

# Bioactive compounds in *Leucocalocybe mongolica* Pharmacological and Therapeutic Applications

Asmaa Hussein Zaki<sup>1-2</sup>, Muhammad Toseef Zahid<sup>1</sup> and Bao Haiying<sup>\*1</sup>

1.Engineering research center of the Chinese ministry of education for edible and medicinal fungi, Changchun, Jilin 130118, china.

2.Department of Agricultural Chemistry, Faculty of Agriculture Minia University, Minia 61111, Egypt.

\*Corresponding Author: Bao Haiying ✉ baohaiying2008@126.com

## Abstract

Medicinal mushrooms have got much attention from biologists in the last decades. *Leucocalocybe mongolica* Imai remains one of the famous clinical and edible mushrooms used in traditional Chinese medicine and Mongolian medicine to treat various diseases. It also commonly consumes in Chinese and Mongolian daily dishes. Numerous biochemical components exist in *L. mongolica*, especially in the fruiting bodies include; polysaccharides, sterol, lectins, laccase, amino acids, terpenes, volatile compounds, and so on. These biomedical components possess a lot of medicinal properties and provide diverse therapeutical effects for human health, such as anti-tumor activity, antiproliferative activity, anti-diabetes properties, hypotensive effect, hepatoprotective effect, antioxidant activity, cardioprotective effect, and so on. However, this review's main objectives are to illustrate some basics about the bioactive components in *L. mongolica* and its pharmacological effects on the human body. In addition to giving forward some suggestions for future research on this medicinal mushroom.

Keywords: *Leucocalocybe mongolica*, bioactive components, pharmacological effect

## 1. Introduction

Mushrooms are a crucial economic section of Chinese biodiversity [1]. The edible mushrooms are good nutritional food due to low calories, carbohydrates, fat, sodium, and cholesterol-free food. These mushrooms can also provide many vital nutrients, such as proteins, vitamin D, fiber, niacin, riboflavin, potassium, and selenium. However, *Leucocalocybe mongolica* is an edible and medicinal mushroom growing in Inner Mongolia's grassland, the north side of China, and Russia's far east. It is called "Kou-mo" or "Bai-mo" in China, while the Mongolian citizens call it huragan-mug. Previously, *Leucocalocybe mongolica* has belonged to the genus *Tricholoma* (Fr.) Staude (*Agaricales Basidiomycota*) [2]. In the last decades, *Tricholoma mongolicum* S. Imai was moved from the genus *Tricholoma* to a newly established genus called *Leucocalocybe*, based on its molecular and morphological features [3]. *L. mongolica* has a short thick stem and white tiny spiny spores, the average size of spores ( $6.0 \times 9.0 \times 5.0$  to  $7.0 \mu\text{M}$ ) (Fig. 1) [3]. However, *L. mongolica* consumes as edible food because it combines exquisite flavor and high nutritional value, which means this

mushroom could be considered a cheap nutritional food source as a feasible alternative to meat for many countries. It possesses great economic and medicinal properties. Recently, *L. mongolica* Imai (LMI) got much attention from researchers due to its rich bioactive components, including polysaccharides, phenolic compounds, sterols, terpenoids, unsaturated fatty acids, alkaloids, and volatile components ..... etc. [6]. *L. mongolica* is a vital source of many biomedical valuable compounds that provide brilliant therapeutic power for disease expectation and control and treat the botulism of injuries and detoxifications [5]. Modern pharmacological studies indicate that *L. mongolica* have different medicinal properties, including antitumor, antiangiogenic, immunomodulatory, antioxidant, anti-antidiabetic, anti-inflammatory hypoglycemic, antifungal, antibacterial, and antiviral [6]. For instance, the fruit bodies have antitumor activity [6]. The Evenks eat it to strengthen the health and decrease the blood temperature.

#### Classification of *Leucocalocybe mongolica*

**Kingdom:** Fungi (Fungi)

**Strain:** Basidiomycota

**Class:** Agaricomycetes

**Subclass:** Agaricomycetidae

**Order:** Agaricales

**Family:** Incertae sedis

**Genus:** *Leucocalocybe*



Fig 1. *Leucocalocybe mongolica* (S.Imai)

## 2. Bioactive compounds

Bioactive compounds play a vital part in our body due to their biological activities and pharmacological applications. *L. mongolica* is rich in chemical components that have multi-medical and nutritional effects. It has dietary compounds vitamins, protein, carbohydrates, fiber, fat, and minerals. an enormous number of bioactive components have been found from *L. mongolica* recently (e.g., polysaccharides, lectins, protein, laccase, phenolic compositions, steroids, and volatile compounds.[7] [8].

### 2.1. Polysaccharides

Polysaccharides are defined as carbohydrate polymers composed of hetero / homo-monosaccharide molecules linked together by glycosidic bonds. However, The polysaccharides exist in mushrooms have multiple pharmacological activities, including anti-tumor [9] , antioxidant [10], antidiabetes [11], anticoagulant[12], anti-inflammatory [13], antiallergic [14], immunomodulatory [15], antifatigue [16], antihepatopathy [17] and antiviral [18]. Other studies showed that polysaccharides isolated from *L. mongolica* Imai have various biomedical activities such as hepatoprotective, immunostimulatory activity, and antitumor anti-radiation, antifatigue, anti-virus , hypoglycemic, anti-coagulation, detoxification, anticancer, in addition to reducing the hazard of atherosclerosis [19] [20],[21],[22],[23],[24]. Its significant antioxidant activity and reducing atherosclerosis risks dose-dependent manner[23][25]. Polysaccharides with relatively high molecular weight have a significantly antiproliferative effect more remarkable than the LMIPs with low molecular weight [26]. The

variation between polysaccharides is based on their chemical compositions, molecular weight, and branching number [27] [28]. The polysaccharide has an indirect antitumor effect in the host cells' immune System by increasing immunity and adverse biological stresses by stimulating some major biological systems [28]. Polysaccharides can also motivate the essential immune System and enhance the antitumor activity by quickening the host's defense mechanisms. Polysaccharides can also activate the effective cells such as macrophages, T-lymphocytes, cytotoxic T- and natural killer cells, B-lymphocytes, T-lymphocytes to express cytokines, like IFN- $\gamma$ , TNF $\alpha$ , and IL-1 $\beta$  [29].

Several researchers used different methods to extract and purify polysaccharides from *Leucocalocybe mongolica* (Table 1). The first method, called Ultrasonic-microwave synergistic extraction (UMSE) uses power of 109.98 W, the ratio of water: the raw material (fruit body) is 21.62 ml/g, for 24.65 min. The yield of crude powder after purification by Sephadex G-100 chromatography was higher than DEAE-Cellulose 52 chromatography purification. The LMIP crude powder contains 73.92% carbohydrates, 1.92% glucuronic acid, and 1.13% proteins. The common monosaccharides in LMIP are glucose, mannose, arabinose, xylose, and rhamnose [23]. The second method is enzyme-assisted extraction using mixture of enzymes (pectinase 2.5%, papain 2.0%, and trypsin 1.5%). The recommended conditions in this method for LMIPs extraction as determined by RSM is 48.4 °C, the pH 5.4 for 132 min., the total LMIPs yield was 24.01%, under the above conditions. The purified LMIP crude powder contained carbohydrates (82.13%), uronic acid (1.85%), and proteins (1.82%). The GC results of polysaccharides showed the composition of rhamnose, arabinose, mannose, glucose, and xylose [26].

**Table (1) Comparison between the different methods for polysaccharides extraction from *L. mongolica***

Method		Ultrasonic-microwave synergistic extraction	enzymes- assisted extraction	cellulase-assisted extraction process	Water Extraction
Extraction agent		Ultrasonic-microwave power (109.98 W)	Pectinase ( 2.5% ), papain (2.0%), trypsin (1.5%)	cellulase 2.0 %	water
Liquid /curde ratio		21.62 ml/g	150 ml/5g	20 ml/g	30 ml/g
Extraction time		24.65 min	132 min	127 min	300 min
Ph		-	pH 5.4	4.0	-
Temperature		-	48.4 °C	50 °C	93 °C
Yield contents as reported by authors	Carbohydrates %	73.92	82.13%	88.64 %	Almost 100%
	Proteins %	1.13	1.82%	1.86 %	-
	glucuronic acid %	1.92	-	-	-
	Uronic acid %	-	1.85%	1.92 %	-
	Total curd	73.92	24.01%	19.01 %	6.64 %.
Refrance		[23]	[28]	[27]	[7]

The third method is cellulase-assisted extraction; the optimal extraction temperature for this method is 50 °C, the cellulase amount is 20g/ Kg mushroom powder, pH of 4.0, and extraction time is 127 min. The predicted polysaccharides yield was 190.1g/ kg crude. The total polysaccharides product under these conditions was 189.6 g/kg. The contents of the whole carbohydrates contents 886.4 g/kg, the uronic acid contents of 19.2 g kg<sup>-1</sup>, and protein contents 18.6 g kg<sup>-1</sup>. According to the GC results, the monosaccharides-content in the extract

were arabinose, xylose, glucose, mannose, and rhamnose [27]. The last extraction method provided by Wang *et al.*, [7], which remains the popular and easier way to extract polysaccharides from *L. mongolica*, the liquid-solid ratio is 30 mL/g. The extraction temperature 93°C, for 300 minutes. The polysaccharide content under these conditions about 6.64%. The LC-MS results demonstrated that the composition of extracted LMPs is D-fructose, trehalose, D-mannose, galactose. and D-xylose [7].

## 2.2. Sterols

### 2.2.1. Ergosterols

Sterols remain one of the major chemical components in all mushrooms with an antitumor effect. Sterols isolated and identified from *L. mongolica* composed of ergosta-4, 6, 8 (14), 22-tetraen-3-one (ET), and (22E, 24R)-ergosta-7, 22-dien-3 $\beta$ , 5 $\alpha$ , 6 $\beta$ -triol (ED) (Fig. 3). However, ergosterol is the highest significant steroid's chemical raw material. It mainly exists in medicinal and edible fungi; one of the primary compounds for synthesizing vitamin D biosynthesis of steroids has recently impressed synthetic chemists. Partially due to the complexity of stereogenicity, the biological profile of its systems, and the way to form these compounds' precision and sensitivity [32], [33], [34]. However, ED and ET's effect on tumor inhibitory rate, tumor weight, and organ indices have been studied in mice [9]. The control group showed an increase in the tumor volume to 2.6 cm or more within 15 days than the lively control group. The tumor weight average in both ED- and ET-treated groups were significantly low than the negative control group depending on the ergosterols dose. Furthermore, treated-groups showed a protecting activity toward the tumor-bearers mice's spleen and thymus comparing with the non-treated group. The macroscopic tumor observation illustrated that ED and ET possess significant tumor inhibitory effect, positively related to the dose concentration increase. The treated groups also have a considerable tumor inhibition rate (91.78%) with reducing the thymus and spleen weights. Moreover, [35] successfully extracted ergosterol (the molecular formula of C<sub>28</sub>H<sub>44</sub>O) and ergosterol peroxide (molecular formula C<sub>28</sub>H<sub>44</sub>O<sub>3</sub>) from *L. mongolica* (Fig. 2).

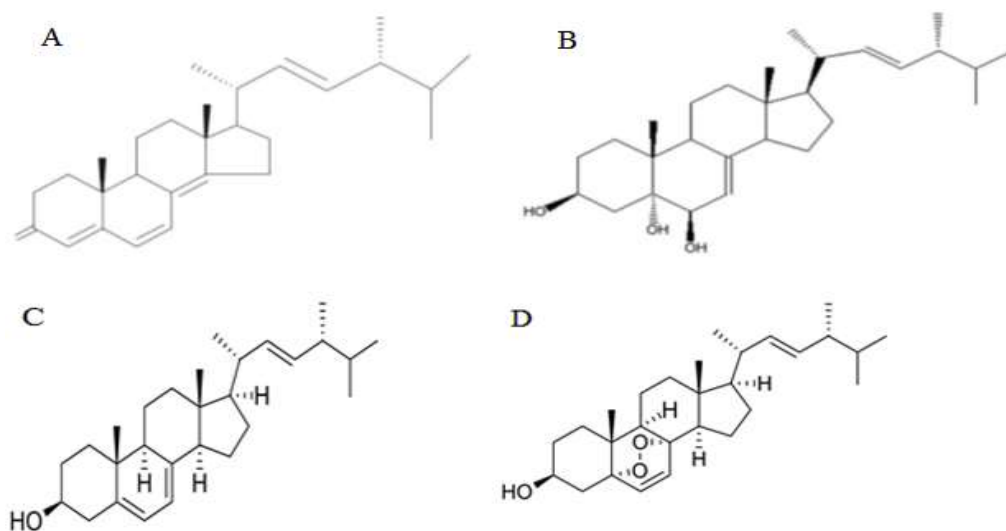


Fig. 2: The chemical structure of ergosterols isolated from *L. mongolica* A: ergosta-4, 6, 8 (14), 22-tetraen-3-one; B: (22E, 24R)-ergosta-7, 22-dien-3 $\beta$ , 5 $\alpha$ , 6 $\beta$ -triol; C: ergosterol; D: ergosterol peroxide [57,58]

### 2.2.2. Terpenes

Terpenoids isolated from mushrooms used as anticancer, anti-proliferation, and anti-inflammatory properties. The anti-inflammatory activity of these fungal terpenoids has been

evaluated using RAW 264.7 cell line *in vitro*. The results of the NF- $\kappa$ B inhibition signaling pathway showed a reduction of the pro-inflammatory mediators' generation [36]. Moreover, triterpenes isolated from other mushroom species gave similar inhibiting activity against the NF- $\kappa$ B signaling pathway [32]. The anticancer properties of mushrooms terpenoids were also studied in the macrophage U937. The results illustrated significant pro-apoptotic and anti-proliferation effects depending on the time and concentration of the extracts associated with cytochrome C release to the cytosol, caspases-3, -8, -9 activation, the probability of mitochondrial membrane losing and PARP damaging. Terpenoid has been isolated from *L. mongolica* using ethanol 77.95% with the extraction temperature is 78.78 °C [37]. However Terpenoids should be examined in an *in vivo* and *in vitro* model for test their potential to as antidiabetic and antitumor or prevent some disease

## 2.3. Peptide and proteins

Mushrooms have a thirty percent protein content higher than ordinary vegetables. Mushrooms produce many bioactive proteins and peptides. Some of these proteins don't have enzymatic activity (e.g., lectins, ribosome-inactivating proteins, and antifungal proteins). Others possess enzymatic activity such as laccases and proteases and an immense amount of single amino acids [38]. Nevertheless, [39] found that the dried mushroom's basidiocarps have higher protein content (42.2%) than the fermented mycelium by liquid fermentation (27.2%) than the fermented solid-mycelium (17.9%).

Protein can be extracted from *L. mongolica* by enzymatic hydrolysis using alcalase with substrate concentration 2% at 55 °C, for 150 min. The enzyme/substrate ratio is 1.5%, the product is about mixture of proteins and polysaccharides. To remove the polysaccharides from this mixture, ethanol 65% can be used with the ratio of 3:1 (ethanol/extract). The final protein concentration under these conditions was about 32.10 % [40]

### 2.3.1. Amino Acids

Various amino acids exist in *L. mongolica*, including glycine, alanine, phenylalanine, leucine, isoleucine, valine, lysine, aspartic acid proline, arginine, glutamate, threonine, serine, tyrosine, histidine, and methionine in the fruiting bodies and the fermented products. Only three amino acids (glutamate, aspartic acid, and alanine) observed significant differences in the concentration with 5.5607%, 3.438%, and 2.0824% in the fruiting bodies, respectively. While the highest amount in the solid-state fermentation were glutamate (1.3740%), then leucine (0.9580%), and proline (0.6676%) [39].

### 2.3.2. Lectins

Lectins are proteins-carbohydrates linked together. They are generally interrelated with glycolipids, polysaccharides, and glycoproteins located on the cell surfaces [41]. The lectins are considered useful tools to study cell surface structures [42]. Lectins have been used in many biological studies, such as in cancer research, bacteriological and embryological studies, tumor cells, membrane glycoconjugates, taxonomical studies, cell sorting, and sorting mutant, and isolation of membranes and serum glycoconjugates,.... etc. Moreover, many lectins showed antitumor and immunomodulatory activities and [43] [44]. Mushroom lectin studies were reported for the first time in the toxicity investigations study of fly agaric (*Amanita*



*muscaria*) in 1910, Lectins were successfully isolated from many mushrooms, i.g., *Inocybe umbrinella* [45], *Amanita pantherina*, *Amanita phalloides* [46], and *Chlorophyllum molybdites* [47]. Beside that Two kinds of lectins (LML-1 and LML-2) isolated from *L.mongolica* mycelium molecular weights of these lectins about 36-38 K.D, both lectins offered hemagglutinating activities and this hemagglutinating activities were lactose-inhibitable. The composition of both two lectins revealed high content of Val, Thr, Gly, Asx, and Glx, while the His and Arg content were very low. the purafction method of lectin from *L. mongolica* fruite body is different to that from mycelia [48]. Both LML-1 and LML-2 caused proliferation inhibition of PU5-1.8 and P815 tumor cell in dose-dependent action but did not show any *in vitro* effect on P388D1 and S-180 tumor growth cell lines[43]. However, LML-1 and LML-2 presented many similar properties, including the inhibition rate by lactose doses, dimeric structure, thermal stability, and equal molecular weights. LML-2 influenced a more significant powerful hemagglutination effect and lower antiproliferative activity toward PU5-1.8 cells, particularly in the serum compared with LML-1[49]

### 2.3.3. Laccase

Laccase (benzenediol: oxygen oxidoreductase) is a polyphenol oxidase that considers a member of the multi-copper oxidases family [50][51][52]. In 1883, laccases were discovered in *Rhus vernicifera* (Japanese lacquer tree). These enzymes are common in some fungi, bacteria, and plants in nature [53][54]. A novel laccase isolated from *L. mongolica* mycelial pellet using different chromatographic fraction methods to reduce all the inactive proteins which contain the laccase (e.g., Ion-exchange chromatographies on Q-Sepharose, CM-cellulose, and DEAE cellulose, and gel purification on Superdex 75) [6]. The extracted laccase from *L. mongolica* was found as one band on the SDS-page (66 kDa molecular mass) within the molecular masse range of other mushroom's laccases. It also is greatly stimulated by Cu<sup>2+</sup> ions and inhibits by Hg<sup>2+</sup> ions [43]. laccase from mushroom possesses some possible pharmacological features and clinical studies' as antiproliferative efficiency against the tumor cells and the inhibition capability against HIV-1 reverse transcriptase. antiproliferative property, also used in industrial, biotechnological, and environmental approaches, particularly in aromatic contaminants enzymatic digestion [55], [56]. In addition *L. mongolica* laccase has antiproliferative activity against HepG 2 cells and MCF 7 tumor cells [49].

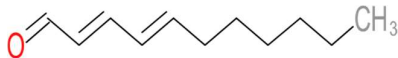
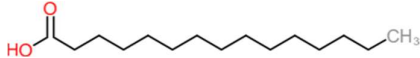
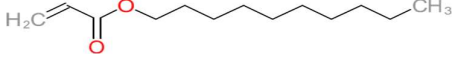
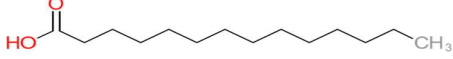
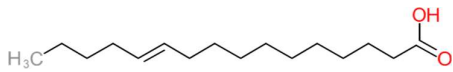
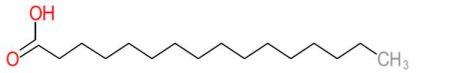
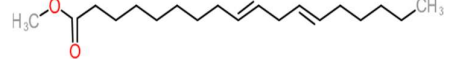
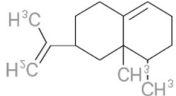
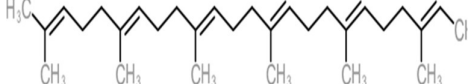
### 2.4. Volatile Compounds

Volatile compounds (VC) are considered the natural flavor components in mushrooms. However, edible mushrooms contain more than 150 volatile compounds. *L. mongolica* flavor is mainly due to the complex of proteins, volatile elements, nucleic acids, and some other flavor compounds [39]. Many kinds of volatile components were detected in *L. mongolica* basidiocarps, include 1-nonanal, pentanal, hexyl alcohol, 3-methylbutanoic acid, 2-methyl pyrazine, heptaldehyde, 3-methylbutyraldehyde, 3-methyl-1-butanol, formic acid, 3-hydroxy-2-butanone, 2-heptanone, heptyl ester, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, 2-acetyl pyrrole, gamma-lactone, benzaldehyde, 4-hydroxynonanoic acid, octanoic acid, 1-octen-3-ol, ethyl-9,12-octadecadienoate, 2-undecanone and 4,5-dihydro-5-methyl-2(3H)-furanone. The volatile components in the Solid-state fermented mushroom 59, 51 Liquid fermentation states, and 40 volatile compounds in the uninoculated control of *L. mongolica*. The similarity of the detected volatile components by GC-MS total ion chromatography was more than 80% between the solid-state fermentation, liquid fermentation states, and the uninoculated control [39].

## 2.5. Other ingredients.

Many other small primary components have been found in *L. mongolica*; the results of GC-MS analysis for petroleum ether extract of *L. mongolica* showed some single molecules like, (E, E)- 2,4-Decadienal, 1R-(1 $\alpha$ ,7 $\beta$ ,8A $\alpha$ )]-1,2,3,5,6,7,8,8a-octahydro-1,8A-dimethyl-7-(1-methyl vinyl) naphthalene, n-Decyl acrylate, Tetradecanoic acid, Pentadecanoic acid, Z-11-Hexadecanoic acid, (E, E)-9,12-Octadecadienoic acid methyl ester, n-Hexadecanoic acid, 2,6,10,14,18,22-Tetracosahexaene, and 11-Octadecenoic acid methyl ester[35] (Table: 2).

**Table:2** Shows some primary components identified by GC-MS analysis of petroleum ether extract of *L. mongolica*.

Compound	Molecular formula	Similarity %	Structure
E,E)-2,4 Decadienal	C <sub>10</sub> H <sub>16</sub> O	95%	
Tetradecanoic acid	C <sub>14</sub> H <sub>28</sub> O <sub>2</sub>	95%	
n-Decyl acrylate	C <sub>13</sub> H <sub>24</sub> O <sub>2</sub>	87%	
Pentadecanoic acid	C <sub>15</sub> H <sub>30</sub> O <sub>2</sub>	99%	
Z-11-Hexadecanoic acid	C <sub>16</sub> H <sub>30</sub> O <sub>2</sub>	91%	
n-Hexadecanoic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	99%	
(E,E)-9,12-Octadecadienoic acid methyl ester	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	99%	
[1R(1 $\alpha$ ,7 $\beta$ ,8A $\alpha$ )]1,2,3,5,6,7,8,8a-octahydro-1,8A-dimethyl-7(1methylvinyl)naphthalne	C <sub>15</sub> H <sub>24</sub>	98%	
2,6,10,14,18,22-Tetracosahexaene	C <sub>24</sub> H <sub>38</sub>	98%	

Similarity % is the percentage of similarity between the GC-MS results and the relative compound in the GC-MS database.

## 3. Medicinal Activities

*L. mongolica* is a rich mushroom in bioactive components that offer health benefits like various cell protections and disease treatment. So, *L. mongolica* has many medicinal properties, including antitumor activity, antiproliferative activity, antioxidant activity, and atherosclerosis risk reduction. And it reduces blood pressure and cholesterol [62].

### 3.1. Antitumor

There were preclinical and clinical studies that mention mushrooms' effect on cancer cells because of their antitumor and anticarcinogenic activities [8]. Many parts in the mushroom showed antitumor activity, especially the fruit body [63]. *Leucocalocybe mongolica* possesses a robust antitumor activity. Research study illustrated that two ergosterols (ET and ED) from *L. mongolica* fruit body were treated tumor-infected mice for 14 days with dose (0.025, 0.05, 0.1 mmol /kg) could enhance the antitumor activity and reduce the generation of tumor angiogenesis through decreased VEGF and Bcl-2 proteins and increase BAX protein expression and by activating the caspase family of proteins in H22 tumor-infected mice. The highest antitumor percentage in the ergosterols-treated groups were 39.741% and 30.26% for ET and ED, respectively. ET and ED caused significant inhibition of the growth of HepG2, MCF-7 and HeLa cells in a dose-dependent matter *in vitro*. The IC50 of ED on the HepG2, MCF-7 and HeLa cells were significantly higher than ET [58]. Another study by [64] revealed that the ergosterol peroxide (5 mg/kg) and petroleum ether extract (35 mg/kg) from *L. mongolica* fruit bodies inhibited the tumor rates in H22-bearing mice by 67.15%, and 69.61%, respectively. Further, the ergosterol and ergosterol peroxide inhibited the division of HepG2 cells and induced apoptosis as well, the ergosterol and ergosterol peroxide showed an apoptotic rate of 41.2% and 42.33%, respectively, at the earlier stage.

The antitumor activity of polysaccharides from *L. mongolica* has been found in several ways, like macrophages activation and stimulating macrophage antigen-presenting activity which plays an essential role in enhancing T-cell propagation and inhibit sarcoma 180 tumor cells growth in rats [65]. Several authors have posited polysaccharides as antitumor agent depends on their chemical compositions, branching, and molecular weight [8], [31]. However, the polysaccharide-treated mice for two weeks with doses (100, 200 and 300 mg/kg) displayed a significant inhibitory effect on tumor growth in H22 tumor-infected mice comparing with the model group. The polysaccharides inhibited the Hela cell's growth in dose-dependent activity [28]. Also, [66] reported that polysaccharides from *L. mongolica* at the doses of 100, 200 and 300 mg/kg for ten days significantly inhibited the tumor cell by downregulation VEGF, Bcl-2 and upregulation of BAX protein as well as increase the apoptosis rate in dose-dependent effect. Overall, Polysaccharides provides the antitumor impact through increasing the expression of BAX protein and decreasing the expression of VEGF and Bcl-2 proteins through activating the caspase family proteins in the tumor-bearing mice, in addition to enhancing the expression rate of PI3K/AKT and p-AKT/AKT [33] (Fig.3 ). The PI3K/AKT rate plays a vital role in the mechanism of antiapoptosis where PI3K provides a phosphate group to AKT forming p-AKT, which can regulate the cell apoptosis's substrate proteins directly or indirectly via phosphorylation [67]. The polysaccharide- peptide complex at dose 10 mg/mL prevented the proliferation of P815 mastocytoma cells *in vitro* [65].



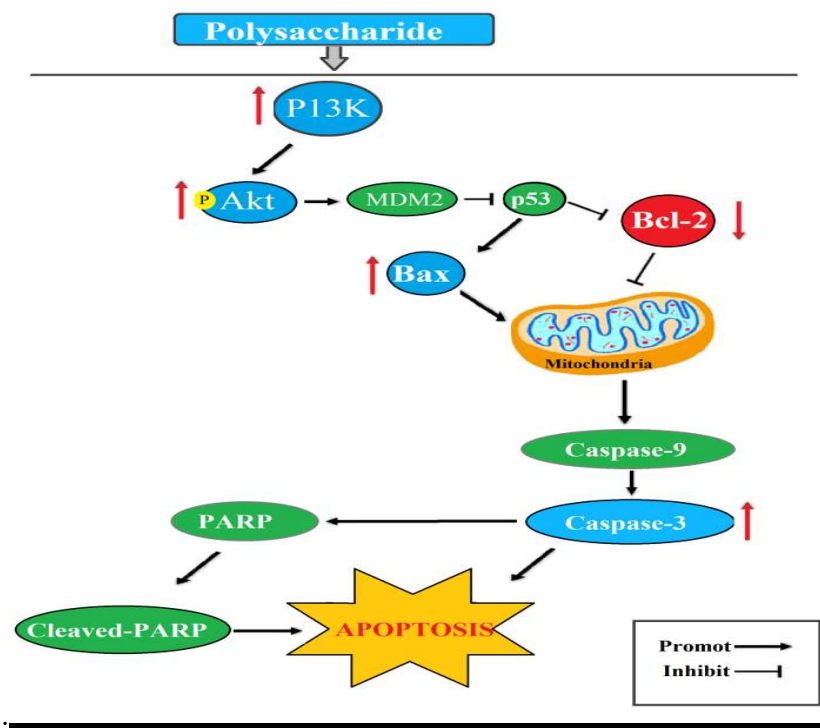


Fig. 3: The antitumor activity mechanisms of polysaccharides.

Some research articles presented that lectins own an antitumor activity. Two lectins extracted from *L. mongolica* demonstrated antiproliferation activities by inhibiting the growth of monocyte-macrophage PU5-1.8 cells in mice by 52 % when treated by (40 µg/mL) for 48 h. but did not offered *in vitro* inhibition for sarcoma 180 cell’s growth [40]. Another experiment by [53] revealed that the treatment of the tumor-bearing mice with lectins (LML1 and LML2) with dose of 5 mg/kg for 20 days decreased the growth of sarcoma 180 cells implanted in mice by 68.84% and 92.93% respectively compared with the tumor-beared control group [7]. Article published by [53] illustrated that *L. mongolica* Laccase exhibited potent anti-proliferation activity against Hep G2 cells, breast cancer cells and inhibited HIV-1 reverse transcriptase; the IC50 values were 1.4 µM, 4.2 µM, 0.65 µM, respectively. Also, LML showed strong suppression toward tumor cell proliferation, especially breast cancer, without any additional cytotoxicity *in vitro* tests [7] and [68]. The lectins from the mycelium dose of 5 mg/kg /day for 20 days enhanced nitrite ion production and activating macrophages in mice to produce macrophage activation and tumor necrosis factors [7]. Overall, *L. mongolica* possesses a strong anticancer activity toward several cancer cell types (Table 3). So, different compounds could become potential candidates for cancer disease treatment.

Table 3: shows antitumor activity of some bioactive compounds from *L. mongolica*.

N0	Bioactive compound	Model	Dose	Results	Reference
1	Ergosterol	HepG2, MCF-7 and HeLa cell in cell line(in vivo)	0, 6.25, 12.5, 25, 50, 100 and 200.0 µg/mL	Ergosterol decreased the growth of HeLa, MCF-7 and HepG2, cell line via increase BAX protein expression and a decrease in VEGF and Bcl-2 protein expression.	[9]
2	Ergosterol (ET and ED)	HeLa, MCF-7, HepG2 and A549 cells(in vitro)	0.025,0.05,0.1mm ol/kg	Ergosterol had significantly inhibited the growth of HeLa, MCF-7	[9]

3	polysaccharide-peptide complex	C57BLI6 mice	10mg/ml	and HepG2, while non-significant inhibition of A549 cells. polysaccharide-peptide complex prevent the proliferation of sarcoma 180 cells in C57BLI6 mice petroleum ether extract and ergosterol peroxide	[60]
4	petroleum ether extract and ergosterol peroxide	H22-bearing mice	35 mg/(kg) and 5 mg/(kg)	displayed tumor suppression rates in H22-bearing mice 69.61% and 67.15%, respectively	[59]
5	Polysaccharide	Human cervix carcinoma cells (Hela cells)	0.8mg/ml	polysaccharide possessed an inhibition effect on the Hela cells growth LMP had antitumor activity against murine	[28]
6	Polysaccharide	murine hepatoma H22 cells in Male Kunming mice	100,200 and300 mg/kg body weight	hepatoma H22 cells in Male Kunming mice by inhibiting VEGF expression and increasing Bax/Bcl-2 ratio. Both lectins provided	[26]
7	Lectins (LML1& LML2)	mouse mon9ocyte-macrophage P8 I5 cells and PU5-1.8	40µg /mL	inhibition in the PUS-1.8 and P815 tumor cell proliferation in a dose-dependent matter. LML1 and LML2	[43]
8	Lectins	Sarcoma 180 cells in the inbred BALB/c and C57BL/6 male mice	5 mg/kg	presented inhibitory effect toward the growth of the implanted sarcoma 180 cells. Laccase had inhibitory effect of breast cancer	[49]
9	Laccase	The MCF7 (breast cancer cells), HepG2 and Hepatoma cells.	0.3 µM, 0.6 µM , 1.25 µM, 2.5 µM, and 5 µM	MCF7 cells , proliferation of hepatoma HepG2 cells and HIV-1 reverse transcriptase.	[6]

3.8. Immune activity

Many previous studies evaluated the effects of lectins on the immune system. For instance, the injections of 10 µg lectins in intraperitoneal tissue for ten days upregulated the transforming growth factor-β, interleukin (IL)-1β, and nitric oxide synthetase expression level [69]. The immunomodulatory properties of *L. mongolica* are due to its rich bioactive compounds. The primary chemical compounds responsible for immune activity in *L. mongolica* are lectins, terpenoids, and polysaccharides [70]. Anywise, T cells are the vital type of white blood cells in the immune system that play a critical part in adaptive immunology response. It’s easy to distinguish T cells from the other lymphocytes by their receptors on the cell surface [65]. The polysaccharide-peptide complex at dose 6 to 50 µg/mL treated for one week had immunomodulatory properties by enhancing nitrate ion production by macrophages activating factor, macrophage production the proliferation of T-cells in the normal BALBIc and C57BLI6 mice [65].

Some authors confirmed that polysaccharides extracted from solid-state fermentation of *L. mongolica* promote the immune system through increasing spleen and thymus weight and enhancing monocyte-macrophage phagocytosis [66]. Another study demonstrated the *in vitro* treatment of polysaccharide-peptide complex at the dose of 10 mg/mL could immune-protection activity by stimulating T-cell proliferation and macrophages to produce nitrite ion inhibiting the P815 mastocytoma cells [65]. Furthermore, *L. mongolica* polysaccharides (100, 200 and 300 mg/kg daily) motivated the immune system by protecting the thymus and spleen of tumor-infected mice comparing with the control group, besides enhancing the production of IL-2, IL-6, IL-1 $\beta$ , and TNF- $\alpha$  [33]. The high dose of polysaccharide from *L. mongolica* fruit bodies could significantly improve mice's immune function though enhance the average content of hemolytin and lysozyme in serum, the monocyte-macrophage phagocytosis, thymus and spleen weights [71]. On the other hand, an earlier study confirmed that, two ergosterols (ET and ED) from *L. mongolica* (100, 200 and 300 mg/kg/d for two weeks) enhanced the immunity via increasing the serum cytokines “TNF- $\alpha$ , IFN- $\gamma$ , IL-2 and IL-6” in H22 tumor-infected mice and recovered both thymus and spleen compared with the negative control group. Also, the water extract, ethyl acetate extract and petroleum ether extract could increase the phagocytic percentage, phagocytic index and hemolysin production of macrophages, increase the levels of cytokines IFN- $\gamma$ , IL-2, IL-4, and IgA, and significantly improve the pathological changes of thymus and spleen tissues in immunosuppressed mice compared with the control group [72]. *L. mongolica* lectins have immune-modulatory activities to motivate the macrophages to produce tumor necrosis factor and nitrite ion (NO $_2^-$ ) in the treated mice. Lectins minimized the mice sarcoma 180 cells growth by 68.8% [7]. According the studies *L. mongolica* plays an important role to enhance immune system.

### 3.3. Hypotensive

Many studies demonstrated that *L. mongolica* lectin has a hypotensive activity for the normal intravenous injected rats. This investigation is similar to several reviews about other edible mushrooms' hypotensive activity [73]. However, exerted lectin from *L. mongolica* mycelium with a dose of (10 mg/kg BW) decreased the mean blood pressure in the arteries via stimulating the macrophages to produce nitric oxide, making effective rat aortic ring relaxation [7]. [74] documented that lectins could reduce blood pressure via enhanced EDRF (endothelium-derived relaxing factor). However, The main EDRF releasing from the endothelium is nitric oxide. The reduction of blood pressure produced by lectin treatment did not return to its initial value, indicating its continued hypotensive activity, which was not affected by the lactose [74] [40].

Lectins from *L. mongolica* provided a significant inhibition toward 3H-NECA binding to the receptors of adenosine A2 in bovine cerebra cortices. In contrast, no binding effect has been found between 3H-PIA and the receptors of adenosine A1. According to the pharmacological results, *L. mongolica* lectin's hypotensive activity may be due to the vasorelaxation through adenosine A2 and/or nitric oxide production receptors. *L. mongolica* lectin created a remarkable relaxing effect on mice's aortic rings. It may occur due to the interaction between LML with the adenosine receptors. Hence, this may explain the hypotensive response in mice [75]. On the other hand, methylene blue can eliminate *L. mongolica* hypotensive activity [7]. In conclusion, although lectin is the only compound studied as a hypotension reduction agent, polysaccharides could also be used due to their ability to increase nitric oxide production.

### 3.2. Anti-diabetic

*Leucocalocybe mongolica* is a high content fiber and low-fat and calories mushroom. It could be an ideal food for regulating the blood's cholesterol level to prevent diabetes, reducing blood pressure and cholesterol [62]. This nutritional fiber binds with cholesterol and lipids, which prevents them from absorbing in the intestine [76]. Previous studies have revealed that a polysaccharide from the fruiting body of *L. mongolica* (10 mg/kg for 13 days) could reduce plasma glucose level in a period-dependent manner, which was remarkably different from those at various time points [77]. Various active components isolated from *L. mongolica* (e.g., polysaccharides, proteins, laccase, lectins, phenolic substances, sterols, alkaloids, and terpenoids) have been illustrated to target different deranged carbohydrate metabolic pathways, thus decrease insulin and blood glucose levels in type two diabetic [78]. However, there are weaknesses in *Leucocalocybe mongolica* bioactive compounds for diabetic studies that need more in-depth work in this research point.

### 3.4. Liver and kidney Protection

Earlier studies have revealed that some polysaccharides from the fruiting body *L. mongolica* at a dose of 100, 200, and 300 mg/kg for two weeks could protect liver and kidney in the tumor-infected mice through decreased AST and BUN values compared with those in the untreated group [33]. However, aspartate aminotransferase (AST) is an essential indication of liver status. The increases in the number of substances of liver production indicate a dysfunction in the liver cell. Another experiment showed that treatment of tumor-bearing mice with ergosterols (ET and ED) at the dose of 100, 200, and 300 mg/kg two weeks enhanced liver and kidney protection through decrease AST and BUN levels compare with the control group in dose-dependent effect [58]. However, several publications informed that lectins in *L. mongolica* possess anti-pathogenic properties [53].

### 3.5. Antioxidant activity

Several studies evaluated the *in vitro* antioxidant assays of polysaccharides from *L. mongolica* using ferric reducing activity power FRAP, DPPH radical scavenging activity, the hydroxyl radical scavenging activity, reducing and infrared spectroscopy analysis assays. The antioxidative analyses indicated significant free radical scavenging activities. The antioxidative activities occurred in a dose-dependent manner [20] [28] [27]. [6] measured the content of flavonoids and phenols in the solid-state fermentation of *L. mongolica*. The flavonoids' content in the extract was 0.183 and 0.267, while phenols were 4.903, 6.975 before and after fermentation, respectively. Some studies have revealed that the *L. mongolica* water extract at a dose 1.2 mL/kg/d for 11 days significantly enhanced the activity of SOD and decreased the MDA value in the plasma [79]. Polysaccharides from *L. mongolica* showed a high antioxidant activity that could significantly inhibit the oxidative modification of LDL, apoptosis of VEC and vascular endothelial damage. Other studies indicated that water extract and ethanolic extract of *L. mongolica* (10, 20, 40 mL/kg) significantly improve the activity of SOD by 66.58% and reduce the content of MDA by 36.89% in the plasma. the antioxidant effect of water extract was higher than the alcohol extract [80].

### 3.7. Cardioprotective

Heart disease is closely linked to atherosclerosis, hypercholesterolemia, and low-density lipoproteins oxidation, so regulating the total cholesterol is necessary to prevent these diseases. However, due to its low fat and high fiber content, edible mushrooms have become an ideal food for the dietary reduction of atherosclerosis. Indeed, oriental medicine prescribes edible mushrooms in a regular antisclerotic and hypocholesterolemic diet [81]. *L. mongolica* fruit bodies' aqueous extract at dose 0.3, 0.6 and 1.2 mL/kg for 11 days simulated cardioprotective through increasing superoxide dismutase (SOD) activity, and decrease the level of creatine kinase, lower lactate dehydrogenase, creatine kinase MB and malondialdehyde in the myocardial tissue induced ischemic mice isoproterenol [79]. Furthermore, *L. mongolica* Imai Polysaccharides possess high antioxidant activity which can inhibit the oxidative modification of LDL and vascular endothelial cells apoptosis. Therefore, LMIPs can reduce the hazard of LDL oxidation, cardiovascular disease, and hypercholesterolemia [27].

### 3.9. Antimicrobial activities

Antimicrobial is a term that involves a wide range of pharmaceutical materials, including; antifungal, antibacterial, anti-parasitic, and antiviral medicines [82]. Bacterial infection treatment is a critical matter. Although many antibiotic drugs are using recently (e.g., oxazolidinones, lincosamides, blactams, quinolones and aminoglycosides), repeating these microbial treatment infections by the same antibiotic makes the microorganism produce new resistant strains to this antibiotic [83]. Therefore, the discovering of new antibiotics remains one of the most fundamental challenges facing scientists. There were five main ways for the antibiotics to exert their effect (1) Alter the cell membrane's function and confusing the permeability so, the cations, nucleic acids, and other pigments get out from the cell and it dies; (2) prevent the cell wall formation by interfering the peptidoglycan synthesis, that will affect the cell wall inflexibility and solidity, which leads to cell death or lysis; (3) Interact with the polymerase complex in DNA replication and transcription. (4) Inhibit the synthesis of nucleotides or their derivatives; (5) Block the DNA expression [84]. Further, some antibiotics can inhibit protein-synthesizing via binding with one or both the 50S and 30S ribosomal subunits.

The antimicrobial activity of the bioactive component is mainly related to molecular weight. The low-molecular-weight compounds in *L. mongolica* are terpenes, steroids, while high-molecular-weight compounds are peptides and proteins. However, the low molecular weight compounds showed less antimicrobial activity than the high-molecular-weight compounds [85]. Some clinical studies indicate that petroleum ether extract of *L. mongolica* fruit bodies at concentration 50, 25 and 12.5 mg/mL exhibited potent antimicrobial activities against *Escherichia coli* ATCC8099 and *Staphylococcus aureus* and there were highly significant differences compared with the control group. The highest inhibitory effect was found in the dose of 50 mg/mL, with the inhibitory effect of 52.78% and 62.05%, respectively. In contrast, both ergosterol and ergosterol peroxide from *L. mongolica* at concentrations of  $2 \times 10^{-2}$  and  $1 \times 10^{-2}$  affected neither *E. coli* ATCC8099 nor *S. aureus* ATCC6538 [59].



#### 4. Conclusion

Mushrooms are a vibrant organism in medicinal and nutritional value. They have a tremendous biological effect on the human body against many severe diseases. In Mongolia and China, *Leucocalocybe mongolica* got increasing importance last decades due to its high protein and fiber content and low amount of fats. Different bioactive compounds have been isolated from *L. mongolica* like polysaccharides, steroids, lectins, protein, and laccase, which demonstrated a significant pharmacological effect toward several diseases *in vivo* and *in vitro* (Fig. 4). However, the polysaccharide is one of the hottest research points in *L. mongolica*. Several studies mentioned that polysaccharides possess many potent clinical activities such as antitumor activity against different cancer cells (i.g. S-180 cells, Hela cells, PUS-1.8 and P815), immune activity, antidiabetic activity, antioxidant activity, and cardioprotective effect. The results showed brilliant effect as an antitumor agent, thus could become potential candidates for the prevention and treatment of cancer disease, but still need further anti-hypotensive and antimicrobial investigations. On the other hand, *L. mongolica* lectin has antitumor, hypotensive activity, immune activity, antidiabetic activity, anti-pathogenic properties. The effect of lectin on liver protection still not clear and need more studies. While, sterols demonstrated antitumor effects against different cancer cell lines HepG2, MCF -7, HeLa cancer cells, sarcoma-180, PU5-1.8, and P815 tumor cells, also used as an antidiabetic agent. Although *L. mongolica* is one of the best edible mushrooms with high nutritional value and multiple medicinal properties, just a few studies have been done on its pharmacological approach, especially its antioxidant activity many ingredients still didn't discover yet. Overall, *L. mongolica* needs more do in-depth research on the anti-diabetic, antioxidant, and antimicrobial activities as well as isolation and purification of the other compounds.

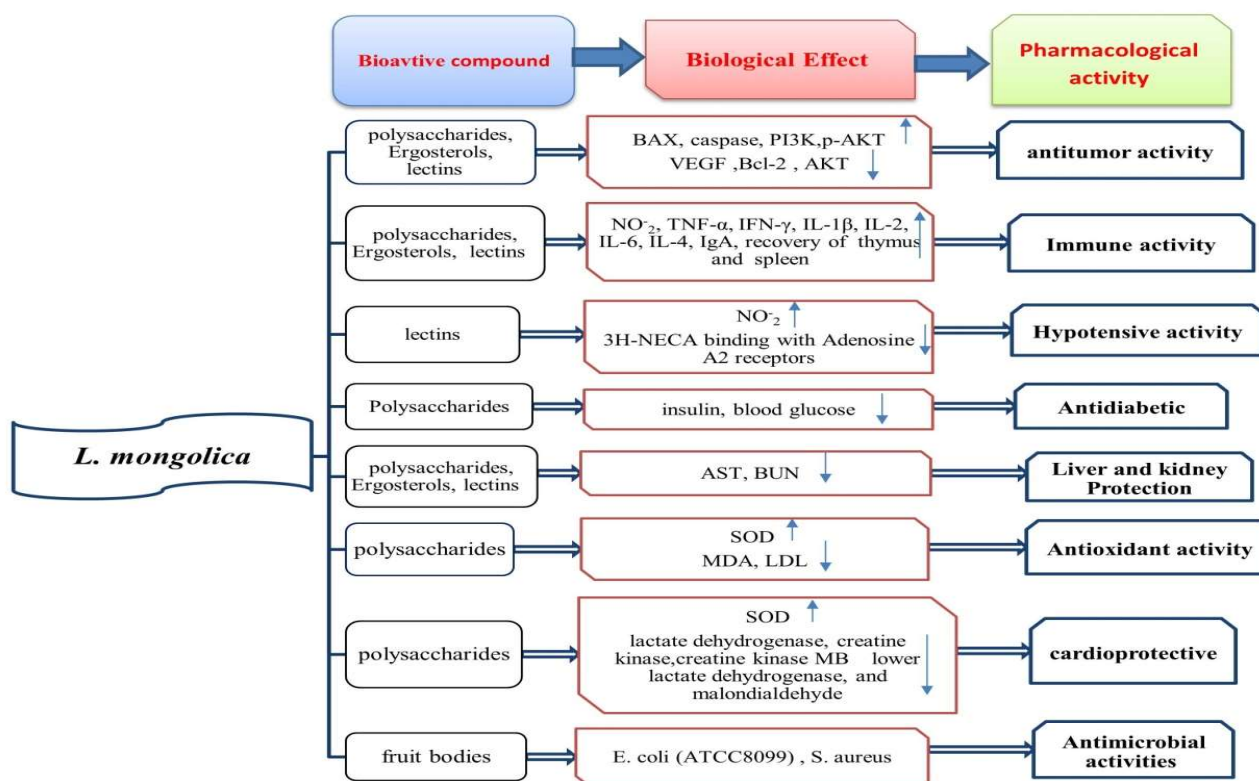


Fig. 4: Mechanisms of the bioactive compounds and their pharmacological activity

**Authors' contributions:** All authors made the same contribution in this study and read and approved the final manuscript

**Funding :** This study was supported by the National Natural Science Foundation of China (0207-202022934)

**Conflicts of Interest:** The authors declare no conflict of interest.

**List of abbreviations:**

The following abbreviations are used in the manuscript:

<i>L. mongolica</i>	<i>Leucocalocybe mongolica</i> ;
LMIPs	<i>Leucocalocybe mongolica</i> polysaccharides
IFN- $\gamma$	Interferon- $\gamma$
TNF $\alpha$	Tumor necrosis factor- $\alpha$ ;
IL-1 $\beta$	Interleukin-1 $\beta$
LML	lectins isolated from the <i>L. mongolica</i>
HIV-1	Human Immunodeficiency Virus
HepG 2	Hepatoma G2
MCF 7	Michigan Cancer Foundation-7
IC <sub>50</sub>	Half maximal inhibitory concentration
PKC	protein kinase C
VC	Volatile compounds
ET	ergosta-4, 6, 8 (14), 22-tetraen-3-one
ED	(22E, 24R)-ergosta-7, 22-dien-3 $\beta$ , 5 $\alpha$ , 6 $\beta$ -triol
AST	aspartate aminotransferase
DPPH	2,2-diphenyl-1-picrylhydrazyl
BUN	blood urea nitrogen
SOD	Superoxide dismutase
MDA	Malondialdehyde
H22	Hepatoma-22

## 5. REFERENCES

1. Liu, P.; Wang, G. X.; Xiang, H.; Yu, F. Q.; Zheng, H.D. Key taxa of larger members in higher fungi of biodiversity from China. *Acta Bot. Yunnanica*. **2003**, *25*, 285–296.
2. Imai, S. *Proc. Imp. Acad. Tokyo* **1937**, *13*, 280–282.
3. Yu, X-D.; Deng, H.; Yao, J -Y. *Leucocalocybe*, a new genus for *Tricholoma ongolicum* (Agaricales, Basidiomycota). *African J. Microbiol. Res.* **2011**, *5*, 5750–5756, doi:10.5897/ajmr11.1228.
4. Wang, X.; Bao, H.; Bau, T. Nutritional value and volatiles of the edible mushroom *leucocalocybe Mongolica*. *Qual. Assur. Saf. Crop. Foods* **2019**, *11*, 679–685, doi:10.3920/QAS2019.1585.
5. Wunir, L. Chun.; Khasbagan Ewenki folk medicinal plants and its comparison with mongolian medicine. *Chinese J. Ethnomedicine Ethnopharmacy* **2009**, *17*, 156–158.
6. Bau, S.; Bao, H. Y.; Bau, T.; Yang, S. D.; Sun, X. Anti-tumor activity of *Tricholoma mongolicum* fruit bodies. *Food Sci.* **2012**, *33*, 280–284.
7. Wang, X.; Bao, H.; Bau, T. Investigation of the possible mechanism of two kinds of sterols extracted from *Leucocalocybe mongolica* in inducing HepG2 cell apoptosis and exerting anti-tumor effects in H22 tumor-bearing mice. *Steroids* **2020**, *163*, 108692, doi:10.1016/j.steroids.2020.108692.
8. Liu Y.; Hesheng.; Wei, Y.; Wang, Y.; Pei, H.; Bao, H. Effects of *Tricholoma mongolica* fruiting body extract on immune function in immunosuppressed mice. *Acta Edible Fungi* **2020**, *27*, 95–104.
9. Zhang, C.; Gao, Z.; Hu, C.; Zhang, J.; Sun, X.; Rong, C.; Jia, L. Antioxidant, antibacterial and anti-aging activities of intracellular zinc polysaccharides from *Grifola frondosa* SH-05. *Int. J. Biol. Macromol.* **2017**, *95*, 778–787, doi:10.1016/j.ijbiomac.2016.12.003.
10. Zhang, Y.; Wang, F. Carbohydrate drugs: current status and development prospect. *Drug Discov. Ther.* **2015**, *9*, 79–87, doi:10.5582/ddt.2015.01028.
11. Sinha, V.R.; Kumria, R. Polysaccharides in colon-specific drug delivery. *Int. J. Pharm.* **2001**, *224*, 19–38, doi:10.1016/S0378-5173(01)00720-7.
12. Dong, B.; Hadinoto, K. Direct comparison between millifluidic and bulk-mixing platform in the synthesis of amorphous drug-polysaccharide nanoparticle complex. *Int. J. Pharm.* **2017**, *523*, 42–51, doi:10.1016/j.ijpharm.2017.03.021.
13. Li, P.; Wang, F. Polysaccharides: Candidates of promising vaccine adjuvants. *Drug Discov. Ther.* **2015**, *9*, 88–93, doi:10.5582/ddt.2015.01025.
14. Do- Amaral, A. E.; Petkowicz, C. L. O.; Mercê, A. L. R.; Iacomini, M.; Martinez, G. R.; Merlin Rocha, M. E.; Cadena, S. M. S. C.; Noletto, G.R. Leishmanicidal activity of polysaccharides and their oxovanadium(IV/V) complexes. *Eur. J. Med. Chem.* **2015**, *90*, 732–741, doi:10.1016/j.ejmech.2014.12.003.

15. Nuti, E., Santamaria, S., Casalini, F., Yamamoto, K., Marinelli, L., La Pietra, V., Novellino, E., Orlandini, E., Nencetti, S., Marini, A. M., Salerno, S., Taliani, S., Da Settimo, F., Nagase, H., & Rossello, A. Arylsulfonamide inhibitors of aggrecanases as potential therapeutic agents for osteoarthritis: Synthesis and biological evaluation. *Eur. J. Med. Chem.* **2013**, 62, 379–394, doi:10.1016/j.ejmech.2012.12.058.
16. An, H.J; Lebrilla, . C.B. Structure elucidation of native n-and o-linked glycans by tandem mass spectrometry (tutorial). *Mass Spectrom. Rev* **2011**, 30, 560–578.
17. Chen, Q.; Mei, X.; Han, G.; Ling, P.; Guo, B.; Guo, Y.; Shao, H.; Wang, G.; Cui, Z.; Bai, Y.; Xu, F. Xanthan gum protects rabbit articular chondrocytes against sodium nitroprusside-induced apoptosis in vitro. *Carbohydr. Polym.* **2015**, 131, 363–369, doi:10.1016/j.carbpol.2015.06.004.
18. Jung, B.; Shim, M. K.; Park, M. J.; Jang, E. H.; Yoon, H. Y.; Kim, K.; Kim, J.H. Hydrophobically modified polysaccharide-based on polysialic acid nanoparticles as carriers for anticancer drugs. *Int. J. Pharm.* **2017**, 520, 111–118, doi:10.1016/j.ijpharm.2017.01.055.
19. Ge, S. M.; Yu, Y. H.; Zhang, Y.F. Study on the extraction and anti-tumor activity from *Tricholoma mongolia* polysaccharide. *Mod. Prev. Med.* **2009**, 36, 3708–3711.
20. Wang, D. W.; Shan, Y. L.; Bau, T. Effect of extraction rate of supercritical CO<sub>2</sub> extraction on *Mongolia* mushroom polysaccharide. *food Sci.* **2006**, 27, 107–110.
21. Wu, X.; Chen, H.; Han, T.; Bai, J.; Zhao, J.; Yuwen, W. Application of response surface methodology for optimization of liquid fermentation medium for polysaccharide production by *Tricholoma Mongolicum* Imai. *Adv. Mater. Res.* **2012**, 550–553, 1831–1836, doi:10.4028/www.scientific.net/AMR.550-553.1831.
22. Bao, L.; Bai, H. P.; Sa, C. F.; Li, R. J.; Xi, L. M. G.; Alatan, G. L.; et al. Antioxidant activity and vasular protecting effect of *Tricholoma mongolia* polysaccharide. *J. Inn. Mong. Univ.* **2014**, 45, 498–501.
23. You, Q.; Yin, X.; Zhang, S.; Jiang, Z. Extraction, purification, and antioxidant activities of polysaccharides from *Tricholoma mongolicum* Imai. *Carbohydr. Polym.* **2014**, 99, 1–10, doi:10.1016/j.carbpol.2013.07.088.
24. Zhao, X.; Bau, T.; Bao, H. Anti-tumor activity of polysaccharides obtained from *Leucocalocybe mongolica* using solid-state fermentation. *Biotechnol. Biotechnol. Equip.* **2020**, 34, 841–849, doi:10.1080/13102818.2020.1807406.
25. Zhao, Y.M.; Song, J.H.; Wang, J.; Yang, J.M.; Wang, Z.B.; Liu, Y.H. Optimization of cellulase-assisted extraction process and antioxidant activities of polysaccharides from *Tricholoma mongolicum* Imai. *J. Sci. Food Agric.* **2016**, 96, 4484–4491, doi:10.1002/jsfa.7662.
26. Wang, J.; Zhao, Y.; Li, W.; Wang, Z.; Shen, L. Optimization of polysaccharides extraction from *Tricholoma mongolicum* Imai and their antioxidant and antiproliferative activities. *Carbohydr. Polym.* **2015**, 131, 322–330, doi:10.1016/j.carbpol.2015.06.009.

27. Ma, L.; Chen, H.; Dong, P.; Lu, X. Anti-inflammatory and anticancer activities of extracts and compounds from the mushroom *Inonotus obliquus*. *Food Chem.* **2013**, *139*, 503–508, doi:10.1016/j.foodchem.2013.01.030.
28. Bohn, J.A.; BeMiller, J.N. (1→3)- $\beta$ -D-Glucans as biological response modifiers: a review of structure-functional activity relationships. *Carbohydr. Polym.* **1995**, *28*, 3–14, doi:10.1016/0144-8617(95)00076-3.
29. Lin, M.; Li, H.; Zhao, Y.; Cai, E.; Zhu, H.; Gao, Y.; Liu, S.; Yang, H.; Zhang, L.; Tang, G. Phylogenetic analysis of the genus *Lactobacillus* and related lactic acid bacteria as determined by reverse transcriptase sequencing of 16S rRNA. *FEMS Microbiol. Lett.* **1991**, *77*, 5–12, doi:10.1111/j.1574-6968.1991.tb04313.x.
30. Wang, X.; Bao, H.; Bau, T. Investigation of the possible mechanism of polysaccharides extracted from *Leucocalocybe mongolica* in exerting antitumor effects in H22 tumor-bearing mice. **2021**, 1–16, doi:10.1111/jfbc.13514.
31. Lin, M.; Li, H.; Zhao, Y.; Cai, E.; Zhu, H.; Gao, Y.; Liu, S.; Yang, H.; Zhang, L.; Tang, G. 2-Naphthoic acid ergosterol ester, an ergosterol derivative, exhibits anti-tumor activity by promoting apoptosis and inhibiting angiogenesis. *Steroids* **2017**, *122*, 9–15, doi:10.1016/j.steroids.2017.03.007.
32. Reguera, L.; Attorresi, C.I.; Ramírez, J.A.; Rivera, D.G. Steroid diversification by multicomponent reactions. *Beilstein J. Org. Chem.* **2019**, *15*, 1236–1256, doi:10.3762/bjoc.15.121.
33. Townsend, D.; Shankland, K.; Weymouth-Wilson, A.; Komsta, Z.; Evans, T.; Cobb, A.J.A. Synthesis of an intriguing steroidal constitutional isomer. *Tetrahedron Lett.* **2020**, *61*, 151942, doi:10.1016/j.tetlet.2020.151942.
34. Chun-lan, T.; Hai-ying, B.A.O.; Tolgor, B.A.U. The chemical constituents and antibacterial activity of the petroleum ether extract of *Tricholoma mongolica* fruit body extract from fruit bodies of *Tricholoma mongolicum*. **2010**, *29*, 619–624. in china
35. Jeong, J.W.; Lee, H.H.; Han, M.H.; Kim, G.Y.; Hong, S.H.; Park, C.; Choi, Y.H. Ethanol extract of *Poria cocos* reduces the production of inflammatory mediators by suppressing the NF- $\kappa$ B signaling pathway in lipopolysaccharide-stimulated RAW 264.7 macrophages. *BMC Complement. Altern. Med.* **2014**, *14*, 1–8, doi:10.1186/1472-6882-14-101.
36. Qingzhi, Y.; Zhiyu, Z.; Wei, Y.; Grassland, X. Optimization of Extraction Technology of the *Tricholoma mongolicum* Triterpenoid by the Method of Response Surface Analysis. **2009**, 2–5.
37. Davarpanah, S. J.; Ahn, J. W.; Ko, S. M.; Jung, S. H.; Park, Y. I.; Liu, J. R.; Jeong, W.J. Stable expression of a fungal laccase protein using transplastomic tobacco. *Plant Biotechnol. Rep.* **2012**, *6*, 305–312, doi:10.1007/s11816-012-0225-4.
38. Wang, D.W.; En-Qi, W.U. Bau, T. Research of Preparation Technology of *Tricholoma mongolicum* Polypeptide. *food science* **2007**, *28*, 245–249.
39. Thakur, A.; Pal, L.; Ahmad, A.; Khan, M.I. Complex carbohydrate specificity of lectin



- from fruiting body of *Ganoderma lucidum*. A surface plasmon resonance study. *IUBMB Life* **2007**, *59*, 758–764, doi:10.1080/15216540701663463.
40. Ganguly, C.; Das, S. Plant lectins as inhibitors of tumor growth and modulators of host immune response. *Chemotherapy* **1994**, *40*, 272–278.
  41. Wang, H.X.; Ng, T.B.; Liu, W.K.; Ooi, V.E.C.; Chang, S.T. Isolation and characterization of two distinct lectins with antiproliferative activity from the cultured mycelium of the edible mushroom *Tricholoma mongolicum*. *Int. J. Pept. Protein Res.* **1995**, *46*, 508–513, doi:10.1111/j.1399-3011.1995.tb01606.x.
  42. Wang, H.X.; Ooi, V.E.C.; Ng, T.B.; Chiu, K.W.; Chang, S.T. Hypotensive and vasorelaxing activities of a lectin from the edible mushroom *Tricholoma mongolicum*. *Pharmacol. Toxicol.* **1996**, *79*, 318–323, doi:10.1111/j.1600-0773.1996.tb00016.x.
  43. Zhao, J.K.; Wang, H.X.; Ng, T.B. Purification and characterization of a novel lectin from the toxic wild mushroom *Inocybe umbrinella*. *Toxicon* **2009**, *53*, 360–366, doi:10.1016/j.toxicon.2008.12.009.
  44. Santhiya, M.; Jansi, M. Screening of wild mushroom *Amanita* species for occurrence of lectins and their partial purification by RP-HPLC. *Middle East J. Sci. Res.* **2013**, *14*, 456–460, doi:10.5829/idosi.mejsr.2013.14.4.7322.
  45. Kobayashi, Y.; Kobayashi, K.; Umehara, K.; Dohra, H.; Murata, T.; Usui, T.; Kawagishi, H. Purification, characterization, and sugar binding specificity of an N-glycolylneuraminic acid-specific lectin from the mushroom *Chlorophyllum molybdites*. *J. Biol. Chem.* **2004**, *279*, 53048–53055, doi:10.1074/jbc.M407997200.
  46. Wang, H.X.; Ng, T.B.; Ooi, V.E.C. Lectin activity in fruiting bodies of the edible mushroom *Tricholoma mongolicum*. *Biochem. Mol. Biol. Int.* **1998**, *44*, 135–141, doi:10.1080/15216549800201142.
  47. Wang, H.X.; Liu, W.K.; Ng, T.B.; Ooi, V.E.C.; Chang, S.T. The immunomodulatory and antitumor activities of lectins from the mushroom *Tricholoma mongolicum*. *Immunopharmacology* **1996**, *31*, 205–211, doi:10.1016/0162-3109(95)00049-6.
  48. Singh Pannu, J.; Kumar Kapoor, R. Microbial Laccases: a Mini-Review on Their Production, Purification and Applications. *Int. J. Pharm. Arch.* **2014**, *3*, 528–536.
  49. Passarini, M.R.Z.; Ottoni, C.A.; Santos, C.; Lima, N.; Sette, L.D. Induction, expression and characterisation of laccase genes from the marine-derived fungal strains *Nigrospora* sp. CBMAI 1328 and *Arthopyrenia* sp. CBMAI 1330. *AMB Express* **2015**, *5*, doi:10.1186/s13568-015-0106-7.
  50. Rezaei, S.; Tahmasbi, H.; Mogharabi, M.; Ameri, A.; Forootanfar, H.; Khoshayand, M.R.; Faramarzi, M.A. Laccase-catalyzed decolorization and detoxification of Acid Blue 92: Statistical optimization, microtoxicity, kinetics, and energetics. *J. Environ. Heal. Sci. Eng.* **2015**, *13*, 1–9, doi:10.1186/s40201-015-0183-1.
  51. Reinhammar, B. Purification and properties of laccase and stellacyanin from *Rhus vernicifera*. *BBA - Bioenerg.* **1970**, *205*, 35–47, doi:10.1016/0005-2728(70)90059-9.

52. Dwivedi, U.N.; Singh, P.; Pandey, V.P.; Kumar, A. Structure-function relationship among bacterial, fungal and plant laccases. *J. Mol. Catal. B Enzym.* **2011**, *68*, 117–128, doi:10.1016/j.molcatb.2010.11.002.
53. Li, M.; Zhang, G.; Wang, H.; Ng, T. Purification and characterization of a laccase from the edible wild mushroom *Tricholoma mongolicum*. *J. Microbiol. Biotechnol.* **2010**, *20*, 1069–1076, doi:10.4014/jmb.0912.12033.
54. Ho Wong, J.; Bun Ng, T.; Jiang, Y.; Liu, F.; Cho Wing Sze, S.; Yanbo Zhang, K. Purification and Characterization of a Laccase with Inhibitory Activity Toward HIV-1 Reverse Transcriptase and Tumor Cells from an Edible Mushroom (*Pleurotus cornucopiae*). *Protein Pept. Lett.* **2010**, *17*, 1040–1047, doi:10.2174/092986610791498966.
55. Wang, H.X.; Ng, T.B. Purification of a novel low-molecular-mass laccase with HIV-1 reverse transcriptase inhibitory activity from the mushroom *Tricholoma giganteum*. *Biochem. Biophys. Res. Commun.* **2004**, *315*, 450–454, doi:10.1016/j.bbrc.2004.01.064.
56. Wu, E. Q.; Tuli, G.E. Research advancement on *Tricholoma mongolicum*. *Edible fungi of china* **2007**, *26*, 3–5.
57. Lu, T.; Bau, T.; Ohga, S. Physiological study of the wild edible mushroom *leucocalocybe mongolica*. *J. Fac. Agric. Kyushu Univ.* **2017**, *62*, 1–8.
58. Suriguge, B.; Bao, H.; Tolgor, B.; Yang, S.D.; Sun, X. Anti-Tumor Activity of *Tricholoma mongolicum* Fruit Bodies. *食品科学* **2012**, *33*, 280–284.
59. Wang, H.X.; Ng, T.B.; Ooi, V.E.C.; Liu, W.K.; Chang, S.T. A polysaccharide-peptide complex from cultured mycelia of the mushroom *Tricholoma mongolicum* with immunoenhancing and antitumor activities. *Biochem. Cell Biol.* **1996**, *74*, 95–100, doi:10.1139/o96-010.
60. Li, W.; Xu, Q.; He, Y. F.; Liu, Y.; Yang, S.- B.; Wang, Z.; Zhang, J.; Zhao, L.C. Anti-tumor effect of steamed *Codonopsis lanceolata* in H22 tumor- bearing mice and its possible mechanism. *Nutrients* **2015**, *7*, 8294– 8307.
61. Sarup Singh, R.; Preet Kaur, H.; Rakesh Kanwar, J. Mushroom Lectins as Promising Anticancer Substances. *Curr. Protein Pept. Sci.* **2016**, *17*, 797–807, doi:10.2174/1389203717666160226144741.
62. Liu, F.; Ng, T. B.; Wang, H, et al Lectin from *L. mongolica* S. Imai (Agaricomycetideae) Mycelia Stimulates Gene Expression of Immunomodulating Cytokines in Mouse Peritoneal Macrophages and Splenocytes. *Int. J. Med. Mushrooms* **2005**, *7*, 243-248.
63. El Enshasy, H. A.; Hatti-Kaul, R. Mushroom immunomodulators: Unique molecules with unlimited applications. *Trends Biotechnol.* **2013**, *31*, 668–677.
64. Qing-zhi, Y.; Xiu-ling, H.E.; Zhi-yu, Z.H. Jiu-sheng, X.Y.W. Effect of *Tricholoma mongolicum* and Its Polysaccharide Extract on Immune Function in Mice. *Prog. Vet. Med.* **2011**, *32*, 47–51.

65. Chiu, K.W.; Lam, A.H.W.; Pang, P.K.T. Cardiovascular active substances from the straw mushroom, *Volvariella volvacea*. *Phyther. Res.* **1995**, *9*, 93–99, doi:10.1002/ptr.2650090203.
66. Kleha, J.F.; Devesly, P.; Johns, A. The effects of lectins on the release of EDRF from rabbit aorta. *Br. J. Pharmacol.* **1991**, *104*, 287–288, doi:10.1111/j.1476-5381.1991.tb12421.x.
67. Topping, D.L. Soluble Fiber Polysaccharides: Effects on Plasma Cholesterol and Colonic Fermentation. *Nutr. Rev.* **1991**, *49*, 195–203, doi:10.1111/j.1753-4887.1991.tb03021.x.
68. Zhang, G.; Huang, Y.; Bian, Y.; Wong, J.H.; Ng, T.B.; Wang, H. Hypoglycemic activity of the fungi *Cordyceps militaris*, *Cordyceps sinensis*, *Tricholoma mongolicum*, and *Omphalia lapidescens* in streptozotocin-induced diabetic rats. *Appl. Microbiol. Biotechnol.* **2006**, *72*, 1152–1156, doi:10.1007/s00253-006-0411-9.
69. Gulati, V.; Dass Singh, M.; Gulati, P. Role of mushrooms in gestational diabetes mellitus. *AIMS Med. Sci.* **2019**, *6*, 49–66, doi:10.3934/medsci.2019.1.49.
70. Zhibao, W.; Hui, Y.; Zaoying, Y.; Jiaming, T.; Limin, Zh.; Xiaohong, T. Effects of Aqueous Extracts of *Tricholoma mongolicum* Fruit Bodies on Selected Myocardial Ischemia Markers in Isoproterenol-Induced Ischemic Mice. *Acta Edulis Fungi.* **2014**, *21*, 47–50.
71. Hui, Z.B.Y. Effect of *Tricholoma mongolicum* on Anti-stress of Mice. *Henan Agric. Sci.* **2014**, *9*, 145–147.
72. Ishikawa, Y.; Marimoto, K.; Hamasaki, T. Flavoglucin, a metabolite of *Eurotium chavalieri*, its oxidation and synergism with tocopherol. *J Am Oil Chem Soc* **1984**, *61*, 1864–1868.
73. Leekha, S.; Terrell, C.L.; Edson, R.S. General principles of antimicrobial therapy. *Mayo Clin. Proc.* **2011**, *86*, 156–167, doi:10.4065/mcp.2010.0639.
74. Gualerzi, C. O.; Brandi, L.; Fabbretti, A.; Pon, C.L. Antibiotics: Targets, mechanisms and resistance. Germany: *John Wiley Sons* **2013**, 133–149.
75. Gallo, G. G.; Lancini, G.; Parenti, F. *Antibiotics: A Multidisciplinary Approach*; 2013;
76. Alves, M.; Ferreira, I.F.R.; Dias, J.; Teixeira, V.; Martins, A.; Pintado, M. A review on antimicrobial activity of mushroom (basidiomycetes) extracts and isolated compounds. *Planta Med.* **2012**, *78*, 1707–1718, doi:10.1055/s-0032-1315370.