins, and lipids. Reactions initiated by peroxynitrite are known to damage cell signalling, eventually leading to conditions including inflammatory disorders, diabetes mellitus, cancer, stroke, cardiovascular, and neurodegenerative diseases.[6]

COX (cyclooxygenase) and LOX (lipoxygenase) are key enzymes in the inflammatory responses, responsible for generating signalling molecules from arachidonic acid. COX enzymes mainly produce prostaglandins, which are associated with inflammation, pain, and fever, while LOX enzymes generate leukotrienes that also promote inflammation. Targeting these enzymes—especially COX-2 and 5-LOX—is a widely used approach in the development of anti-inflammatory drugs.[7] Nitric oxide (NO), a powerful signalling molecule synthesized by Inducible nitric oxide synthase (iNOS), also plays a major role in the inflammatory process. Although NO can have both protective and harmful effects during inflammation, NO generated by iNOS is typically linked with damaging outcomes in chronic inflammatory conditions. It acts as a key mediator by promoting vasodilation, attracting immune cells, and mediating inflammatory responses.[8] Downregulation of these pro-inflammatory mediators, like COX, LOX, and iNOS, helps to regulate the progression of inflammation and related conditions.

A growing body of evidence suggests that inflammation and diabetes are closely linked, with inflammatory pathways playing a pivotal role in the development of diabetes. Many antidiabetic agents, pioglitazone, metformin, as well as insulin, exert inherent anti-inflammatory effects as part of their primary mechanisms and are known to reduce levels of inflammatory markers.[9] Type 2 diabetes mellitus is also driven by increased oxidative stress triggered by various metabolic conditions, alongside a complex inflammatory response that often occurs without noticeable clinical symptoms. This underlying subclinical inflammation results from the body's defensive anti-inflammatory mechanisms.[10]

In the last few years, Gas Chromatography-Mass Spectrometry (GC-MS) has become a powerful tool in the separation and identification of volatile organic components in plant and non-plant species. In the last few years, it has been widely used for volatile secondary metabolite profiling of mushrooms.[11] Its high sensitivity (often down to parts-per-billion or lower), specificity, and ability to match spectra to libraries make it ideal for detecting trace compounds and elucidating molecular structures.

Phellinus spp. is one of the largest genera of Hymenochaetaceae with approximately 220 species. *P. vaninii*, *P. buamii*, *P. linteus*, and *P. igniarius* are considered as precious food supplements and medicinal ingredients in China, Korea, Japan, and other Asian countries for over 2000 years. Studies on this mushroom species in the last few decades have confirmed the occurrence of bioactive primary and secondary metabolites with multiple health-promoting benefits, including antioxidant, immunomodulatory, antitumor, anti-inflammatory, antimicrobial, and antidiabetic effects.[12] *Phellinus rimosus (P.rimosus)* is a wood-rotting parasitic macrofungus found growing on jackfruit tree trunks in Kerala, India. It is a less investigated medicinal mushroom. In vitro antioxidant activity as well as hepatoprotective activity of ethyl acetate extract of *P. rimosus* in animal models were reported by Ajith and Janardhanan.[13] Authors have also reported the in vitro free radical scavenging and in vivo anti-inflammatory activity of the methanolic extract of *P. rimosus*.[14] Ethyl acetate extract of *P. rimosus* was found to possess antioxidant and antimutagenic activity against the mutagen, 1-aminopyrene, treated with nitrite.[15] In our previous studies, water-soluble high molecular weight polysaccharides isolated from *P. rimosus* have revealed profound antioxidant, anti-inflammatory, anti-arthritic, hypoglycemic & hypolipidemic effects, anti-tumor, anti-cancer, and apoptotic properties.[16-20]

2. Objectives

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The present investigation was carried out to identify the bioactive components present in the ethanolic extract of *P. rimosus,* using GC-MS analysis. The study further focused on the in vitro antioxidant, anti-inflammatory, and antidiabetic effects of the mushroom ethanolic extract. This is the first-ever report presenting the GC-MS analysis of the ethanolic extract of *P.rimosus* and its biological properties using in vitro models.

3. Methods

Preparation of Ethanolic Extract of *P. rimosus*

Sporocarps of *Phellinus rimosus* growing on the trunks of *Artocarpus heterophyllus* (jackfruit tree) were collected from the outskirts of Thrissur, Kerala, India. The mushroom was identified, and a voucher specimen was deposited in the Herbarium of the Centre for Advanced Studies in Botany, University of Madras, Chennai, India (HERB.MUBL-3171). The sporocarps were cut into small pieces and at 40–50 °C for 3-4 days and powdered. The powdered material was first defatted using petroleum ether for 8–10 hours in a Soxhlet apparatus, followed by extraction with 99% ethanol at 50–70 °C for 9–10 hours. Whatman No.1 filter paper was used to filter the extract, which was then dried at a low temperature of 30–40 °C using a rotary evaporator. The dried extract was kept airtight in a container at 4 °C until further use.

Characterization of Ethanolic Extract of *P. rimosus* Using GC-MS

GC-MS analysis was employed to monitor the occurrence of various volatile components in the ethanolic extract of *P.rimosus.* The analysis was carried out at the Centre for Analytical Instrumentation- Kerala (CAI-K), Kerala State Council for Science, Technology and Environment (KSCSTE)- Kerala Forest Research Institute (KFRI), Peechi-680653, Kerala, India.

GC-MS used in this analysis employed a fused silica column, and the components were separated using helium as a carrier gas at a constant flow of 1ml/min. The 1µl sample extract was injected into the GC-MS instrument (GC SPECIFICATION: Shimadzu Nexis GC-2030; AUTO SAMPLER: AOC-30/20i). The initial temperature was set at 100°C, whereas the injector temperature was 250°C, and throughout the process, temperature flow was set at a rate of 10°C/min. The actual separation was observed at the 35th minute, for which the final temperature was adjusted to 280°C and run for 15 min. The mass spectra obtained from GC-MS analysis of the *P.rimosus* were interpreted using the National Institute of Standards and Technology (NIST) database. The spectra of unknown components in the ethanolic extract were matched with those of known compounds in the NIST 20 library. Components present in the ethanolic extract were identified based on their name, molecular weight, and chemical structure.

Antioxidant Assays of the Ethanolic Extract of *P. rimosus*

Total Antioxidant Capacity Assay (TACA) Total Antioxidant Capacity Assay (TACA) was conducted following the method described by Prieto *et al.*[21] Different concentrations of the extract (200–2000 µg) were mixed with 1 ml of TACA reagent, followed by the incubation of the mixtures for 90 min. at 95°C. Reading was taken at 695nm once the reaction mixture had cooled down to room temperature. Vitamin C served as the standard reference. The percentage inhibition of the treated against the Control was calculated using the following equation.

% Inhibition = (T-C)/ T×100

(T= Absorbance of Treated, and C= Absorbance of Control)

Total Reducing Power Assay (TRPA)

Various concentrations of the extract were combined with 2.5 ml of phosphate buffer (200 mM, pH 6.6) and 2.5 ml of 1% potassium ferricyanide, followed by incubation for 20 min. at 50°C. After incubation, 2.5 ml of 10% trichloroacetic acid was added, and the mixtures were centrifuged for 10 minutes. The upper phase (5 ml) was then mixed with 5 ml of distilled water and 1 ml of 0.1% ferric chloride. The absorbance of the final solution was measured at 700 nm. Vitamin C was used as the standard. The reducing power was calculated using the equation:

% activity = (T- C)/ T×100

(T = Absorbance of Treated, and C = Absorbance of the Control)

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Anti-Inflammatory Analysis of Ethanolic Extract of *P. rimosus*

Cell Lines

RAW 264.7(Macrophage) cell line was initially procured from the National Centre for Cell Sciences (NCCS), Pune, India, and maintained in Dulbecco's Modified Eagle Medium, DMEM (Sigma Aldrich, USA). The DMEM medium in 25 cm^2 tissue culture flask with antibiotic solution (Penicillin (100U/ml), Streptomycin (100µg/ml), Amphotericin B (2.5µg/ml)), supplemented with L-glutamine, sodium bicarbonate (Merck, Germany) and 10% Fetal Bovine serum (FBS), was used for cell line cultivation. A humidified 5% CO_2 incubator (NBS Eppendorf, Germany) at 37ºC was used to get 60% confluent growth of the cell line, which was then activated by the addition of 1 µL lipopolysaccharide (LPS: 1µg/ml). LPS-stimulated RAW cells were exposed to different concentrations (25, 50, 100µg/ml) of ethanolic extract of *P.rimosus* and incubated for 24 hours. After incubation, the anti-inflammatory assays were performed using the cell lysate.

Inhibition of Cyclooxygenase (COX) Activity

The COX activity was assayed by the method of Walker and Gierse[22]. 100µl cell lysate was incubated with Tris-HCl buffer (pH 8), glutathione 5 mM/L, and hemoglobin 5 mM/L for 1 minute at 25°C. The reaction was initiated by the addition of arachidonic acid 200 mM/L and terminated after 20 minutes incubation at 37°C, by the addition 200µL of 10% trichloroacetic acid in 1 N hydrochloric acid. The tubes were centrifuged, then 1% thiobarbituric acid (200µL) was added and boiled for 20 minutes. It was followed by 3 min centrifugation at 4 °C. The absorbance was taken at 632 nm to determine the COX activity, and the percentage inhibition was calculated using the following equation. Diclofenac was used as the standard.

% inhibition = (C-T)/C × 100 (C= Absorbance of Control and T= Absorbance of Treated)

Inhibition of Lipoxygenase (LOX) Activity

The reaction mixture (2 ml final volume) contained Tris-HCl buffer (pH 7.4), 50 µL of cell lysate, and sodium linoleate (200 µL) and the formation of 5-hydroxyeicosatetraenoic acid was read as the LOX activity which was monitored as an increase of absorbance at 234 nm (Agilent Cary 60).[23] Diclofenac was used as the standard. The percentage inhibition of the enzyme was calculated using the equation:

% inhibition = (C-T)/C × 100

(C= Absorbance of Control and T= Absorbance of Treated)

Inhibition of Inducible Nitric Oxide Synthase (iNOS) Activity

Nitric oxide synthase was determined by the method described by Salter *et al*[24]. The cell lysate was homogenized in 2ml of HEPES (4-(2-Hydroxyethyl) piperazine-1-ethanesulfonic acid) buffer. The assay system contained 0.1ml -2µmol/L L-Arginine, 0.1ml-4µmol/L manganese chloride, 0.1ml-10mmol/L, 30µg dithiothreitol (DTT), 0.1ml- 1mmol/L NADPH, 0.1ml-4µmol/L tetrahydropterin, 0.1 ml 10µmol/L oxygenated haemoglobin and 0.1ml cell lysate. An increase in absorbance was recorded at 401nm, and enzyme activity was determined. Diclofenac was used as the standard.

% inhibition = (C-T)/C × 100

(C= Absorbance of Control and T= Absorbance of Treated)

Antidiabetic Activity of Ethanolic Extract of *P. rimosus.*

In Vitro Yeast Sugar Uptake Assay

Commercial baker's yeast was washed by repeated centrifugation (3,000×g, 5 min) in distilled water until the supernatant fluids were clear, and a 10% (v/v) suspension was prepared in distilled water. Various concentrations of the extract (200-2000µg) were added to 1ml of glucose solution (25mM) and further incubated for 10 minutes at 37°C. The reaction was started by adding 100µl of yeast suspension, vortexed, and further incubated at 37°C for 60 minutes. After 60 minutes, the tubes were centrifuged (2500rpm, for 5 minutes) and glucose was estimated in the supernatant by using Benedict reagent, absorbance measured in a UV Spectrophotometer at 520nm against the blank. Metronidazole was used as a standard. The percentage increase in the uptake of glucose was calculated.

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% increase in glucose uptake = (C- T)/C × 100

(C= Absorbance of Control and T= Absorbance of Treated)

Alpha-Amylase Inhibitory Assay

0.5ml different concentrations (200-2000µg) of ethanolic extract was mixed with 0.5ml of α-Amylase solution (0.5mg/ml) and 0.002M sodium phosphate buffer (pH 6.9 with 0.006M NaCl). The mixture was incubated at room temperature for 10 min, and 0.5ml of starch solution (1%) in 0.002M sodium phosphate buffer (pH 6.9 with 0.006M NaCl) was added. The resulting mixture was incubated at room temperature for 10 minutes, and the reaction was terminated using 1-2 drops of iodine solution. The mixture was then diluted with 10ml of distilled water, and absorbance was measured in a UV Spectrophotometer at 540nm. Acarbose was used as a standard drug.

% inhibition of α-Amylase = (C-T)/C × 100

(C= Absorbance of Control and T= Absorbance of Treated)

Statistical Analysis

All data presented in this study are from triplicate analysis, and the given values are Mean ± SD.

4. Results

Preparation of Ethanolic Extract of *P. rimosus*

The sporocarps of *P. rimosus* were dried and finely ground using a mixer grinder, resulting in a smooth, dark brown mushroom powder. From 150 g of defatted *P. rimosus* powder, 4.69 g of ethanolic extract was obtained, corresponding to a 3.13% yield.

Characterization of Ethanolic Extract of *P. rimosus* by GC-MS

GC-MS study was employed for the characterization of the of *P. rimosus* ethanolic extract and a total of 32 distinct peaks were detected in the chromatogram. Upon analysis, dotriacontane was identified at four different retention times and 16-hentriacontanol at two different retention times, indicating repeated detections of the same compounds. After eliminating these duplicates, the extract was found to contain 28 unique compounds. These were identified by matching their mass spectra and retention times against the spectrum of known components using the NIST 20 library. **Table 1** lists each identified compound with its retention time (RT), percent peak area, molecular formula (MF), and molecular weight (MW). **Figure 1** represents the mass spectra for all identified compounds.

Table 1. GC-MS Profiling of the Ethanolic Extract of *P. rimosus*

Peak	Name of the Compound	*RT (min)	Peak Area %	*MF	*MW
1	Azulene	8.345	5.31	$C_{10}H_8$	128
2	1-Tetradecene	11.160	0.51	$C_{14}H_{28}$	196
3	1-(4-Ethoxyphenyl)propan-1-ol	12.300	0.66	$C_{11}H_{16}O_2$	180
4	2,4-Di-tert-butylphenol	12.700	0.59	$C_{14}H_{22}O$	206
5	Benzoic acid, 4-ethoxy-, ethyl ester	12.909	1.53	$C_{11}H_{14}O_3$	194
6	Diethyl Phthalate	13.679	10.68	$C_{12}H_{14}O_4$	222
7	Benzoic acid, 2,4-dihydroxy-3,6-dimethyl-, methyl ester	15.150	2.55	$C_{10}H_{12}O_4$	196
8	Benzene, 1,2,4,5-tetrachloro-3,6-dimethoxy-	15.236	1.39	$C_8H_6C_{l4}O_2$	274
9	Phenol, 2,3,5,6-tetrachloro-4-methoxy-	15.577	0.93	$C_7H_4C_{l4}O_2$	260
10	Tetradecanoic acid	15.659	5.85	$C_{14}H_{28}O_2$	228

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11	Benzene1, 2,3,4-tetrachloro-5-methoxy-6-nitro-	15.919	0.85	$C_7H_3Cl_4NO_3$	289
12	1-Nonadecene	16.108	1.43	$C_{19}H_{38}$	266
13	Benzenamine, 2,3,4,5-tetrachloro-6-methoxy-	17.167	2.31	$C_7H_5Cl_4NO$	259
14	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	17.966	0.95	$C_{17}H_{24}O_3$	276
15	n-Hexadecanoic acid	18.966	9.55	$C_{16}H_{32}O_2$	256
16	Hexadecanoic acid, ethyl ester	19.704	5.66	$C_{18}H_{36}O_2$	284
17	Oleic Acid	23.137	3.33	$C_{18}H_{34}O_2$	282
18	Linoleic acid ethyl ester	23.620	1.33	$C_{20}H_{36}O_2$	308
19	(E)-9-Octadecenoic acid ethyl ester	23.774	2.56	$C_{20}H_{38}O_2$	310
20	Octadecanoic acid, ethyl ester	24.371	1.96	$C_{20}H_{40}O_2$	312
21	Heneicosane	26.602	1.72	$C_{21}H_{44}$	296
22	Dotriacontane	31.068	27.17	$C_{32}H_{66}$	450
23	Nonacosanal	29.027	1.73	$C_{29}H_{58}O$	422
24	Bis(2-ethylhexyl) phthalate	30.750	1.19	$C_{24}H_{38}O_4$	390
25	16-Hentriacontanol	35.324	4.46	$C_{31}H_{64}O$	452
26	Hexatriacontane	35.028	4.57	$C_{36}H_{74}$	506
27	Tetracontane	36.483	3.18	$C_{40}H_{82}$	562
28	Tetrapentacontane	38.120	2.06	$C_{54}H_{110}$	758
			100.00		

*RT- Retention time; MF- Molecular Formula; MW- Molecular Weight

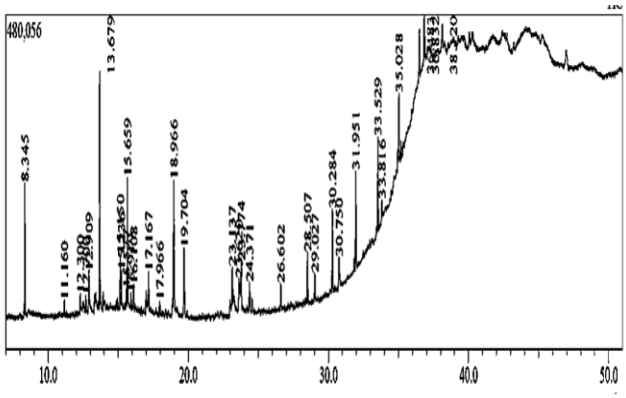

Figure 1. GC-MS Chromatogram of Ethanolic Extract of *P. rimosus*

The most abundant compounds found in the ethanolic extract were Dotriacontane (27.17%), Diethyl Phthalate (10.68%), n-Hexadecanoic acid (9.55%), Tetradecanoic acid (5.85%), Hexadecanoic acid, ethyl ester (5.66%), and Azulene (5.31%). (E)-9-Octadecenoic acid ethyl ester (2.56%), Benzoic acid, 2,4-dihydroxy-3,6-dimethyl-, methyl ester (2.55%), Benzenamine, 2,3,4,5-tetrachloro-6-methoxy- (2.31%), Tetrapentacontane (2.06%), Octadecanoic acid, ethyl ester (1.96%), Nonacosanal (1.73%), Heneicosane (1.72%), Benzoic acid, 4-ethoxy-, ethyl ester (1.53%), 1-Nonadecene (1.43%), Benzene, 1,2,4,5-tetrachloro-3,6-dimethoxy- (1.39%), Linoleic acid ethyl ester (1.33%), Bis(2-ethylhexyl) phthalate (1.19%), were found in moderate amounts. The compounds found in minimal amounts were 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione (0.95%), Phenol, 2,3,5,6-tetrachloro-4-methoxy- (0.93%), Benzene, 1,2,3,4-

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tetrachloro-5-methoxy-6-nitro- (0.85%), 1-(4-Ethoxyphenyl)propan-1-ol (0.66%), 2,4-Di-tert-butylphenol (0.59%), and, 1-Tetradecene (0.51%).

Antioxidant Potential of Ethanolic Extract of *P. rimosus*

Total Antioxidant Capacity Assay (TACA)

Figure 2 shows the dose-dependent antioxidant capacity of the extract. In TACA, the ability of the drug to reduce molybdenum (VI) to molybdenum (V) is measured based on the formation of green phosphate-molybdate complex in acidic conditions. At the maximum tested dose of 2000 µg/ml, the ethanolic extract showed a remarkable activity of 90.15%. In comparison, Vitamin C showed 99% activity at its maximum tested dose of 100 µg/ml. The IC_{50} values of the ethanolic extract of *P. rimosus* and Vitamin C were found to be 171 µg/ml and 52 µg/ml, respectively.

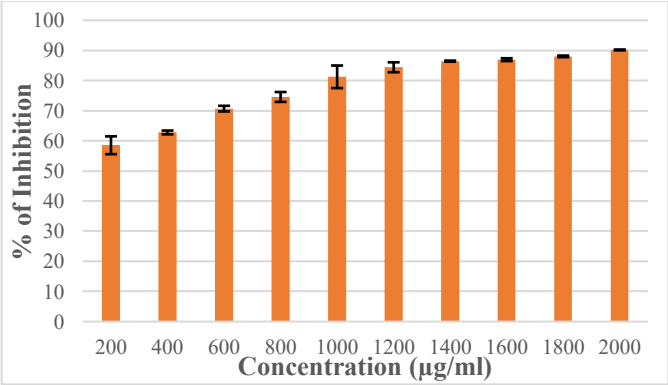

Figure 2. Antioxidant Potential of Ethanolic Extract of *P. rimosus* by TACA. Values are mean ± SD, n=3.

Total Reducing Power Assay (TRPA)

In the TRPA, the *P.rimosus* extract also showed reducing power in a dose-dependent manner **(Figure 3)**. The TRPA is based on the principle that compounds with reducing potential react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which subsequently reacts with ferric chloride to produce a ferric-ferrous complex exhibiting an absorption maximum at 700 nm. The ethanolic extract demonstrated 88.54% activity at the highest tested concentration of 2000 µg/ml, which was comparable to the standard reference compound, Vitamin C. Vitamin C exhibited 99% activity at its maximum tested concentration of 100 µg/ml. The IC_{50} values for *P. rimosus* extract and Vitamin C were 262.54 µg/ml and 50.43 µg/ml, respectively.

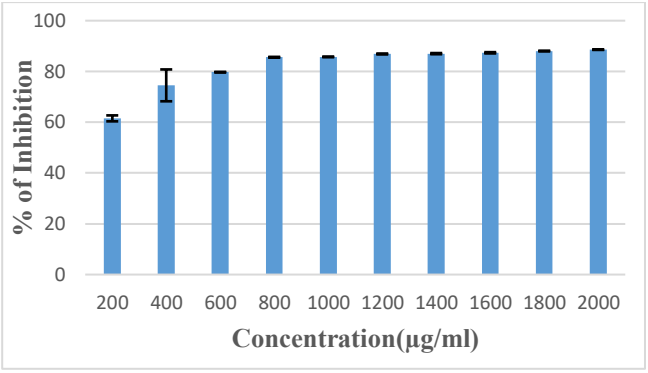

Figure 3. Antioxidant Efficiency of Ethanolic Extract of *P. rimosus* by TRPA. Values are mean ± SD, n=3.

Anti-inflammatory Potential of Ethanolic Extract of *P. rimosus*

Inhibitin of Cyclooxygenase (COX) Activity

The anti-inflammatory potential of *P. rimosus* was evaluated through the Cyclooxygenase (COX) inhibition assay using varying concentrations of its ethanolic extract. At a concentration of 25 µg/ml, the extract exhibited 24.20% inhibition of COX activity, which increased to 48.27% at 50 µg/ml and further to 62.02% at 100 µg/ml, and was comparable to that of the reference drug used in this assay. The results indicate that *P. rimosus* possesses significant anti-inflammatory activity in a dose-dependent manner, highlighting its potential as a natural source for anti-inflammatory agents **(Table 2).**

Table 2. COX Inhibition Activity of the Ethanolic Extract of *P. rimosus.*

Drug Conc. (µg)	**% of Inhibition**	
	P.rimosus	**Diclofenac**
LPS	0	0
25	24.21 ± 0.615	35.08 ± 0.492
50	48.28 ± 0 .772	58.37 ± 0.590
100	62.03 ± 0.147	78.80 ± 0.393

Values are mean ± SD, n=3.

Inhibition of Lipoxygenase (LOX) Activity

In LOX inhibition assay, the ethanolic extract also exhibited a dose-dependent activity **(Table 3)**. At a concentration of 25 µg/ml, the extract exhibited 24.77%

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inhibition of LOX activity, which increased to 49.29% at 50 µg/ml and reached 62.47% at 100 µg/ml. Activity was comparable to that of the standard Diclofenac. These findings reinforce the dose-dependent anti-inflammatory potential of *P. rimosus*, as evidenced by its inhibitory effects on both COX and LOX pathways. The extract exhibited a similar range of inhibitory activity against both COX and LOX enzymes.

Table 3. LOX Inhibition Activity of the Ethanolic Extract of *P. rimosus.*

Drug Conc. (µg)	**% of Inhibition**	
	P.rimosus	**Diclofenac**
LPS	0	0
25	24.76± 0.592	33.89 ± 0.710
50	49.29 ± 0 .592	55.89 ± 0.828
100	62.47 ± 1.597	81.88 ± 0.887

Values are mean ± SD, n=3.

Inhibition of Inducible Nitric Oxide Synthase (iNOS)

As presented in **Table 4**, the ethanolic extract demonstrated a dose-dependent iNOS inhibition. At 25 µg/ml, the extract showed 19.38% inhibition, which increased to 41.06% at 50 µg/ml and reached 54.01% at 100 µg/ml, which was comparable to the activity of the standard used. These results further support the dose-dependent anti-inflammatory potential of *P. rimosus*, as evidenced by its consistent activity across multiple inflammatory pathways, including COX, LOX, and iNOS. However, the extract was more efficient in the inhibition of COX and LOX compared to iNOS, indicating a relatively stronger effect on prostaglandin and leukotriene pathways.

Table 4. iNOS Inhibition Activity of the Ethanolic Extract of *P. rimosus.*

Drug Conc. (µg)	**% of Inhibition**	
	P.rimosus	**Diclofenac**
LPS	0	0
25	19.38 ± 0.958	31.97 ± 1.149
50	41.06 ± 0.952	51.36 ± 0.574
100	54.61 ± 0.950	78.18 ± 0.574

Values are mean ± SD, n=3.

Antidiabetic Potential of Ethanolic Extract of *P. rimosus*

Yeast Glucose Uptake Assay

The mechanism of glucose transport across the yeast cell membrane is widely recognized as a valuable in vitro method for screening the hypoglycaemic potential of various compounds. In this assay, the amount of glucose remaining in the medium after a specific incubation period serves as an indicator of glucose uptake by yeast cells. In our study, there was no significant reduction in glucose levels in the test samples compared to the control, indicating that the ethanolic extract of *P. rimosus* does not enhance glucose uptake by yeast cells.

α-Amylase Inhibitory Assay

Alpha-Amylase plays a crucial role in the digestion of dietary starch, breaking it down into oligosaccharides that are further hydrolyzed into absorbable monosaccharides at the intestinal brush border. Inhibiting this enzyme is considered an effective strategy for managing diabetes by reducing postprandial glucose levels. In the present study, the ethanolic extract of *P. rimosus* exhibited a concentration-dependent inhibition of alpha-Amylase activity, as shown in **Figure 4**. The maximum inhibitory effect observed was 91.89% at a concentration of 2000 µg/ml, which was comparable to the activity of the standard drug used. The IC_{50} value for *P. rimosus* extract was calculated as 169.03 µg/ml, compared to 84.58 µg/ml for acarbose.

Monosaccharides/ disaccharides were differentiated from polysaccharides (starch) by using the iodine reagent. While monosaccharides and disaccharides remain colorless in the presence of iodine, the appearance of a blue-black or purple color indicates the presence of starch, confirming a positive result. In this study, an increase in blue coloration with higher extract concentrations suggested the inhibition of starch breakdown, further supporting the alpha-Amylase inhibitory effect of the extract. The ability to inhibit the action of carbohydrate-digesting enzyme α-Amylase may be one of the reasons for the antidiabetic potential of *P. rimosus.*

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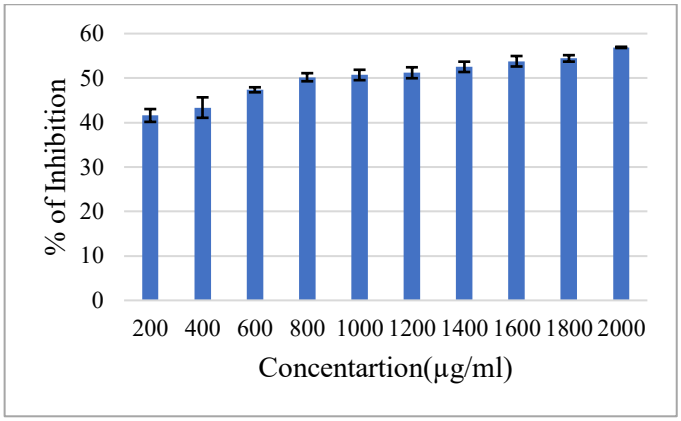

Figure 4. Antidiabetic Potential of Ethanolic Extract of *P. rimosus* by α-Amylase Inhibition Assay. Values are mean ± SD, n=3.

5. Discussion

Phellinus species are widely distributed and cultivated across East Asia, particularly in China, Japan, and Korea, where they serve as valuable raw materials for medicines, cosmetics, and functional foods. Extensive pharmacological research has demonstrated that the bioactive compounds in *Phellinus* spp. offer a broad range of health benefits, including antitumor, gut microbiota regulation, anti-inflammatory, immunomodulatory, hypoglycemic, hypolipidemic, antioxidant, antimicrobial, antiviral, anti-arthritic, hepatoprotective, anticancer, and pro-apoptotic effects. [12, 16-19, 25-28]

The volatile and semi-volatile organic compounds like organic acids, phenols, long-chain and branched hydrocarbons, alcohols, ketones, esters, etc. can be effectively analysed by GC-MS, a powerful technique that combines the high resolution separation of GC with the structure identification capacity of MS.[29] The integration of the NIST 20 database with GC-MS further enhances the accuracy and reliability of compound identification by enabling precise spectral matching with an extensive library of known chemical profiles. Several studies have employed GC-MS analysis to identify various bioactive compounds in wild mushrooms, such as fatty acids, amino acids, sugar alcohols, and polysaccharides.[30]

Many of the components identified in *P.rimosus* using GC-MS are already reported to possess significant medicinal properties. Methanolic extract of Kei apple (*Dovyalis caffra*), rich in dotriacontane, showed antioxidant potential and anti-anticancer activity against HepG2 cells. Molecular docking studies by Qanash *et al.*[31] further supported the therapeutic relevance of dotriacontane from Kei apple. Mishra *et al.*[32] identified dotriacontane as a major component in the acetone extract of *Curcuma raktakanta*, a lesser-known species from Kerala, India, by GC-MS analysis. In this study, dotriacontane significantly reduced the viability of cancer cell lines of breast, cervical, and glioma, with the strongest effect on C-6 glioma cells, in a dose-dependent manner. Though the anti-inflammatory activity of dotriacontane has not been much studied, its antioxidant and anti-cancer properties show its potential to be an effective anti-inflammatory agent.

Although phthalate esters (PAEs) are often considered synthetic additives or man-made pollutants, their natural production by plants, algae, bacteria, and fungi suggests ecological significance. PAEs exhibit allelopathic, antimicrobial, and insecticidal properties, which might enhance the competitiveness of plants, algae, and microorganisms to better accommodate biotic and abiotic stress. [33,34] Diethyl phthalate (DEP), identified through GC-MS analysis of mushroom extracts, has demonstrated antimicrobial activity against *E. coli*[35] and quorum-sensing inhibitory effects as well as cytotoxicity against MDA-MB-231 breast cancer cells.[34] The naturally occurring PAEs have been well documented to exhibit a wide range of properties, including antidiabetic, antimicrobial, anti-inflammatory, antitumor, antioxidant, apoptosis-inducing, cell cycle arrest-inducing, and cytotoxic effects. These findings underscore the potential of exploring natural PAEs as promising candidates for drug discovery.[36]

n-Hexadecanoic acid (palmitic acid) exhibits a wide spectrum of pharmacological properties, including antiviral, anti-inflammatory, analgesic effects, and the ability to regulate lipid metabolism.[37] Purushothaman *et al.*[38] demonstrated, through molecular docking studies, that HDA isolated from the mangrove plant *Excoecaria agallocha* L. induced remarkable anti-inflammatory properties by interacting with the enzymes COX-1 and COX-2. Additionally, palmitic acid (PA) has been reported to induce cell cycle arrest and trigger apoptosis in human neuroblastoma and breast cancer cells [39], highlighting its anticancer potential. Ganesan *et al.*,[40] reported the free radical scavenging activities of *n*-hexadecanoic acid

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isolated from the leaves of *Ipomoea eriocarpa* through assays like DPPH, ABTS, reducing power, superoxide, and nitric oxide inhibition. Tetradecanoic acid (myristic acid) exhibited antimicrobial activity by disrupting microbial membranes, anti-inflammatory effects through downregulation of pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α, and showed potential anticancer activity by modulating cellular transformation and virulence pathways. [41-43]

Nisa *et al.,*[44] reported the isolation and characterization of Hexadecanoic acid, ethyl ester from *Arisaema flavum* (Forssk.) Schott. and found that it possesses significant anticancer activity against breast cancer cell line MCF-7. Antioxidant, anti-inflammatory, hypocholesterolemic, anti-cancer, antifungal, antitumor, antibacterial, antiarthritic, and anti-coronary activities are other pharmacological properties associated with Hexadecanoic acid, ethyl ester.[45,46] The anti-inflammatory pathway of azulene is considered complex as it involves interaction with multiple biological pathways. One of the key mechanisms involved is the inhibition of the enzyme COX-2, which participates in the biosynthesis of prostaglandins, key mediators of inflammatory processes. [47,48]

The presence of major components such as Dotriacontane, Diethyl Phthalate, *n*-Hexadecanoic acid, Tetradecanoic acid, Hexadecanoic acid ethyl ester, and Azulene, along with other active constituents in moderate and low concentrations, which have been already reported in the literature for their pharmacological potential, may be responsible for the significant biological activities shown by the ethanolic extract from *P. rimosus.*

Research indicates that both oxidative stress and inflammation are involved in the onset of insulin resistance, a hallmark of type 2 diabetes (T2D).[49] These processes activate stress-related kinases such as c-Jun N-terminal kinase (JNK) and IκB kinase (IKK), which interfere with insulin signalling by inhibiting insulin receptor substrate-1 (IRS-1). OS is also a key contributor to dysfunction in various organs targeted by diabetes.[50] Chronic oxidative stress and persistent inflammation not only promote insulin resistance but also lead to sustained hyperglycemia. Elevated blood glucose levels can, in turn, increase free radical formation and impair the body's antioxidant defenses, initiating persistent inflammation. This interconnected cycle, linking oxidative stress, inflammation, insulin resistance, and hyperglycemia, intensifies the risk of complications associated with diabetes, such as diabetic nephropathy, retinopathy, hepatic injury, as well as brain and heart damage.[49]

Inhibiting COX-2 and 5-LOX is a well-established approach in anti-inflammatory drug development.[7] Nitric oxide (NO) and inducible nitric oxide synthase (iNOS) play multifaceted roles in inflammation. Elevated expression of iNOS is associated with many human ailments. Targeting iNOS to reduce NO production may also represent a promising anti-inflammatory approach.[29] Kim *et al.* [51] reported that treatment with *Phellinus igniarius* in RAW 264.7 macrophage cells led to a reduction in nitric oxide (NO) production, enhanced free radical scavenging, and a significant downregulation of mRNA expression for IL-5, IL-1α, IL-1β, iNOS, TNF-α, and COX-2. In the current study, the ethanolic extract of *P.rimosus* effectively downregulated key inflammatory mediators, including COX, LOX, and iNOS, which may have played a crucial role in its marked anti-inflammatory activity.

Mushrooms are a less tapped, but a significant reservoir of biologically active metabolites with a wide spectrum of medicinal properties. A mushroom-based formulation containing ethanolic extracts of *Morchella esculenta* and *Daedaleopsis nitida* exhibited significant antidiabetic effects in streptozotocin-induced diabetic rats. [52] Similarly, Wu and Zu[53] evaluated the antidiabetic potential of 70% ethanol extracts from several mushroom species—including *Auricularia auricula-judae*, *Hericium erinaceus*, *Ganoderma lucidum*, *Tremella fuciformis*, *Lentinus edodes*, *Russula sanguinea*, *Grifola frondosa*, and *Agrocybe aegerita,* and reported promising results. In a study by Yang *et al.,*[54] the ethyl acetate extract of *Phellinus baumii* demonstrated antidiabetic activity, primarily attributed to its antioxidant properties that protected pancreatic β-cells from oxidative stress-induced damage. Similarly, *Phellinus igniarius* was reported to exhibit antioxidant and anti-inflammatory effects in RAW 264.7 mouse macrophages by suppressing pro-inflammatory cytokines, leading to reduced synthesis of prostaglandin E2 (PGE2) and nitric oxide (NO).[51] In the antidiabetic analysis, the ethanolic extract of *Phellinus rimosus* did not enhance glucose uptake by yeast cells. However, α-Amylase inhibitory activity shown by the ethanolic extract suggests that the antidiabetic effects may

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be due to the inhibition of carbohydrate-digesting enzymes rather than glucose uptake enhancement.

6. Conclusion

The ethanolic extract of *P.rimosus* demonstrated potent anti-inflammatory, antioxidant, and antidiabetic activities—three interlinked pharmacological effects that collectively contribute to its therapeutic potential. Its ability to scavenge free radicals, along with the significant downregulation of key inflammatory mediators such as COX, LOX, and iNOS, suggests a dual mechanism in mitigating oxidative stress and inflammation. These effects, in turn, appear to play a crucial role in alleviating hyperglycemic conditions, underscoring the extract's ability to manage diabetes. α-Amylase inhibitory activity of the mushroom also adds to its antidiabetic potential. However, further detailed analysis is necessary to elucidate the exact mechanism of action of the antidiabetic activity of this mushroom. However, the present in vitro investigation highlights that the mushroom *Phellinus rimosus* is a promising candidate for the development of natural therapeutic agents.

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References

1. Singh, G. K. K. Antimicrobial and Phytochemical screening of selected wild mushrooms naturally found in Garhwal Himalayan region, Uttarakhand, India. *JABET* **2022**, *5* (2), 417. https://doi.org/10.5455/jabet.2022.d125.
2. Anusiya, G.; Prabu, U. G.; Yamini, N. V.; Sivarajasekar, N.; Rambabu, K.; Bharath, G.; Banat, F. A review of the therapeutic and biological effects of edible and wild mushrooms. *Bioengineered* **2021**, *12* (2), 11239–11268. https://doi.org/10.1080/21655979.2021.2001183.
3. Badalyan, S. M.; Barkhudaryan, A.; Rapior, S. Recent progress in research on the pharmacological potential of mushrooms and prospects for their clinical application. In *Springer eBooks*; **2019**; pp 1–70. https://doi.org/10.1007/978-981-13-6382-5_1.
4. Kothiyal, G.; Singh, K.; Kumar, A.; Juyal, P.; Guleri, S. Wild macrofungi (mushrooms) diversity occurrence in the forest of Uttarakhand, India. *Int. J. Bot. Stud.* **2022**, *7* (1), 567–578.
5. Beckman, J. S.; Koppenol, W. H. Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. *AJP Cell Physiology* **1996**, *271* (5), C1424–C1437. https://doi.org/10.1152/ajpcell.1996.271.5.c1424.
6. Stadler, K. Peroxynitrite-Driven Mechanisms in diabetes and insulin resistance – the latest advances. *Curr Med Chem* **2011**, *18* (2), 280–290. https://doi.org/10.2174/092986711794088317.
7. Rudrapal, M.; Eltayeb, W. A.; Rakshit, G.; El-Arabey, A. A.; Khan, J.; Aldosari, S. M.; Alshehri, B.; Abdalla, M. Dual synergistic inhibition of COX and LOX by potential chemicals from Indian daily spices investigated through detailed computational studies. *Sci Rep* **2023**, *13* (1). https://doi.org/10.1038/s41598-023-35161-0.
8. Zamora, R.; Vodovotz, Y.; Billiar, T. R. Inducible nitric oxide synthase and inflammatory diseases. *Mol Med* **2000**, *6* (5), 347–373. https://doi.org/10.1007/bf03401781.
9. Tsalamandris, S.; Antonopoulos, A. S.; Oikonomou, E.; Papamikroulis, G.-A.; Vogiatzi, G.; Papaioannou, S.; Deftereos, S.; Tousoulis, D. The role of inflammation in Diabetes: Current concepts and future Perspectives. *Eur Cardiol* **2019**, *14* (1), 50–59. https://doi.org/10.15420/ecr.2018.33.1.
10. Skrha, J. Diabetes mellitus 2. typu jako subklinický zánet [Type 2 diabetes mellitus as a subclinical inflammation]. Cas. Lék. Čes. **2010**, *149* (6), 277–281.
11. Oni, J. O.; Akomaye, F. A.; Markson, A. A.; Egwu, A. C. GC-MS analysis of bioactive compounds in some wild-edible mushrooms from Calabar, Southern Nigeria. *Eur. J. Biol. Biotechnol.* **2020**, *1* (6). https://doi.org/10.24018/ejbio.2020.1.6.129.
12. Zhang, H.; Jiang, F.; Li, L.; Liu, X.; Yan, J.-K. Recent advances in the bioactive polysaccharides and other

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www.jchr.org

JCHR (2025) 15(5), 219-232 | ISSN:2251-6727

key components from *Phellinus* spp. and their pharmacological effects: A review. *Int J Biol Macromol*. **2022**, *222*, 3108–3128. https://doi.org/10.1016/j.ijbiomac.2022.10.085.

13. Ajith, T. A.; Janardhanan, K. K. Antioxidant and antihepatotoxic activities of *Phellinus rimosus* (Berk) Pilat. *J Ethnopharmacol*. **2002**, *81* (3), 387–391. https://doi.org/10.1016/s0378-8741(02)00042-9.
14. Ajith, T. A.; Janardhanan, K. K. Antioxidant and anti-inflammatory activities of methanol extract of *Phellinus rimosus* (Berk) Pilat. *Indian J. Exp. Biol.* **2001,** *39*, 1166–1169.
15. Ajith, T. A.; Janardhanan, K. K. Antimutagenic effect of *Phellinus rimosus* (Berk) Pilat against chemical induced mutations of histidine-dependent *Salmonella typhimurium* strains. *Food Chem. Toxicol.* **2011**, *49*, 2676–2680. https://doi.org/10.1016/j.fct.2011.07.022.
16. Meera, C. R.; Janardhanan, K. K.; Karunagaran, D. Anti-proliferative and apoptotic activities of the medicinal mushroom *Phellinus rimosus* (Berk) Pilat on HCT 116 human colon cancer cells. *Int. J. Med. Mushrooms* **2018**, *20* (10), 935–945. https://doi.org/10.1615/IntJMedMushrooms.. 2018028343
17. Meera, C. R.; Janardhanan, K. K. Antitumor Activity of a Polysaccharide−Protein Complex Isolated from a Wood-Rotting Polypore Macro Fungus *Phellinus rimosus* (Berk) Pilat. *J Environ Pathol Toxicol Oncol*. **2012**, *31* (3), 223–232. https://doi.org/10.1615/jenvironpatholtoxicoloncol.v31.i3.40.
18. Janardhanan, K. K.; Vishwakarma, R. A.; Meera, C. R.; Nitha, B. Anti-inflammatory and Free Radical Scavenging Activities of Polysaccharide - Protein Complex Isolated from *Phellinus rimosus* (Berk.) Pilát (Aphyllophoromycetideae). *Int J Med Mushrooms*. **2009a**, *11* (4), 365–373. https://doi.org/10.1615/intjmedmushr.v11.i4.30.
19. Meera, C. R.; Smina, T. P.; Nitha, B.; Mathew, J.; Janardhanan, K. K. Antiarthritic Activity of a Polysaccharide-Protein Complex Isolated from *Phellinus rimosus* (Berk.) Pilát (Aphyllophoromycetideae) in Freund's Complete Adjuvant - Induced Arthritic Rats. *Int J Med Mushrooms* **2009b**, *11* (1), 21–28. http://doi.org/10.1615/IntJMedMushr.v11.i1.30
20. Rony, K. A.; Ajith, T. A.; Janardhanan, K. K. Hypoglycemic and Hypolipidemic Effects of the Cracked-Cap Medicinal Mushroom *Phellinus rimosus* (Higher Basidiomycetes) in Streptozotocin-Induced Diabetic Rats. *Int J Med Mushrooms* **2015**, *17* (6), 521–531. https://doi.org/10.1615/intjmedmushrooms.v17.i6.30.
21. Prieto, P.; Pineda, M.; Aguilar, M. Spectrophotometric Quantitation of Antioxidant Capacity through the Formation of a Phosphomolybdenum Complex: Specific Application to the Determination of Vitamin E. *Anal Biochem*. **1999**, *269* (2), 337–341. https://doi.org/10.1006/abio.1999.4019.
22. Walker, M. C.; Gierse, J. K. In vitro assays for cyclooxygenase activity and inhibitor characterization. In *Methods in Molecular Biology*; *Methods Mol Biol*. 2010; Vol. 644, pp 131–144.
23. Axelrod, B.; Cheesbrough, T. M.; Laakso, S. Lipoxygenase from Soybeans: EC 1.13.11.12 Linoleate: Oxygen Oxidoreductase. *Methods Enzymol.* **1981**, *71*, 441-451. https://doi.org/10.1016/0076-6879(81)71055-3.
24. Salter, M.; Duffy, C.; Garthwaite, J.; Strijbos, P. J. Ex vivo measurement of brain tissue nitrite and nitrate accurately reflects nitric oxide synthase activity in vivo. *J. Neurochem.* **1996**, *66* (4), 1683–1690. https://doi.org/ 10.1046/j.1471-4159.1996.66041683.x
25. Zheng, M.; Wang, L.; Sun, Y.; Pi, X.; Zhang, W.; Gao, P.; Lu, S.; Liu, W. Hypoglycemic effect of the *Phellinus baumii* extract with α-glucosidase-inhibited activity and its modulation to gut microbiota in diabetic patients. *Biomed. Pharmacother.* **2023**, *158*, 114130. https://doi.org/10.1016/j.biopha.2022.114130
26. Zheng, S.; Deng, S.; Huang, Y.; Huang, M.; Zhao, P.; Ma, X.; Wen, Y.; Wang, Q.; Yang, X. Anti-diabetic activity of a polyphenol-rich extract from *Phellinus igniariusin* KK-Ay mice with spontaneous type 2 diabetes mellitus. . *Food Funct*. **2017**, *9* (1), 614–623. https://doi.org/10.1039/c7fo01460k.
27. Lu, T.-L.; Huang, G.-J.; Lu, T.-J.; Wu, J.-B.; Wu, C.-H.; Yang, T.-C.; Iizuka, A.; Chen, Y.-F. Hispolon from *Phellinus linteus* has antiproliferative effects via MDM2-recruited ERK1/2 activity in breast and

bladder cancer cells. *Food Chem Toxicol*. **2009**, *47* (8), 2013–2021. https://doi.org/10.1016/j.fct.2009.05.023.

28. Mizuno, T. Development of an Antitumor Biological Response Modifier from *Phellinus linteus* (Berk. et Curt.) Teng (Aphyllophoromycetideae) (Review). *Int J Med Mushrooms*. **2000**, *2* (1), 21-33. https://doi.org/10.1615/intjmedmushr.v2.i1.20.
29. Kothiyal, G.; Singh, K.; Kishor, D.; Kamal, R.; Kumar, D.; Guru, S.; Rai, R. GC-MS Analysis for Determination of Bioactive Compounds of *Phellinus pectinatus*: A Species of Wild Mushroom from Uttarakhand Himalaya, India. *J. Chem. Health Risks* 2024, *14* (3), 2005–2013.
30. Smiderle, F. R.; Carbonero, E. R.; Mellinger, C. G.; Sassaki, G. L.; Gorin, P. A. J.; Iacomini, M. Structural characterization of a polysaccharide and a β-glucan isolated from the edible mushroom *Flammulina velutipes*. *Phytoche.* **2006**, *67* (19), 2189–2196. https://doi.org/10.1016/j.phytochem.2006.06.022.
31. Qanash, H.; Yahya, R.; Bakri, M. M.; Bazaid, A. S.; Qanash, S.; Shater, A. F.; M, A. T. Anticancer, antioxidant, antiviral and antimicrobial activities of Kei Apple (Dovyalis caffra) fruit. *Sci Rep*. **2022**, *12* (1). https://doi.org/10.1038/s41598-022-09993-1.
32. Mishra, S.; Verma, S. S.; Rai, V.; Awasthee, N.; Arya, J. S.; Maiti, K. K.; Gupta, S. C. *Curcuma raktakanda* Induces Apoptosis and Suppresses Migration in Cancer Cells: Role of Reactive Oxygen Species. *Biomolecules* **2019**, *9* (4), 159. https://doi.org/10.3390/biom9040159.
33. Huang, L.; Zhu, X.; Zhou, S.; Cheng, Z.; Shi, K.; Zhang, C.; Shao, H. Phthalic Acid esters: Natural sources and biological activities. *Toxins* **2021**, *13* (7), 495. https://doi.org/10.3390/toxins13070495.
34. Rashiya, N.; Sangavi, J.; Padmini, N.; Langeswaran, K.; Alagarsamy, A.; Selvakumar, G.; Saravanan, M. In silico and in vitro analysis of diethyl phthalate as a quorum sensing inhibitor and its antitumor evaluation against MDA-MB-231 cell lines. *Mol Divers*. **2025**. https://doi.org/10.1007/s11030-025-11202-w.
35. Aruna, V. Antimicrobial activity of diethyl phthalate isolated from marine *Streptomyces* MW09-1: an in silico approach. *J. Adv. Sci. Res.* 2020, *11* (Suppl 6), 158-165.
36. Roy, R. N. Bioactive natural derivatives of phthalate ester. *Crit Rev Biotechnol*. **2020**, *40* (7), 913–929. https://doi.org/10.1080/07388551.2020.1789838.
37. Librán-Pérez, M.; Pereiro, P.; Figueras, A.; Novoa, B. Antiviral activity of palmitic acid via autophagic flux inhibition in zebrafish (Danio rerio). *Fish Shellfish Immunol*. **2019**, *95*, 595–605. https://doi.org/10.1016/j.fsi.2019.10.055.
38. Purushothaman, R.; Vishnuram, G.; Ramanathan, T. Anti-inflammatory efficacy of n-Hexadecanoic acid from a mangrove plant *Excoecaria agallocha* L. through in silico, in vitro and in vivo. *Pharmacol Res Nat Prod*. **2025**, 100203. https://doi.org/10.1016/j.prenap.2025.100203.
39. Hsiao, Y.-H.; Lin, C.-I.; Liao, H.; Chen, Y.-H.; Lin, S.-H. Palmitic Acid-Induced Neuron Cell Cycle G2/M Arrest and Endoplasmic Reticular Stress through Protein Palmitoylation in SH-SY5Y Human Neuroblastoma Cells. *Int J Mol Sci*. **2014**, *15* (11), 20876–20899. https://doi.org/10.3390/ijms151120876.
40. Ganesan, T.; Subban, M.; Leslee, D. B. C.; Kuppannan, S. B.; Seedevi, P. Structural characterization of n-hexadecanoic acid from the leaves of *Ipomoea eriocarpa* and its antioxidant and antibacterial activities. *Biomass Convers Biorefin*. **2022**, *14* (13), 14547–14558. https://doi.org/10.1007/s13399-022-03576-w.
41. Desbois, A. P.; Smith, V. J. Antibacterial free fatty acids: activities, mechanisms of action and biotechnological potential. *Appl Microbiol Biotechnol*. **2009**, *85* (6), 1629–1642. https://doi.org/10.1007/s00253-009-2355-3.
42. Alonso-Castro, A. J.; Serrano-Vega, R.; Gutiérrez, S. P.; Isiordia-Espinoza, M. A.; Solorio-Alvarado, C. R. Myristic acid reduces skin inflammation and nociception. *J. Food Biochem.* **2021**, *46* (1),e14013. https://doi.org/10.1111/jfbc.14013.
43. Huang, Q.; Chen, C.; Zhang, Z.; Xue, Q. Anti-inflammatory effects of myristic acid mediated by the NF-κB pathway in lipopolysaccharide-induced BV-2 microglial cells. *Mol.Omics* **2023**, *19* (9), 726–734. https://doi.org/10.1039/d3mo00063j.
44. Nisa, S.; Bibi, Y.; Masood, S.; Ali, A.; Alam, S.; Sabir, M.; Qayyum, A.; Ahmed, W.; Alharthi, S.; Santali, E. Y.; Alharthy, S. A.; Bawazir, W. M.; Almashjary, M. N. Isolation, characterization and

anticancer activity of two bioactive compounds from *Arisaema flavum* (Forssk.) Schott. *Molecules* **2022**, *27* (22), 7932. https://doi.org/10.3390/molecules27227932.

45. Gupta, V.; Tyagi, S.; Tripathi, R. Hexadecanoic acid methyl ester, a potent hepatoprotective compound in leaves of *Pistia stratiotes* L. *Appl Biol Chem J*. **2023**, 118–120. https://doi.org/10.52679/tabcj.2023.0012.
46. Gopalasamy, R.; Mohan, V. GC-MS analysis of bioactive components of *Hugonia mystax* L. bark (Linaceae). *J. Pharm. Biomed. Sci.* **2013**, *29*, 818-824.
47. Slon, E.; Slon, B.; Kowalczuk, D. Azulene and its derivatives as potential compounds in the therapy of dermatological and anticancer diseases: new perspectives against the backdrop of current research. *Molecules* **2024**, *29* (9), 2020.https://doi.org/10.3390/molecules29092020.
48. Bakun, P.; Czarczynska-Goslinska, B.; Goslinski, T.; Lijewski, S. In vitro and in vivo biological activities of azulene derivatives with potential applications in medicine. *Med Chem Res*. **2021**, *30* (4), 834–846. https://doi.org/10.1007/s00044-021-02701-0.
49. Papachristoforou, E.; Lambadiari, V.; Maratou, E.; Makrilakis, K. Association of Glycemic Indices (Hyperglycemia, Glucose Variability, and Hypoglycemia) with Oxidative Stress and Diabetic Complications. *J Diabetes Res*. **2020**, *2020*, 1–17. https://doi.org/10.1155/2020/7489795.
50. Sibony, R. W.; Segev, O.; Dor, S.; Raz, I. Overview of oxidative stress and inflammation in diabetes. *J Diabetes*. **2024**, *16* (10). https://doi.org/10.1111/1753-0407.70014.
51. Kim, E.-H.; Choi, Y.-S.; Kim, Y.-M. Antioxidative and anti-inflammatory effect of *Phellinus igniarius* on RAW 264.7 macrophage cells. *J Exerc Rehabil*. **2019**, *15* (1), 2–7. https://doi.org/10.12965/jer.1938010.005.
52. Saleem, B.; Hussain, G.; Rasul, A.; Anwar, H.; Hassan, M. Antidiabetic potential of mushroom-based herbal formulation in Streptozotocin-Induced diabetic rats. *Scientifica* **2024**, *2024* (1). https://doi.org/10.1155/2024/7468975.
53. Wu, T.; Xu, B. Antidiabetic and antioxidant activities of eight medicinal mushroom species from China. *Int J Med Mushrooms*. **2015**, *17* (2), 129–140. https://doi.org/10.1615/intjmedmushrooms.v17.i2.40.
54. Yang, K.; Zhang, S.; Ying, Y.; Li, Y.; Cai, M.; Guan, R.; Hu, J.; Sun, P. Cultivated fruit body of *Phellinus baumii*: A potentially sustainable antidiabetic resource. *ACS Omega* **2020**, *5* (15), 8596–8604. https://doi.org/10.1021/acsomega.9b04478.