Research Article

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In vitro exploration of *Hypsizygus ulmarius* (Bull.) mushroom fruiting bodies: Potential antidiabetic and anti-inflammatory agent

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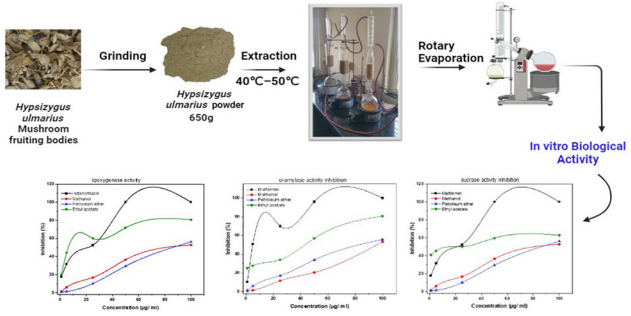
Abstract: The growing interest in exploring mushrooms and their bioactive components as potential therapies for diabetes and inflammatory conditions has prompted our investigation. In this study, we examined the methanolic extract, as well as the petroleum ether and ethyl acetate fractions, derived from the fruiting bodies of *Hypsizygus ulmarius* and assessed the potential *in vitro* anti-inflammatory and anti-diabetic effects. The inhibition of salivary α-amylase, salivary sucrase, and α-glucosidase enzymes by the methanolic extract and its fractions was used to measure the level of antidiabetic activity. Further, the inhibitory effects of the enzymes lipoxygenase (LOX), cyclooxygenase (COX), and myeloperoxidase (MPO) were tested to assess the anti-inflammatory efficacy of the methanolic extract and its fractions. The fraction containing ethyl acetate has been demonstrated to have the highest level of *in vitro* antidiabetic effect, exhibiting IC_{50} values of 44.93, 27.70, and 44.75 µg/ml for salivary α-amylase, salivary sucrase, and α-glucosidase enzymes, respectively. Moreover, the fraction of ethyl acetate revealed the greatest *in vitro* anti-inflammatory action, with IC_{50} values of 25.67 µg/ml for LOX, 34.04 µg/ml for COX, and 38.71 µg/ml for MPO.



Graphical abstract

Keywords: *Hypsizygus ulmarius* (Bull.) mushroom, methanol extract, petroleum ether fraction, ethyl acetate fraction, antidiabetic, anti-inflammatory

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1 Introduction

Edible mushrooms have gained more attention from researchers in recent years because of their potential as a source of medication [1] rather than their nutritional worth, flavor, or texture. Many of the cultured broth, cultured mycelia, and fruiting bodies of mushrooms exhibit a variety of biological actions, including immunomodulating and cancer-fighting abilities due to the presence of substances with biological activity including steroids, alkaloids, lactones, triterpenes, phenolic compounds, and flavonoids as well as polysaccharides, especially β-glucans polysaccharide–protein complexes and polysaccharide–protein complexes [2–4].

Furthermore, a variety of drugs made from mushrooms possess approximately 130 pharmacological functions, such

as treating diseases caused by *Helicobacter pylori*, including immunological disorders, especially in immunosuppressed and immunodeficient individuals, stomach ulcers caused by *H. pylori* and chronic gastritis. In addition, it plays a role in the treatment of cancers, anemia, bacterial, parasitic, and fungal infections, free radical, chronic fatigue syndrome, hypercholesterolemic, diabetic and cardiovascular diseases, Epstein-Barr virus, an acquired immunodeficiency syndrome, Herpes simplex virus, chronic hepatitis B, C, and D infections, patients receiving chemotherapy or radiation therapy and people with dementia (especially Alzheimer's disease) [5–7].

Diabetes mellitus, commonly referred to as diabetes, is a chronic metabolic disorder, indicated by raised blood glucose levels (hyperglycemia). There are two most common types of diabetes mellitus. If there is a complete deficiency of insulin secretion as a result of the autoimmune destruction of beta-pancreatic cells, it is known as type I diabetes but type II diabetes mellitus occurs when there is insulin dysfunction, insufficient insulin secretion by the pancreatic beta cells, and delayed insulin secretion due to insulin resistance. In addition to type 1 and type 2 diabetes, diabetes mellitus can occur due to a genetic abnormality, other pathologic disorders, clinical conditions, or gestational diabetes mellitus. It was predicted that 536.6 million people in 2021 will be diabetic; also by the year 2045, 783.2 million people will have diabetes [8–12].

There are many enzymes responsible for the degradation of carbohydrates and increasing glucose levels in the blood involved in diabetes, such as the salivary α-amylase enzyme, which starts the starch's hydrolysis into maltose. Further breakdown of the starch is completed by pancreatic amylase later in digestion, while hydrolysis of sucrose into glucose and fructose occurs by salivary sucrase enzyme and α-glucosidase enzyme releases glucose from maltose and sucrose [13–19].

Many studies were conducted on mushrooms to investigate their ability to control diabetes mellitus and its complications [20–23]. In a study conducted by Shoba and Krishnakumari [21], methanol and extracts of water of fresh fruiting bodies of *Pleurotus eous* displayed an inhibitory effect toward α-amylase with IC_{50} values of 460 and 500 µg/ml, respectively. Also, they showed inhibition activity against α-glucosidase with IC_{50} values of 325 and 280 µg/ml, respectively.

Moreover, the inhibitory effect of *Ganoderma neojaponicum* fruiting bodies in hot aqueous extracts (at various times) was observed. The maximum inhibitory effect on α-amylase and α-glucosidase enzymes was achieved by the purified polysaccharide fraction with IC_{50} values of 12.85 and 165.08 µg/ml, respectively [22].

Also, the antidiabetic effects of 80% hot water and methanol extracts from *Trametes pubescens* fruiting bodies using α-amylase and α-glucosidase assays were revealed [23]. The methanol extract showed a higher activity on α-amylase with inhibition activity of more than 50% at a concentration of 0.25 mg/ml, while both extracts have the same inhibition activity on α-glucosidase (more than 50%) in the concentration range of 0.5–2.0 mg/ml.

Inflammation is a biological process that occurs due to harmful stimuli or injury; for instance ROS, microbes, irritation from heat, or injured cells, ionizing radiation, or UV light [24]. There is much evidence indicating that inflammation plays a role in the development and regulation of various stages of cancer progression as well as the pathogenesis of mitochondrial dysfunction and many degenerative diseases, such as diabetes, dementia, arthritis, and multiple sclerosis [25–27]. Myeloperoxidase (MPO), lipoxygenase (LOX), and cyclooxygenases (COXs) are the three principal enzymes that mediate inflammation. These enzymes are also involved in inflammation and various degenerative disorders [28–32].

Arachidonic acid and linoleic acid, two poly-unsaturated fatty acids, are oxygenated by the LOX enzyme to produce physiologically active metabolites that are involved in inflammatory and immunological responses [33–35]. It is an essential enzyme in the biosynthesis of leukotrienes, which are crucial players in a number of inflammatory disorders [36–38].

The proinflammatory heme protein enzyme MPO, which is produced by activated leukocytes, is important for the host's defense in inflammatory areas because it produces halogenating chemicals like hypochlorous acid and hypobromous acid [39,40].

Moreover, inflammatory-related disorders such as cancer, renal disease, atherosclerosis, acute coronary syndromes advance, and ischemic heart disease more quickly respond to high concentrations of MPO-related halogenating species [31,41,42].

Prostaglandin (PG) endoperoxide synthase, or COX, is a kind of oxidoreductase enzyme necessary for the generation of biological modulators from arachidonic acid, including PGs, prostacyclins, and thromboxane. This enzyme has a role in inflammation, fever, pain, cancer, and tumorigenesis [43–46].

One of the most common medications for treating inflammation is the NSAID; however, several studies have shown that long-term use can have negative effects on the gastrointestinal tract, pushing for the development of safe, practical anti-inflammatory medications derived from natural sources, like mushrooms [47].

The anti-inflammatory activity of mushroom extracts has been documented by many researchers. Kamiyama et al. assessed the anti-inflammatory effects of *Trametes*

versicolor fruiting bodies using a LOX enzyme activity assay. They observed that (steam distillation) dichloromethane extract showed the highest LOX inhibitory percentage activity at 81.3%, followed by acetone extract at 50%, while the acetone extract from soxhlet extraction demonstrated potent anti-inflammatory effects at 70.6% [48]. Another study found that the acetone extract of mycelia showed the highest inhibition percentage on LOX activity, with 89.1% inhibition, followed by the aqueous extract of supernatant with 88.2% inhibition [49]. Nowadays, researchers are more interested in studying natural product extracts that contain a variety of active pharmacological substances and a variety of extraction techniques such as percolation, maceration, and extraction methods by using pressurized liquid, microwave-assisted, or Soxhlet extraction. The Soxhlet extraction technique exhibited a high extraction efficiency and required only a small amount of solvent and less time to run [50,51]. Therefore, our current research focuses on the anti-diabetic and anti-inflammatory effects of the methanolic extract, petroleum ether, and ethyl acetate fractions extracted from the fruiting bodies of *Hypsizygus ulmarius*. The level of antidiabetic activity of methanolic extract and its fractions was identified by the inhibition of salivary α-amylase, salivary-sucrase, and α-glucosidase enzymes. In the same way, the effects of LOX, COX, and MPO enzyme inhibitors were studied to find out how well the methanolic extract and its fractions fight inflammation.

2 Materials and methods

The materials utilized during the investigation work included samples of diabetic enzymes, specifically salivary α-amylase and salivary sucrose, which were collected from a volunteer's saliva after a meal. α-Glucosidase, a pure enzyme, was purchased as a pure enzyme (yeast, *Saccharomyces cerevisiae*, Sigma Aldrich G5003-100UN). For anti-inflammatory enzymes, LOX, MPO, and COX were isolated from white blood cells obtained from a volunteer. Standard metformin (Glycomet 500 tablets) was sourced from USV Ltd, while standard indomethacin (Indocid tablets) was obtained from Cipla Ltd.

The fungus *Hypsizygus ulmarius* (Bull.) (Elm Oyster mushroom) culture was kindly provided by the Indian Institute of Horticultural Research Kandapura, Bengaluru, Karnataka, India. The fungus was maintained at 'S' Mushroom Agritech, Hyderabad, Telangana state, India, where it was cultured to produce fruiting bodies. The collected mushroom was reported by the supplier to grow at 25°C and was dried properly by the solar method (dehydrated *Hypsizygus ulmarius* (Bull.) fruiting bodies).

2.1 Preparation of extracts

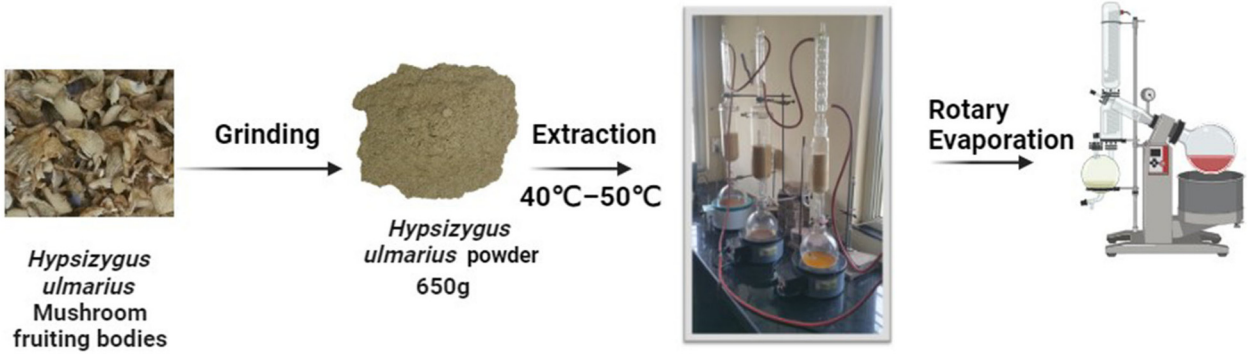
The dried mushroom was ground with an electric mixer grinder. About 650 g of the dried powder was extracted with 5 L of methanol (40–50°C) utilizing Soxhlet extractor (14 cycles). After extraction, a rotary evaporator was used to evaporate the extract [52] (Scheme 1).

2.2 Fractionation of the methanolic extract

About 90 g of the methanolic extract was fractionated using petroleum ether and ethyl acetate. A rotary evaporator was used to evaporate the extracted fractions.

2.3 Antidiabetic activity

The in vitro antidiabetic activities of the methanol extract, petroleum, ether, and ethyl acetate fractions were evaluated by the inhibition of salivary α-amylase activity, salivary sucrase activity, and α-glucosidase activity assays.



Scheme 1: Preparation of a methanol extract from *Hypsizygus ulmarius* (Bull.) fruiting bodies.

2.3.1 Inhibition of salivary α-amylase activity

The salivary α-amylase inhibition study was conducted according to the method of Dehghan et al. [53]. The enzyme salivary amylase was collected from a volunteer soon after the meal and was diluted (1:5) in 0.1 M phosphate-buffered saline (pH 6.9). The enzyme solution (500 µg equivalent) was pre-incubated with varying concentrations of methanol extract, petroleum ether, and ethyl acetate fractions (1, 5, 25, 50, and 100 µg/ml) for 10 min at 37°C. The enzymatic reaction was allowed to continue for 20 min at 37°C, and then a 0.1% starch solution was added to the incubation medium. After adding the 3,5-dinitrosalicylic acid (DNS, 1%) reagent to the reaction mixture, the tubes were kept in a boiling water bath for 10 min. The tubes were cooled until they were at room temperature and the absorbance was measured at 540 nm. Metformin was utilized as a positive control.

2.3.2 Inhibition of salivary sucrase activity

Sucrase activity in saliva was determined according to Karjalainen et al. [54]. The following is the protocol: increasing concentrations of the methanolic extract and its fractions (1, 5, 25, 50, and 100 µg/ml), 0.3 M sucrose, and 6.7 mM Na-acetate buffer (pH 4.7) were incubated with 200 µl of saliva for 2 h at 30°C. To stop the reaction, 0.5 ml solution of 1% DNS was added. The total amount of reducing sugars, or glucose and fructose, was determined colorimetrically at 540 nm after the reaction mixture was heated for 5 min at 100°C. By deducting the amount of nonenzymatic "free" glucose from the amount of glucose present in the reaction mixture that was incubated, the amount of glucose that was enzymatically liberated was calculated.

2.3.3 Inhibition of α-glucosidase activity

A pure enzyme system was utilized to investigate the inhibitory effects of the methanolic extract and its fractions on α-glucosidase activity [55]. In summary, the tested extracts were incubated with 0.2 units of α-glucosidase at increasing concentrations (1, 5, 25, 50, and 100 µg/ml) for 10 min. The addition of 4-nitrophenyl α-D-glucopyranoside solution in maleate buffer (0.1 M, pH 6) started the reaction. The reaction mixture was incubated at 37°C for 30 min. The addition of a 2 M NaOH solution stopped the reaction. At 400 nm, the enzyme's activity was determined with a spectrophotometer. Metformin was utilized as a positive control.

2.4 Anti-inflammatory activity

In vitro, the anti-inflammatory activity of the methanol extract, petroleum ether, and ethyl acetate fractions was evaluated by the inhibition of LOX, MPO, and COX enzymes.

2.4.1 Anti-inflammatory assay procedures

2.4.1.1 Isolation of human white blood cells

A crude white blood cell fraction was isolated from human blood according to Sengar et al. [56]. About 10 ml of human blood was collected into an EDTA-coated sample tube and centrifuged at 2,000 rpm for 10 min at room temperature. The supernatant plasma was aspirated out using a micropipette. The intermediate buffy coat containing white blood cells was isolated using a micropipette into a fresh sample tube. This was used as the enzyme sample for COX, MPO, and LOX activity assays.

2.4.1.2 Inhibition of LOX activity

The LOX inhibition assay was assessed using the Anthon and Barrett method [57]. In short, 0.2 unit of LOX was incubated with test extracts at different concentrations (1, 5, 25, 50, and 100 µg/ml) for 10 min, and subsequently, 1 ml of linoleic acid (50 µM) was added. The increase in the absorbance was measured at 234 nm using a spectrophotometer. The positive control was indomethacin.

2.4.1.3 Inhibition of MPO activity

The MPO inhibition test was performed using the method of Jyothilakshmi et al. [58]. In the presence of hydrogen peroxide, MPO oxidizes guaiacol into tetraguaiacol, which is measured at 460 nm using a colorimeter. The inhibitory properties of the extracts were estimated by incubating them at increasing concentrations (1, 5, 25, 50, and 100 µg/ml) for 10 min with the enzyme mixture. Indomethacin was used as a reference drug.

2.4.1.4 Inhibition of COX activity

COX inhibition was quantified [58] using the thiobarbituric acid reactive substance assay. Hemoglobin (5 mM), glutathione (5 mM), and the enzyme in Tris-HCl buffer (pH 8) were all included in the assay mixture. Subsequently, the enzyme mixture was supplemented with test extracts at varying concentrations (1, 5, 25, 50, and 100 µg/ml). Arachidonic acid was then added as the substrate, and the

mixture was incubated for 20 min at 37°C. Indomethacin was utilized as a reference standard medicine.

2.5 Percent of inhibition

The formula to calculate the percent of inhibition is a crucial tool in scientific research, particularly in studies involving enzyme inhibition or assessing the impact of inhibitory substances. The following formula was applied to compute the % inhibition:

$$\text{Percent of inhibition} = (A_{\text{control}} - A_{\text{sample}})/(A_{\text{control}}) \times 100,$$

where A_{control} is the absorbance of the control (enzyme mixture without extract) and A_{sample} is the absorbance of samples (enzyme mixture with extract).

2.6 IC_{50} value calculation

IC_{50} values were calculated using Microsoft Excel software. The higher the enzymes' inhibitory activity, the lower their IC_{50} values.

2.7 Statistical analysis

The mean ± standard error is used to express the experimental data. To perform statistical analyses, *t*-tests, and one-way ANOVA were used. When $p \leq 0.05$, the difference was considered statistically significant. The software used for this analysis was SPSS ver. 20.0.

3 Results and discussion

After extraction, the yield of the methanolic extract from fruiting bodies of *H. ulmarius* was 133 g (20.46%) (dark brown, semisolid, and viscous). Only 90 g from 133 g was fractionated by petroleum ether and ethyl acetate to yield 8 g (8.88%) (dark brown, semisolid, and sticky) and 4 g (4.44%) (dark brown and waxy), respectively.

3.1 Inhibition of salivary α-amylase

Table 1 displays the IC_{50} and inhibitory activity results for salivary α-amylase by the methanolic extract, petroleum ether, and ethyl acetate fractions.

As shown in Table 1 and Figure 1, the methanol extract and its fractions possess inhibitory activity against salivary α-amylase. Compared to the standard metformin drug (IC_{50} = 15.67 ± 0.003 µg/ml), the ethyl acetate fraction had the maximum inhibitory activity percentage at concentrations of 50 µg/ml (57.04%) and 100 µg/ml (80.74%) with IC_{50} of 44.93 ± 0.007 µg/ml, followed by petroleum ether fraction with inhibitory activity at a concentration of 100 µg/ml (55.74%) with IC_{50} of 86.09 ± 0.007 µg/ml, and methanol extract with IC_{50} of 97.76 ± 0.007 µg/ml at a concentration of 100 µg/ml (53.3% inhibition activity).

Table 1: IC_{50} values and salivary α-amylase inhibition activity by metformin, extracts, and their fractions

Standard, extracts, and their fractions	IC_{50} (µg/ml) ± standard error
Metformin	15.67 ± 0.003
Methanol	97.76 ± 0.007*
Petroleum ether	86.09 ± 0.007*
Ethyl acetate	44.93 ± 0.007*

Note: All values are expressed as mean ± SEM (n = 3). *When compared to a standard dose of indomethacin, the difference is statistically significant ($p \leq 0.05$).

3.2 Inhibition of salivary sucrase

Table 2 lists the IC_{50} and inhibitory activity of the methanolic extract and its fractions on the salivary sucrase enzyme. According to the results of a test on salivary sucrase enzyme inhibition, the methanolic extract and its fractions exhibit an affinity for suppressing the sucrase enzyme in saliva. As shown in Table 2 and Figure 2, the ethyl acetate fraction has the highest inhibitory action with

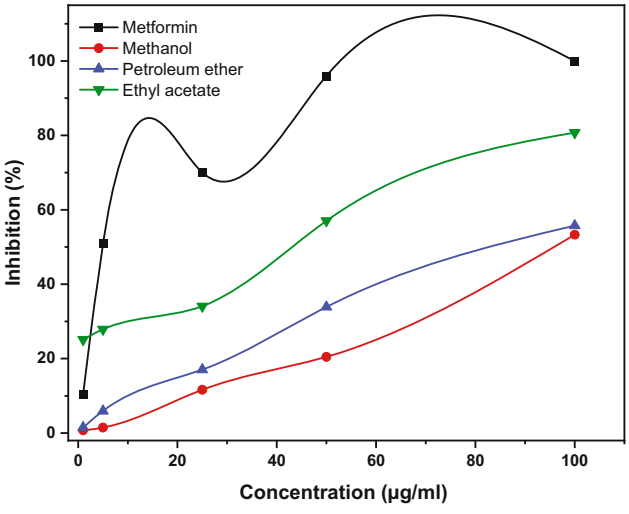


Figure 1: Percentage of salivary α-amylase inhibitory activity by metformin, extracts, and their fractions.

Table 2: IC_{50} values of salivary sucrase inhibition activity by metformin, extracts, and their fractions

Standard, extracts, and their fractions	IC_{50} (µg/ml) ± standard error
Metformin	23.87 ± 0.010
Methanol	88.48 ± 0.006*
Petroleum ether	88.97 ± 0.003*
Ethyl acetate	27.70 ± 0.003*

Note: All values are expressed as mean ± SEM (n = 3). *When compared to a standard dose of indomethacin, the difference is statistically significant ($p \leq 0.05$).

Table 3: IC_{50} values of α-glucosidase inhibition activity by metformin, extracts, and their fractions

Standard, extracts, and their fractions	IC_{50} (µg/ml) ± standard error
Metformin	30.82 ± 0.003
Methanol	98.32 ± 0.025*
Petroleum ether	90.10 ± 0.009*
Ethyl acetate	44.75 ± 0.006*

Note: All values are expressed as mean ± SEM (n = 3). *When compared to a standard dose of indomethacin, the difference is statistically significant ($p \leq 0.05$).

IC_{50} = 27.70 ± 0.003 µg/ml at concentrations of 25, 50, and 100 µg/ml, and inhibition activities of 50.1, 59.78, and 62.9%, respectively. Additionally, the IC_{50} values for the methanol extract and petroleum ether fraction are 88.48 ± 0.006 and 88.97 ± 0.003 µg/ml, respectively. The methanol extract and petroleum ether fraction showed inhibition activity at a concentration of 100 µg/ml with 52.79 and 56.19% inhibition activities, respectively. The conventional drug metformin demonstrated the highest level of inhibitory behavior, exhibiting an IC_{50} of 23.87 ± 0.010 µg/ml.

3.3 Inhibition of α-glucosidase

Table 3 shows the IC_{50} values for the α-glucosidase enzyme inhibition activity of petroleum ether, ethyl acetate fractions, and the methanolic extract. Results demonstrated that the methanol extract and its fractions have inhibition activity on the α-glucosidase enzyme. In contrast to the conventional metformin drug (IC_{50} = 30.82 ± 0.003 µg/ml), ethyl acetate fraction recorded the highest inhibitory activity with 50.72 and 60.09% at concentration of 50 and 100 µg/ml and with IC_{50} of 44.75 ± 0.006 µg/ml. Also, the petroleum ether fraction and methanol extract showed inhibitory activity of 53.57 and 51.89% at a concentration of 100 µg/ml and IC_{50} values of 90.10 ± 0.009 and 98.32 ± 0.025 µg/ml, respectively, as shown in Table 3 and Figure 3. Generally, the inhibition activities of the methanol extract and its fractions on the enzymes α-amylase, sucrase, and α-glucosidase were lower than the usual metformin medication.

3.4 Anti-inflammatory activity

3.4.1 Inhibition of LOX activity

Table 4 shows data on the inhibition activity and 50% inhibitory concentration (IC_{50}) of the methanolic extract and its

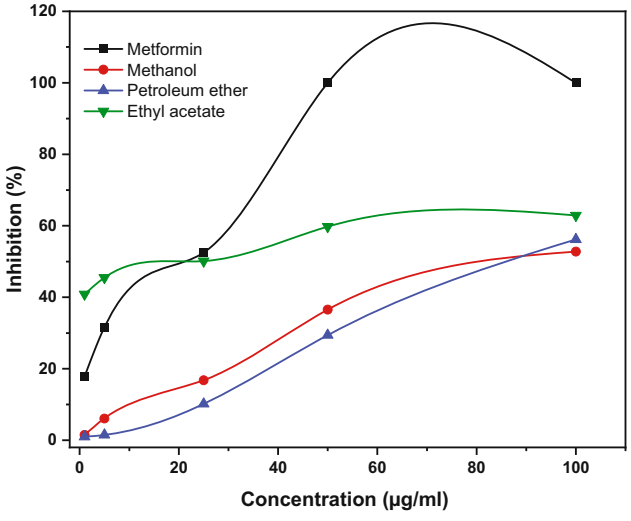


Figure 2: Percentage of salivary sucrase enzyme inhibition activity by metformin, extracts, and their fractions.

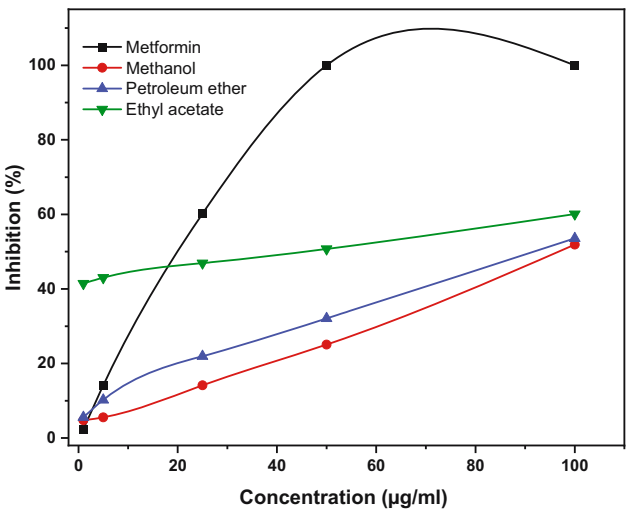


Figure 3: Percentage of α-glucosidase enzyme inhibition activity by metformin, extracts, and their fractions.

Table 4: IC_{50} values of LOX enzyme inhibition activity by indomethacin, extracts, and their fractions

Standard, extracts, and their fractions	IC_{50} (µg/ml) ± standard error
Indomethacin	23.87 ± 0.010
Methanol	88.48 ± 0.006*
Petroleum ether	88.97 ± 0.003*
Ethyl acetate	25.67 ± 0.003*

Note: All values are expressed as mean ± SEM (n = 3). *When compared to a standard dose of indomethacin, the difference is statistically significant ($p \leq 0.05$).

Table 5: IC_{50} values of MPO inhibition activity by indomethacin, extracts, and their fractions

Standard, extracts, and their fractions	IC_{50} (µg/ml) ± standard error
Indomethacin	24.53 ± 0.007
Methanol	120.34 ± 0.006*
Petroleum ether	65.83 ± 0.012*
Ethyl acetate	38.71 ± 0.006*

Note: All values are expressed as mean ± SEM (n = 3). *When compared to a standard dose of indomethacin, the difference is statistically significant ($p \leq 0.05$).

fractions on the LOX enzyme. Table 4 and Figure 4 exhibit the maximum inhibition activity of the LOX enzyme by the ethyl acetate fraction with an IC_{50} value of 25.67 ± 0.003 µg/ml and at concentration of 25, 50 and 100 µg/ml with inhibition percentages of 60.18, 71.73 and 80.62%, respectively.

Next, the IC_{50} values for the methanol extract and petroleum ether fraction were found to be 88.48 ± 0.006 and 88.97 ± 0.003 µg/ml, respectively, with inhibition percentages of 52.79 and 56.19% at a concentration of 100 µg/ml. The highest level of inhibitory activity was demonstrated by the standard indomethacin medication, with an IC_{50} of 23.87 ± 0.010 µg/ml.

3.4.2 Inhibition of MPO activity

The IC_{50} values and MPO enzyme inhibition activities for the fractions of petroleum, ethyl acetate, and methanolic extract are presented in Table 5. The results in Table 5 and Figure 5 indicate that the methanolic extract and its fractions possess an inhibitory effect on MPO activity. The ethyl acetate fraction showed the maximum inhibitory effect with inhibition percentages of 50.22, 52.17, 76.74, and 50.77% at concentrations of 5, 25, 50, and 100 µg/ml, respectively, and with an IC_{50} value of 38.71 ± 0.006 µg/ml. The petroleum ether fraction and methanol extract follow, with IC_{50} values of 65.83 ± 0.012 and 120.34 ± 0.006 µg/ml and inhibition percentages of 73.48 and 60.47%, respectively, at a concentration of 50 µg/ml. Also, it has been noted that at a higher concentration (100 µg/ml), the methanol extract and its fractions showed a decrease in inhibition activity, which probably means that a higher concentration of extracts has a lower inhibition activity (decreased gradually). The highest level of inhibitory effectiveness was seen with the drug standard indomethacin, which had an IC_{50} of 24.53 ± 0.007 µg/ml.

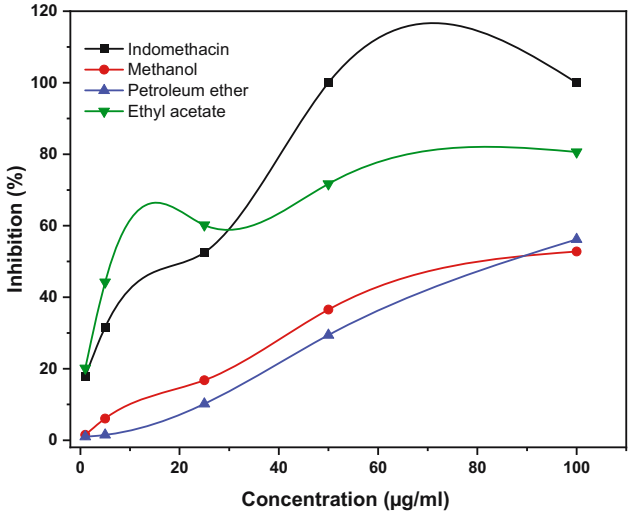


Figure 4: Percentage of LOX enzyme inhibition activity by indomethacin, extracts, and their fractions.

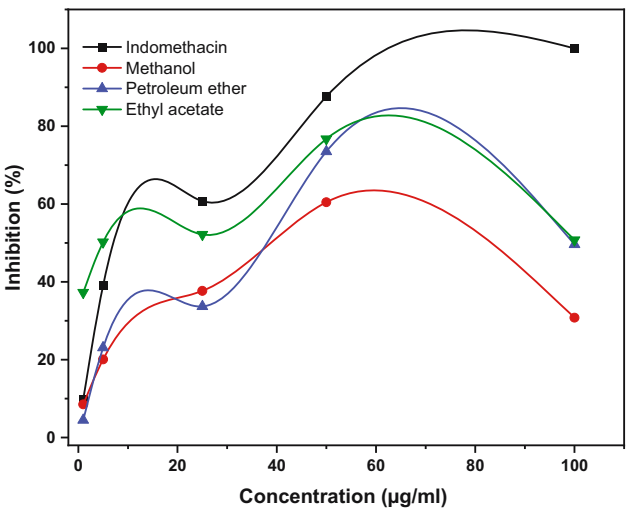


Figure 5: Percentage of MPO enzyme inhibition activity by indomethacin, extracts, and their fractions.

Table 6: IC_{50} values of COX inhibition activity by indomethacin, extracts, and their fractions

Standard, extracts and their fractions	IC_{50} (µg/ml) ± standard error
Indomethacin	28.97 ± 0.006
Methanol	100.15 ± 0.067*
Petroleum ether	89.36 ± 0.006*
Ethyl acetate	34.04 ± 0.006*

Note: All values are expressed as mean ± SEM (n = 3). *When compared to a standard dose of indomethacin, the difference is statistically significant ($p \leq 0.05$).

3.4.3 Inhibition of COX activity

Table 6 illustrates the IC_{50} values and the inhibitory activity of COX by the methanol extract and its fractions. Based on Table 6 and Figure 6 data, the COX activity was inhibited by the methanolic extract and its fractions. Ethyl acetate fraction, according to the results, exhibited the greatest inhibiting effect, with an IC_{50} of 34.04 ± 0.006 µg/ml and inhibition percentages of 55.52, 63.48, and 72.07% at concentrations of 25, 50, and 100 µg/ml, respectively. It was followed by petroleum ether fraction and methanol extract with IC_{50} values of 89.36 ± 0.006 and 100.15 ± 0.067 µg/ml, respectively, and inhibition percentages of 53.76 and 50.79% at a concentration of 100 µg/ml. The most effective inhibitor was standard indomethacin, with an IC_{50} of 28.97 ± 0.006 µg/ml. Generally, the inhibitory activity of the methanol extract and its fractions on LOX, COX, and MPO enzymes were lower than the standard indomethacin drug.

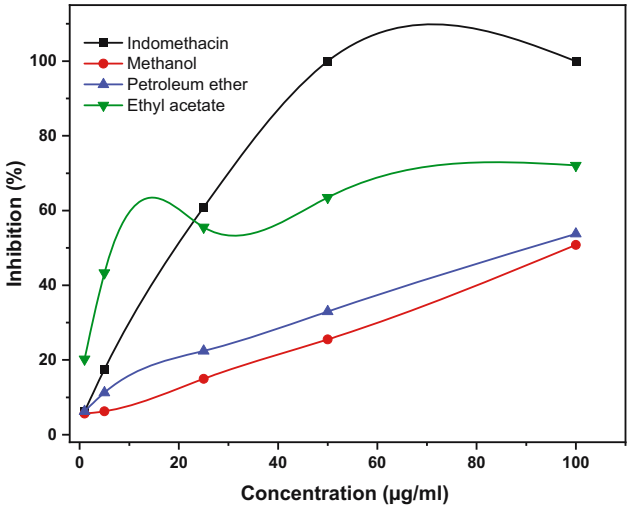


Figure 6: Percentage of COX enzyme inhibition activity by indomethacin, extracts, and their fractions.

Several studies have investigated the ability of mushrooms to control diabetes mellitus and its complications [20]. The results of these study showed higher antidiabetic activity in comparison to a previous study that found the fresh fruiting bodies of *Pleurotus eous* in both methanol and aqueous methanol extracts inhibited the activity of α-amylase with IC_{50} values of 460 and 500 µg/ml, respectively. Additionally, they demonstrated inhibitory efficacy on α-glucosidase with corresponding IC_{50} values of 325 and 280 µg/ml. [21]. Also, another study demonstrated the antidiabetic activity of *Daedaleopsis confragosa* in hot water (60°C), methanol, and ethanol extracts [59]. In their investigation, the strongest α-amylase inhibitory activity was recorded in the methanol extract, followed by hot water and ethanol extracts with IC_{50} of 34.63, 37.18, and 48.19 µg/ml, respectively, which is higher in activity compared to our methanol extract result.

Moreover, the inhibitory effects of *Auricularia auricula-judae* *n*-hexane, chloroform, ethyl acetate, and 80% methanol extracts on α-amylase activity were all observed [60]. The ethyl acetate extract showed the greatest inhibitory activity with IC_{50} of 14.05 mg/l, followed by the chloroform extract with IC_{50} of 14.3 mg/l and n-hexane extract with IC_{50} of 26.8 mg/l. 80%. The methanol extract has no inhibitory activity on the α-amylase enzyme. Compared to the results obtained from these study the ethyl acetate fraction from our results exhibited a lower activity against α-amylase (IC_{50} 44.93 µg/ml) and our methanol extract showed a higher activity (IC_{50} 97.76 µg/ml). Also, the α-amylase and α-glucosidase inhibitory effects of the methanolic extract of *Pleurotus florida* have been observed [61]. However, the methanol extract of *Pleurotus florida* displayed inhibition activity on α-amylase enzyme with IC_{50} of 35.96 µg/ml, which is higher than our result, but lower results on α-glucosidase enzyme with IC_{50} of 202.02 µg/ml were observed compared to our results.

Additionally, it has been demonstrated that a wide range of mushroom species have anti-inflammatory properties through pure culture mycelia, fruiting bodies, and culture broth extracts. Various bioactive metabolites, including phenolic compounds, flavonoids, alkaloids, steroids, β-glucan, polysaccharides, proteoglycan, lectin, triterpenoids, terpenes, schizophyllan, etc., are responsible for this activity [62,63].

Compared to our results, an investigation revealed that the *Geastrum fimbriatum* ethanol extract had anti-inflammatory effects on LOX activity at a concentration of 100 µg/ml, the extract of ethanol showed only 12.92% inhibition on the LOX enzyme activity, which is lower than our ethyl acetate fraction that showed an inhibition of 80.62% at a concentration of 100 µg/ml [64]. Furthermore, the anti-inflammatory activity of ergothioneine, a

mushroom-derived amino acid, from *Grifola frondosa, Lentinula edodes, Coprinus comatus,* and *Pleurotus comucopiae* has been investigated using the MPO activity assay [65]. *C. comatus* was the most effective mushroom extract among the four water extracts, showing 90% inhibition of MPO activity at a concentration of 1.0 mg/ml. This is less active than our ethyl acetate fraction, which at 50 µg/ml only demonstrated a 76.74% inhibition activity.

Also, the methanolic extracts of a 335 Korean native mushroom sample have been investigated for their anti-inflammatory properties using LOX activity assay [66]. Among 335 mushroom samples, the highest inhibition activity at a concentration of 100 µg/ml was shown by *Phellinus baumii* (100.0%) and *Inonotus mikadoi* (85.2%), which is greater than our result achieved by the ethyl acetate fraction (80.62%). In contrast, our results achieved by the ethyl acetate fraction was greater than the result obtained from *Collybia maculata* (73.3%), *Phellinus gilvus* (66.7%), *Phellinus linteus* (59.5%), *Strobilomyces confuses* (52.4%) and *Tylopilus neofelleus* (51.6%), respectively. Also, a fraction isolated from the methanolic extract of *P. baumii* (inoscavin A) demonstrated strong LOX inhibitory activity with an IC_{50} value of 6.8 µM, which is better than our results recorded by the ethyl acetate fraction (IC_{50} = 25.67 µg/ml).

Furthermore, the presence of bioactive phytochemical components, such as phenolic compounds, flavonoids, alkaloids, tannins, coumarin, steroid, anthraquinone, and saponin, maybe the reason for the antidiabetic and anti-inflammatory properties of our extracts, as reported by many researchers [67–74].

4 Conclusions

H. ulmarius fruiting bodies possess a variety of phytochemical compounds. The results from the present study indicated that the methanolic extract and its fractions demonstrated antidiabetic and anti-inflammatory activities (inhibitory activity on salivary α-amylase, salivary sucrose, α-glucosidase, LOX, COX, and MPO enzymes). For both diabetic-correlated enzymes (salivary α-amylase, salivary sucrase, and α-glucosidase) and inflammatory-involved enzymes (LOXs, COX, and MPO), ethyl acetate fraction recorded the highest inhibitory action.

The findings of this study supported the hypothesis that several natural extracts may possess potential sources of active pharmaceutical compounds, which can be used in the treatment of different types of diseases and infections.

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References

[1] Wasser SP. Current findings, future trends, and unsolved problems in studies of medicinal mushrooms. Appl Microbiol Biotechnol. 2011;89:1323–32.

[2] Okonkwo BO, Afieroho OE, Ahanonu ED, Okwubie L, Abo KA. Pleurotus pulmonarius (Fr.) Quel.(Pleurotaceae): *In vitro* antioxidant evaluation and the isolation of a steroidal isoprenoid. J Appl Biol Biotechnol. 2019;7(4):32–8.

[3] Barbieri R, Coppo E, Marchese A, Daglia M, Sobarzo-Sánchez E, Nabavi SF, et al. Phytochemicals for human disease: An update on plant-derived compounds antibacterial activity. Microbiol Res. 2017;196:44–68.

[4] De Silva DD, Rapior S, Sudarman E, Stadler M, Xu J, Aisyah Alias S, et al. Bioactive metabolites from macrofungi: ethnopharmacology, biological activities and chemistry. Fungal Diversity. 2013;62:1–40.

[5] Chang S-T, Wasser SP. The role of culinary-medicinal mushrooms on human welfare with a pyramid model for human health. Int J Med Mushrooms. 2012;14(2):95–134.

[6] Lo H-C, Wasser SP. Medicinal mushrooms for glycemic control in diabetes mellitus: history, current status, future perspectives, and unsolved problems. Int J Med Mushrooms. 2011;13:5.
[7] Dai Y-C, Yang Z-L, Cui B-K, Yu C-J, Zhou L-W. Species diversity and utilization of medicinal mushrooms and fungi in China. Int J Med Mushrooms. 2009;11(3):287–302.
[8] Zheng Y, Bai L, Zhou Y, Tong R, Zeng M, Li X, et al. Polysaccharides from Chinese herbal medicine for anti-diabetes recent advances. Int J Biol Macromol. 2019;121:1240–53.
[9] Ganesan K, Chung SK, Vanamala J, Xu B. Causal relationship between diet-induced gut microbiota changes and diabetes: a novel strategy to transplant Faecalibacterium prausnitzii in preventing diabetes. Int J Mol Sci. 2018;19(12):3720.
[10] Ozougwu J, Obimba K, Belonwu C, Unakalamba C. The pathogenesis and pathophysiology of type 1 and type 2 diabetes mellitus. J Physiol Pathophysiol. 2013;4(4):46–57.
[11] DeFronzo RA. The triumvirate: β-cell, muscle, liver: a collusion responsible for NIDDM. Diabetes. 1988;37(6):667–87.
[12] Sun H, Saeedi P, Karuranga S, Pinkepank M, Ogurtsova K, Duncan BB, et al. IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. Diabetes Res Clin Pract. 2022;183:109119.
[13] Ademiluyi AO, Oboh G. Soybean phenolic-rich extracts inhibit key-enzymes linked to type 2 diabetes (α-amylase and α-glucosidase) and hypertension (angiotensin I converting enzyme) in vitro. Exp Toxicol Pathol. 2013;65(3):305–9.
[14] Smith M, Dion G. Morton the mouth, salivary glands and oesophagus. The Digestive System. Edinburgh, UK: Churchill Livingstone Elsevier; 2010. p. 19–38.
[15] Hämäläinen M, Karjalainen S, Söderling E. A simple test for the determination of salivary sucrase activity. Caries res. 1988;22(17):4–6.
[16] Morholt E, Brandwein PF, Joseph A. A sourcebook for the biological sciences. Br J Educ Stud. 1967;15:1.
[17] Wani NA, Tirumale S. *In-vitro* inhibitory effect of different extracts of chaetomium cupreum on alpha glucosidase, alpha amylase and Hepg2 cancer cells. J Pharmacogn Phytochem. 2017;5:71–8.
[18] Teeri TT. Engineering of enzymes of carbohydrate metabolism. Curr Opin Biotechnol. 1991;2(4):614–21.
[19] Benmohamed M, Guenane H, Messaoudi M, Zahnit W, Egbuna C, Sharifi-Rad M, et al. Mineral profile, antioxidant, anti-inflammatory, antibacterial, anti-urease and anti-α-amylase activities of the unripe fruit extracts of pistacia atlantica. Molecules. 2023;28(1):349.
[20] Garduño-Diaz SD, Khokhar S. Prevalence, risk factors and complications associated with type 2 diabetes in migrant South Asians. Diabetes/Metab Res Rev. 2012;28(1):6–24.
[21] Shoba K, Krishnakumari S. In vitro studies on antioxidant and antidiabetic activity of Pleurotus eous mushroom in methanol and aqueous extract. Asian J Pharm Clin Res. 2018;11:259–85.
[22] Subramaniam S, Raman J, Sabaratnam V, Heng CK, Kuppusamy UR. Functional properties of partially characterized polysaccharide from the medicinal mushroom Ganoderma neo-japonicum (Agaricomycetes). Int J Med Mushrooms. 2017;19:10.
[23] Im KH, Nguyen TK, Choi J, Lee TS. In vitro antioxidant, anti-diabetes, anti-dementia, and inflammation inhibitory effect of Trametes pubescens fruiting body extracts. Molecules. 2016;21(5):639.
[24] Edwards T. Inflammation, pain, and chronic disease: an integrative approach to treatment and prevention. Altern Ther Health Med. 2005;11(6):20–8.
[25] Moro C, Palacios I, Lozano M, D'Arrigo M, Guillamón E, Villares A, et al. Anti-inflammatory activity of methanolic extracts from edible mushrooms in LPS activated RAW 264.7 macrophages. Food Chem. 2012;130(2):350–5.
[26] Perwez Hussain S, Harris CC. Inflammation and cancer: an ancient link with novel potentials. Int J Cancer. 2007;121(11):2373–80.
[27] López-Armada MJ, Riveiro-Naveira RR, Vaamonde-García C, Valcárcel-Ares MN. Mitochondrial dysfunction and the inflammatory response. Mitochondrion. 2013;13(2):106–18.
[28] Atay Balkan İ, Gören AC, Kırmızıbekmez H, Yeşilada E. Evaluation of the in vitro anti-inflammatory activity of Nerium oleander L. flower extracts and activity-guided isolation of the active constituents. Rec Nat Prod. 2018;12(2):128–41.
[29] Amdekar S, Roy P, Singh V, Kumar A, Singh R, Sharma P. Anti-inflammatory activity of lactobacillus on carrageenan-induced paw edema in male wistar rats. Int J Inflamm. 2012;752015.
[30] Anilkumar M. 10. Ethnomedicinal plants as anti-inflammatory and analgesic agents. In Ethnomedicine: A Source of Complementary Therapeutics. Research Sign Post; 2010. p. 267–93.
[31] Loria V, Dato I, Graziani F, Biasucci LM. Myeloperoxidase: a new biomarker of inflammation in ischemic heart disease and acute coronary syndromes. Mediators Inflamm. 2008;2008:135625.
[32] Ding XZ, Hennig R, Adrian TE. Lipoxygenase and cyclooxygenase metabolism: new insights in treatment and chemoprevention of pancreatic cancer. Mol Cancer. 2003;2(1):1–2.
[33] Rådmark O, Samuelsson B. 5-Lipoxygenase: mechanisms of regulation1. J lipid Res. 2009;50:S40–5.
[34] Catalano A, Procopio A. New aspects on the role of lipoxygenases in cancer progression. Histol Histopathol. 2005;20:969–75.
[35] Brash AR. Lipoxygenases: occurrence, functions, catalysis, and acquisition of substrate. J Biol Chem. 1999;274(34):23679–82.
[36] Dobrian AD, Lieb DC, Cole BK, Taylor-Fishwick DA, Chakrabarti SK, Nadler JL. Functional and pathological roles of the 12-and 15-lipoxygenases. Prog Lipid Res. 2011;50(1):115–31.
[37] Rackova L, Oblozinsky M, Kostalova D, Kettmann V, Bezakova L. Free radical scavenging activity and lipoxygenase inhibition of Mahonia aquifolium extract and isoquinoline alkaloids. J Inflamm. 2007;4(1):1–7.
[38] Schneider I, Bucar F. Lipoxygenase inhibitors from natural plant sources. Part 1: Medicinal plants with inhibitory activity on arachidonate 5-lipoxygenase and 5-lipoxygenase [sol] cyclooxygenase. Phytother Res: Int J Devoted Pharmacol Toxicol Eval Nat Prod Derivatives. 2005;19(2):81–102.
[39] Gaut JP, Yeh GC, Tran HD, Byun J, Henderson JP, Richter GM et al. Neutrophils employ the myeloperoxidase system to generate antimicrobial brominating and chlorinating oxidants during sepsis. Proc Natl Acad Sci. 2001;98:11961–6.
[40] Harrison J, Schultz J. Studies on the chlorinating activity of myeloperoxidase. J Biol Chem. 1976;251(5):1371–4.
[41] Asahi T, Kondo H, Masuda M, Nishino H, Aratani Y, Naito Y, et al. Chemical and immunochemical detection of 8-halogenated deoxyguanosines at early stage inflammation. J Biol Chem. 2010;285(12):9282–91.
[42] Takeshita J, Byun J, Nhan TQ, Pritchard DK, Pennathur S, Schwartz SM, et al. Myeloperoxidase generates 5-chlorouracil in human atherosclerotic tissue: a potential pathway for somatic mutagenesis by macrophages. J Biol Chem. 2006;281(6):3096–104.
[43] Barot M, Patel M, Kwatra D, Mitra AK. Transporter–metabolism interplay in the eye. Ocular Transporters Receptors. Cambridge, UK: Woodhead Publishing; 2013. p. 229–48.

[44] Gandhi J, Khera L, Gaur N, Paul C, Kaul R. Role of modulator of inflammation cyclooxygenase-2 in gammaherpesvirus mediated tumorigenesis. Front Microbiol 2017;8:538.
[45] Sobolewski C, Cerella C, Dicato M, Ghibelli L, Diederich M. The role of cyclooxygenase-2 in cell proliferation and cell death in human malignancies. Int J Cell Biol. 2010;215158.
[46] Vane J. A mechanism of action for aspirin-like drugs: the inhibition of prostaglandin synthesis. Nature. 1971;231:232–5.
[47] Ong C, Lirk P, Tan C, Seymour R. An evidence-based update on nonsteroidal anti-inflammatory drugs. Clin Med Res. 2007;5(1):19–34.
[48] Kamiyama M, Horiuchi M, Umano K, Kondo K, Otsuka Y, Shibamoto T. Antioxidant/anti-inflammatory activities and chemical composition of extracts from the mushroom Trametes versicolor. Int J Nutr Food Sci. 2013;2(2):85–91.
[49] Yahaya YA, Don MM. Evaluation of Trametes lactinea extracts on the inhibition of hyaluronidase, lipoxygenase and xanthine oxidase activities in vitro. J Phys Sci. 2012;23:1–15.
[50] Messaoudi M, Rebiai A, Sawicka B, Atanassova M, Ouakouak H, Larkem I, et al. Effect of extraction methods on polyphenols, flavonoids, mineral elements, and biological activities of essential oil and extracts of mentha pulegium L. Molecules. 2021;27(1):11.
[51] Zhang Q-W, Lin L-G, Ye W-C. Techniques for extraction and isolation of natural products: A comprehensive review. Chin Med. 2018;13:1–26.
[52] Jose GS, Radhamany P. In vitro antioxidant activities, total phenolics and flavonoid of wild edible mushroom Macrolepiota mastoidea (Fr.) Singer. Int J Pharm Pharm Sci. 2013;5(2):161–6.
[53] Dehghan F, Khodaei F, Afshar L, Shojaei F, Poorhakimi E, Soori R, et al. Effect of competition on stress salivary biomarkers in elite and amateur female adolescent inline skaters. Sci Sports. 2019;34(1):e37–44.
[54] Karjalainen S, Karjalainen M, Forrester D. Physiologic variation of sucrase activity and microbial counts in human saliva. Eur J Oral Sci. 1992;100(2):111–6.
[55] Honda M, Hara Y. Inhibition of rat small intestinal sucrase and α-glucosidase activities by tea polyphenols. Biosci Biotechnol Biochem. 1993;57(1):123–4.
[56] Sengar D, Jerome F, Douglas R. A simple method for separation of buffy coat from peripheral blood of chickens. Can J Comp Med. 1968;32(4):593.
[57] Anthon GE, Barrett DM. Colorimetric method for the determination of lipoxygenase activity. J Agric Food Chem. 2001;49(1):32–7.
[58] Jyothilakshmi M, Jyothis M, Latha MS. Anti-inflammatory efficacy of the rhizome of Zingiber zerumbet–an in vitro study using THP1 cell line. J Med Plants Stud. 2016;4:103–6.
[59] Chandrawanshi NK, Tandia DK, Jadhav S. Determination of antioxidant and antidiabetic activities of polar solvent extracts of Daedaleopsis confragosa (Bolton) J. Schröt. Res J Pharm Technol. 2018;11(12):5623–30.
[60] Elkhateeb WA, El-Hagrassi A, Fayad W, El-Manawaty M, Zaghlol G, Daba G, et al. Cytotoxicity and hypoglycemic effect of the Japanese Jelly mushroom Auricularia auricula-judae. Chem Res J. 2018;3:123–33.
[61] Prabu M, Kumuthakalavalli R. Antidiabetic potential of the oyster mushroom Pleurotus florida (Mont.) singer. Int J Curr Pharm Res. 2017;9(4):51–4.
[62] Elsayed EA, El Enshasy H, Wadaan MA, Aziz R. Mushrooms: a potential natural source of anti-inflammatory compounds for medical applications. Mediators Inflamm. 2014;805841.
[63] Kim S-E, Hwang BS, Song J-G, Lee SW, Lee I-K, Yun B-S. New bioactive compounds from Korean native mushrooms. Mycobiology. 2013;41(4):171–6.
[64] Sarac N, Hakan A, Baygar T, Aysel U. In vitro anticoagulant and antiinflammatory activities of Geastrum fimbriatum Fr., namely as Earthstar fungus. Int J Second Metab. 2019;6(1):1–9.
[65] Asahi T, Wu X, Shimoda H, Hisaka S, Harada E, Kanno T, et al. A mushroom-derived amino acid, ergothioneine, is a potential inhibitor of inflammation-related DNA halogenation. Biosci Biotechnol Biochem. 2016;80(2):313–7.
[66] Lee SW, Song J-G, Hwang BS, Kim D-W, Lee Y-J, Woo E-E, et al. Lipoxygenase inhibitory activity of Korean indigenous mushroom extracts and isolation of an active compound from Phellinus baumii. Mycobiology. 2014;42(2):185–8.
[67] Ballard CR, Junior MRM. Health benefits of flavonoids. Bioactive Compounds. Duxford, UK: Elsevier; 2019. p. 185–201.
[68] de Paulo Farias D, de Araujo FF, Neri-Numa IA, Pastore GM. Antidiabetic potential of dietary polyphenols: A mechanistic review. Food Res Int. 2021;145:110383.
[69] Jing W, Xiaolan C, Yu C, Feng Q, Haifeng Y. Pharmacological effects and mechanisms of tannic acid. Biomed Pharmacother. 2022;154:113561.
[70] Khouzani MA, Mogharabi M, Faramarzi MA, Mojtabavi S, Azizian H, Mahdavi M, et al. Development of coumarin tagged 1, 2, 3-triazole derivatives targeting α-glucosidase inhibition: Synthetic modification, biological evaluation, kinetic and in silico studies. J Mol Struct 2023;1282:135194.
[71] Lull C, Wichers HJ, Savelkoul HF. Antiinflammatory and immunomodulating properties of fungal metabolites. Mediators Inflamm. 2005;2005:63–80.
[72] Prasathkumar M, Anisha S, Khusro A, Essa MM, Chidambaram SB, Qoronfleh MW, et al. Anti-pathogenic, anti-diabetic, anti-inflammatory, antioxidant, and wound healing efficacy of Datura metel L. leaves. Arab J Chem. 2022;15(9):104112.
[73] Vyas P, Yadav DK, Khandelwal P. Tectona grandis (teak)–A review on its phytochemical and therapeutic potential. Nat product Res. 2019;33(16):2338–54.
[74] Xin D, Li H, Zhou S, Zhong H, Pu W. Effects of anthraquinones on immune responses and inflammatory diseases. Molecules. 2022;27(12):3831.