International Journal of Zoology and Applied Biosciences **ISSN: 2455-9571**
Volume 7, Issue 6, pp: 41-48, 2022 (Special Issues)
https://doi.org/10.55126/ijzab.2022.v07.i06.sp011
http://www.ijzab.com

Research Article

IN VITRO ANTIOXIDANT ACTIVITIES AND CHARACTERIZATION OF ETHANOLIC POLYSACCHARIDE FROM *HYPSIZYGUS ULMARIUS* MUSHROOM

***Alamelu Thimmaraju and Sudha Govindan**

Department of Biochemistry, Periyar University, Salem, Tamil Nadu, India

Article History: Received 2nd November 2022; Accepted 17th December 2022; Published 24th December 2022

ABSTRACT

The *Hypsizygus ulmarius* ethanol polysaccharide (HUEP)and its water-soluble edible mushroom, both recognized for their therapeutic powers and for providing crucial enzymes for industry, were extracted used ethanol in this work. Investigated were the in vitro antioxidant capacities of decreasing power, ABTS, and the DPPH assays. The study's goal was to ascertain the chemical composition of various molecules, including uronic acid, protein, and carbohydrate. UV, X-ray Diffraction, and Fourier transform infrared spectroscopy (FTIR). In these assays with lower EC_{50} values, the study found that HUEP exhibited the strongest antioxidant activity. Various polysaccharide extracts of the mushroom may be used as a readily available food source that is high in natural antioxidants, as a potential food supplement, or even as a medicinal agent, according to the findings of the current study. The results of various in vitro assay systems showed that the polysaccharide ethanolic extract of HUEP has strong antioxidant properties. Extracts from polysaccharides could be useful for creating food additives that are antioxidants.

Keywords: *Hypsizygus ulmarius*, Antioxidant, Polysaccharide, Mushroom, Ethanolic extract.

INTRODUCTION

The fruiting body of a fungus that produces mushrooms normally grows above ground on soil and bears spores. Many nations enjoy eating fresh and preserved mushrooms as a delicacy, especially for their distinct flavour and texture (Kalač, (2013). There are thought to be 150,000 kinds of mushrooms on earth, yet only 10% of them are known to science (Wasser, 2011). At least 2000 of them are edible, and more than 200 are harvested from the wild and used for a variety of traditional medical treatments (Sánchez, 2004). Consumer demand for functional foods has increased in recent years as a result of growing awareness of human health, nutrition, and illness prevention. In reality, mushrooms have gained popularity as useful foods and a source of bioactive compounds. Research on a variety of mushrooms has been conducted by the scientific community in an effort to find new therapeutic options. In several therapies, it has been discovered that mushrooms are medically effective, including antitumor. In the (Greeshma *et al*., 2016), reported of *H. ulmarius* exhibits antioxidant, anti-inflammatory, and anti-tumor activities when ethanol is produced from the plant. Mushrooms offer a wealth of bioactive substances, many of which have therapeutic properties; they are valued for their ability to improve health and are widely consumed in Asian nations. Previous studies (Daba, & Ezeronye, 2003; Milano *et al*., 2020; Guo et al., 2020; Jeong *et al*., 2010), have demonstrated the potential uses of mushroom polysaccharides for immune system stimulation, anticancer therapy, and the management and prevention of hyperglycemia and hypercholesterolemia. Other researchers asserted that oyster mushrooms may be considered as functional foods because of their positive effects on human health (Synytsya *et al*., 2009; Patel *et al*., 2012). The *H. ulmarius* ethanol polysaccharide (HUEP) technique recovers bioactive compounds from the sample matrix using hot water extraction temperatures at (100˚C). The sample matrix is extracted using the HUEP technique to retrieve bioactive compounds. The HUEP boosted aqueous extraction's

*Corresponding Author: Alamelu Thimmaraju, Research Scholar, Department of Biochemistry, Periyar University, Salem, Tamil Nadu, India Email: alamelumaha123@gmail.com.

extraction efficiency similar to other conventional extraction techniques by raising the ionization constant, which led to the production of more free ions (H^+ and OH). HUEP offer more advantages than conventional technologies, including highly effective extraction and environmentally friendly features. Water's diffusivity efficiency significantly increases in subcritical settings, boosting extraction recovery yield and accelerating extraction (Zhang *et al*., 2019). In the evaluation of the literature, there were no studies about HUAE and the chemical modification of oyster mushroom extraction. Therefore, it is believed that employing HUAE will make it easy and secure to recover the source of mushroom extraction (Chen *et al*., 2016).The yield and structural features of polysaccharides as well as biological activity may be significantly influenced by the extraction technique. Due to the simple use, eco friend, and hot water extraction. Since ancient times, technology has generally been used for the extraction and processing of polysaccharides. It is the most practical and conventional method and is commonly applied in companies (Yan *et al*., 2018). To our knowledge, no thorough research has been done on the physicochemical properties and biological analysis of polysaccharides from *H. ulmarius*, nor has the connection between HUEP's chemical structure and antioxidant activity. Therefore, the objective of this study was to use the hot water extraction method to ethanol polysaccharide from *H. ulmarius*. We assessed the chemical profile, UV, and XRD. Additionally investigated in vitro was polysaccharide's antioxidant activity.

MATERIALS AND METHODS

Themushroom (*H. ulmarius*) was bought from VG. Mushroom Salem, and the 5% phenol sulfuric acid, DPPH, and ABTS were bought from Sigma Chemical Co. (st. Louis, MO, USA).

Hot water extraction

In order to create the pre-extract, 50g of *Hypsizygus ulmarius* mushroom powder was defatted with 250 mL of petroleum ether and put in a magnetic stirr for 8 hours in two days later. It was filtered using Whatman No. 4 filter paper and allowed to dry at room temperature after adding 750ml of distilled water, boiling at 90°C for three hours, and then repeating three times. After the combined supernatant was concentrated, dried at 60°C in an oven, and the extraction was precipitated (ethanol(95%, *v/v*) and kept at 4°C over night) to extract polysaccharide given the designation HUEP, it was then filtered and centrifuged for 15 min at 3000 rpm (Thimmaraju & Govindan, 2022).The formula below was used to compute the ethanol extraction Yield (%):[Weight of dried Aqueous extraction weight of raw materials (g)] x 100% = Aqueous extraction yield (%w/w).

Characterization of ethanol polysaccharide

Spectral analysis of FTIR

The ethanol polysaccharide IR spectra were collected using a Bruker-Vector 22 spectrometer (Bruker, Inc.). 400 to 4000 cm-1 is the frequency range (Rheinstetten Germany). 1mg of the material with KBr powder thoroughly crushed and made into 1mm pellets for FTIR analysis.

Ultraviolet Analysis and X-ray Diffractions

In Millipore water, HUEP was solubilized before being scanned with a UV spectrophotometer (UV-1800, Shimadzu). An X-ray diffract was used to find the XRD spectrum (Siemens D 5000, Germany).

Physicochemical properties

The Brad Ford approach was used to calculate the protein content using bovine serum albumin as the reference (Zor & Selinger 1996). To detect uronic acid, D-glucuronic acid was employed as the reference in a modified carbazole approach (Bitter, 1962). The phenol-sulphuric acid method was employed to ascertain the total sugar content of the HUEP used glucose as the reference material (Dubios *et al*., 1951).

Antioxidant activities

Antioxidant assay of DPPH

A freshly prepared 1ml of DPPH solution was combined with HUEP (0.5 to 2.5 mg/ml) at various concentrations totaling around 2.0 ml or a positive control (VC) (with a final concentration of 0.2mM). After incubation, the resultant solution was read at 517 nm for absorbance (Shimada *et al*., 1992; Subramsnian *et al* 2020). The scavenging ability is calculated using the formula [(A0-A1)/A0] x 100, where A0 represents the absorbance of the control and A1 represents the absorbance of the sample.

Antioxidant assay of ABTS

The aforementioned method was used to evaluate HUEP's capacity to scavenge ABTS+ cation radicals (Re *et al*., 1999). In order to create the ABTS+ cation, 2.045 mM potassium per sulphate and 7 mM ABTS cation solution were combined and incubated for 16 hours. In the evening, at 25°C (room temperature). The ABTS+ cation solution was diluted with 80% ethanol to achieve an absorbance of 0.700 0.02 at 734 nm. The absorbance was measured at 734 nm after mixing 10L of 1-5mg/ml HUAE for 6 min with 1ml of diluted ABTS+ cation solution. The quenching activity of the ABTS+ cation radical is computed using the formula [(A0-A1)/A0] x100%, where A0 is the absorbance of ABTS + water and A1 is ABTS + HUEP.

Reducing Power

Using (Deng *et al.*, 2011) the decreasing power of HUEP was evaluated. The reaction mixtures contained potassium ferricyanide (1%, w/v), phosphate buffer, and HUEP (1–5 mg/mL) (pH 6.6, 0.2M). After 20 min at 50˚C, 205 mL of 10% w/v trichloroacetic acid was added to the mixture to stop the reaction. The mixture was then centrifuged at 1200 rpm, 10 min. 2.5 mL of the supernatant were taken in total, and 0.5mL of deionized water and 0.1% (w/v) $FeCl_3$ were added. After 15 min of incubation at room temperature, the absorbance was assessed at 700 nm using Vc as a positive control.

Statistical Analysis

The results are provided as means with standard deviations (SD). Because each trial was conducted in triplicate, One-way analysis of variance (ANOVA) was carried out using the SPSS program. The Duncan multiple range tests were used to determine the mean differences. All results with a p value of <0.05 or above were deemed statistically significant.

RESULT AND DISCUSSION

Depending up on the different ionic groups that the sample contains. Since the sample was called *Hypsizygus ulmarius* ethanol polysaccharide (HUEP) and the extraction's goal was to solubilize the sample in deionized water, they were utilizing hot water extraction of ethanol precipitated and HUEP yield was 16 %. The chemical composition's hot water extraction (HUEP) yielded an estimated total carbohydrate content of 102.6% (Table-1). According to the degree of polymerization, the derivatives of polyhydroxylated aldehydes or ketones that make up mushroom carbohydrates can be categorized as mono, di, oligo, or polysaccharides (Nasrollahzadeh *et al*., 2021; Re *et al*., 1999; He *et al*., 2020). They are one of the essential elements of higher fungi and typically range from 30.5 to 86% (Reddy, 2016; Kalac 2013). Glucose, fructose, maltose, rhamnose, arabinose, sucrose, and xylose have been identified as the primary carbohydrates in mushrooms (Kaliyaperumal *et al*., 2018; Khan *et al*., 2018). However, a large number of mushrooms are made up of glucans, which are linear or linear polysaccharides integrated by glucose with linkage (1-3) and (1-6) present as part of fruiting bodies (Kalac 2013; Sanchez 2017).

The estimated total protein percentage of HUEP's was 59.7 % (Table 1). Numerous physical, chemical, and biological features are given to these biomolecules by these nitrogen compounds (Bonomi & Iametti, 2018; Lin *et al*., 2013). Recent research has shown that the protein output of macro-high fungus is one of the most nutrient-dense components when compared to other vegetable protein sources (Singh & Passari, 2018). Edible mushrooms typically contain between 10 and 35% of protein by dry weight as a part of an intricate network of fungus cells (Reddy, 2016b; Sanchez 2017).Along with some enzymes like laccase or inactivated ribosomal enzyme, the majority of the proteins connected to mushrooms are lectins. As shown in (Table-1) (Wang et al., 2021). HUEP had a greater concentration of uronic acid was 77.76% than *Monascus pupureus* mycelium crude polysaccharide (MPS) (7.57% by 19.45%). The amount of uronic acid in polysaccharides has been found to be directly correlated with their antioxidant capabilities (Ma et al., 2013). Because of the abundance of uronic acid and perceived carbohydrates, the antioxidant capacity may be increased.

Table 1. Characterization of ethanol polysaccharide.

Physicochemical Properties	HUEP (%)
Total carbohydrate	102.6
Total Protein	59.70
Uronic acid	77.76

It is common practice to use FT-IR spectroscopy to identify different functional groups, including C-H, N-H, O-H, and C-O Figure 1. The hydroxyl OH stretching vibration was indicated by the oddly large peak at 3429.92 cm^{-1}. The weak band 2940 cm^{-1} is related to vibrations of the hydroxyl group, as well as vibrations of the C-H ring and its bending and stretching (Nie *et al.*, 2018). These extractions contain bound proteins that exhibit an amide group vibration at 1569 cm^{-1}. Sulphate groups' (SO3) absorption peak at 1299 cm^{-1} supported asymmetrical S=O stretching vibrations (Qian *et al.*, 2009). The stretching vibration of C-O and C-H was suggested by the HUEP absorption peak at 1416 cm-1 (Liang *et al.*, 2019). The absorption peak in the 1200-1000 cm^{-1} region, which is brought on by C-O stretching vibrations, and the peaks suggest that HUEP may have contained pyranose monomer.

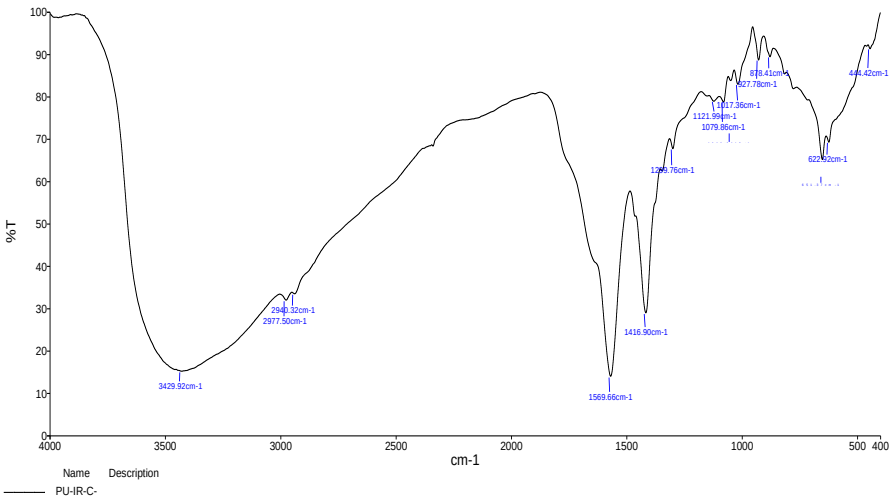

Figure 1.FTIR spectra of HUEP in the range of 400-3500 cm^{-1}.

The UV spectrum shows Figure-2 (a) of absorption peak at 400nm, which is consistent with the findings of Table 1 about the protein, content and suggests that HUEP may contain significant amounts of protein.

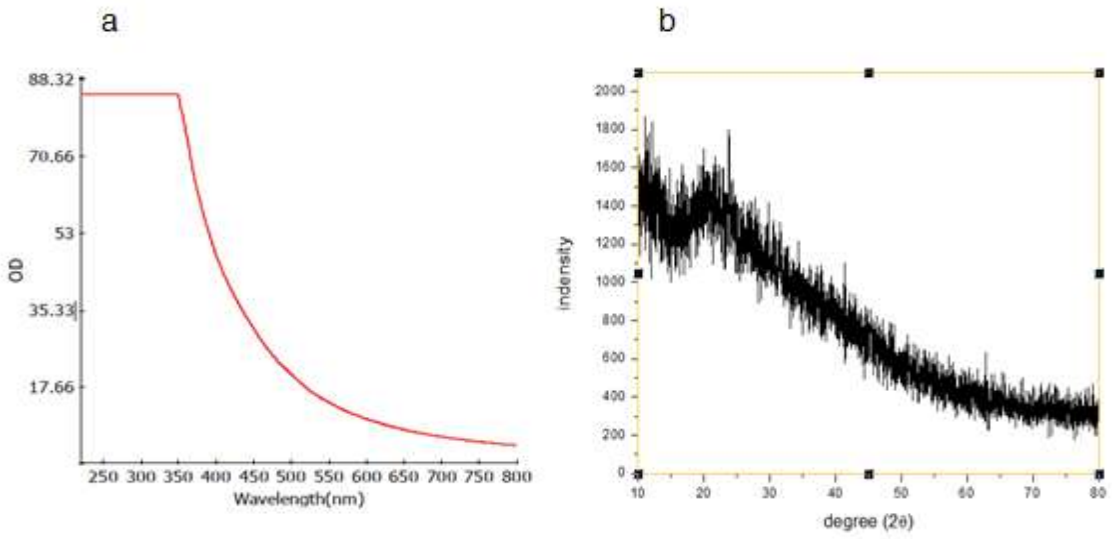

Figure 2. (a) UV spectra of HUAE (b) XRD pattern of HUAE.

XRD pattern Figure 2 (b) of the range of the HUEP's, which was between 10 and 17.5° (b). The amorphous nature of HUAE is demonstrated by the broad curve and absence of a hurried peak, which is consistent with past research (Qian *et al*., 2009b). Antioxidant assays using three methods for removing free radicals are based on various reaction mechanisms, and the outcomes are frequently not the same. Therefore, in order to evaluate the antioxidant capacity of the samples in an objective manner, it is required to test several oxidants. Here, we evaluated using seven modelsystems.

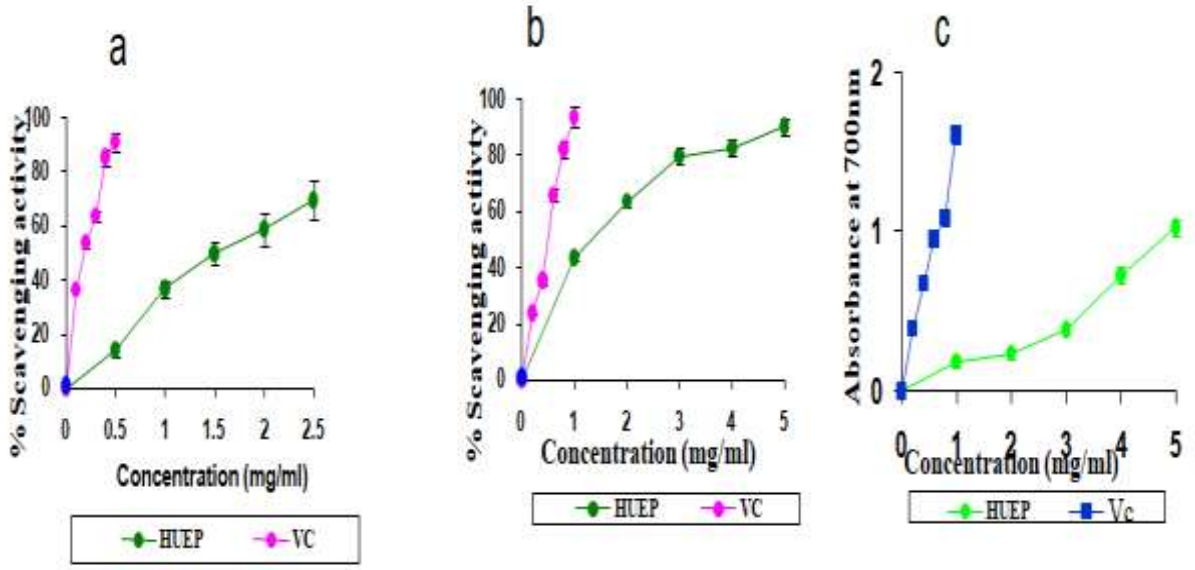

Figure3. In *vitro* antioxidant assays of HUEP. DPPH (a), ABTS+ (b) and Reducing Power (c) Results were expressed as mean ± SD (n=3). Mean values within each graph and show significant differences ($p<0.05$) between concentrations.

Table2. EC_{50} value of HUEP and the different antioxidant assays.

Antioxidant Assays	HUEP	Vc
DPPH	3.031	0.390
ABTS	1.04	0.245
Reducing Power	0.926	0.289

DPPH approach based on the transformation of purple-tinted free radicals from original colour to yellow in the presence of antioxidants, this analysis was conducted. The decrease in absorbance shows that hydrogen has a quenching impact on free radicals (Liu *et al*., 2020). The DPPH free radical scavenging activity of HUEP, which exhibits a dilution-dependent response, is based on this. HUAE's quenching rate on DPPH radicals shown in (Table 2) rise from 13.69% to 69.02% at 0.5-2.5 mg/mL (r^2=0.961, Y= 16.35x+5.88). HUAP has a considerably very low IC_{50} value (0.2-1 mg/mL). By providing DPPH with hydrogen or electrons, aqueous extraction creates stable molecules.

Additionally, aqueous HUEP can prevent the synthesis of hydroxyl radicals (Wang *et al*., 2020b). The ABTS+ performance is often used to assess the in vitro antioxidant capacity of natural substances. When potassium per sulphate and ABTS are combined, the result is the blue-green cation ABTS free radical. The system is then bleached as a result of the antioxidant component's interaction with the ABTS free radical, confirming the antioxidant capabilities of HUEP. (Figure. 2 b) contrasts the HUEP's ability to scavenge ABTS with that of vitamin C radicals. The maximum amount of HUEP that could effectively scavenge the ABTS radical was (43-90%) in the concentration range of 1 to 5 mg/mL. HUEP's (IC_{50} = 1.04 mg/ml) capacity to scavenge the ABTS radical is inferior to that of vitamin C (IC50 = 0.245 mg/ml) (Table 2) (Kallithraka *et al*., 2001). HUEP had a low concentration and quenching rate of antioxidant assay of ABTS. Ability to decrease other chemicals is a key indicator of a compound's potential antioxidant effect (Xu et al., 2011). Investigations into HUEP's and Vitamin C's ability to decrease blood pressure are shown in (Figure. 2 c). HUEP's are far more effective at decreasing than vitamin C. The results showed that IC_{50} valueof (0.926 mg/mL) HUEP's might possess antioxidant properties.

CONCLUSION

The results of this study unambiguously showed that *H. ulmarius* ethanol extract exhibited significant antioxidant activity against various ethanol polysaccharides using hot water. UV and FT-IR spectra revealed that HUEP had a large amount of protein and several types of glycosidic linkages. Several in vitro tests were conducted to gauge HUEP's antioxidant performance. HUEP displayed significant antioxidant activity, as evidenced by the outcomes of the ABTS, DPPH, and reducing power tests, all of which revealed a dose-dependent relationship. These results demonstrated that the aqueous extract of *H. ulmarius* has potent antioxidant activity and may be a unique natural antioxidant in food and medicine.

ACKNOWLEDGMENT

The authors express sincere thanks to the head of the Department of Biochemistry, Periyar University, Salem, Tamil Nadu, India for the facilities provided to carry out this research work.

REFERENCES

Bitter, T. (1962). A modified uronic acid carbazole reaction. *Annals Biochemistry*., *4*, 330-334.

Bonomi, F., &Iametti, S. (2018). Proteins Foods. Reference Module in Chemistry, *Molecular Sciences and Chemical Engineering*. 978-0-12-409547- 2.14544-5.

Chen, G., Yuan, Q., Saeeduddin, M., Ou, S., Zeng, X., & Ye, H. (2016). Recent advances in tea polysaccharides: Extraction, purification, physicochemical characterization and bioactivities. *Carbohydrate Polymers*, *153*, 663-678.

Daba, A. S., &Ezeronye, O. U. (2003). Anti-cancer effect of polysaccharides isolated from higher basidiomycetes mushrooms. *African Journal of Biotechnology*, *2*(12), 672-678.

Deng, P., Zhang, G., Zhou, B., Lin, R., Jia, L., Fan, K., & Zhang, J. (2011). Extraction and in vitro antioxidant activity of intracellular polysaccharide by Pholiotaadiposa SX-02. *Journal of Bioscience and Bioengineering*, *111*(1), 50-54.

Dubios, M. K., Gilles, J. K., Robers, P. A., & Smith, F. (1951). Calorimetric determination of sugar and related substance. *Analytical Chemistry*, *26*, 351-356.

Greeshma, P., Ravikumar, K. S., Neethu, M. N., Pandey, M., Zuhara, K. F., &Janardhanan, K. K. (2016). Antioxidant, anti-inflammatory, and antitumor activities of cultured mycelia and fruiting bodies of the elm oyster mushroom, Hypsizygusulmarius (Agaricomycetes). *International Journal of Medicinal Mushrooms*, *18*(3).

Guo, W. L., Deng, J. C., Pan, Y. Y., Xu, J. X., Hong, J. L., Shi, F. F., &Lv, X. C. (2020). Hypoglycemic and hypolipidemic activities of Grifolafrondosa polysaccharides and their relationships with the modulation of intestinal microflora in diabetic mice induced by high-fat diet and streptozotocin. *International Journal of Biological Macromolecules*, *153*, 1231-1240.

He, J., Evans, N. M., Liu, H., & Shao, S. (2020). A review of research on plant-based meat alternatives: Driving forces, history, manufacturing, and consumer attitudes. *Comprehensive Reviews in Food Science and Food Safety*, *19*(5), 2639-2656.

Jeong, S. C., Jeong, Y. T., Yang, B. K., Islam, R., Koyyalamudi, S. R., Pang, G., & Song, C. H. (2010). White button mushroom (Agaricusbisporus) lowers blood glucose and cholesterol levels in diabetic and hypercholesterolemic rats. *Nutrition Research*, *30*(1), 49-56.

Kalač, P. (2013). A review of chemical composition and nutritional value of wild growing and cultivated mushrooms. *Journal of the Science of Food and Agriculture*, *93*(2), 209-218.

Kalač, P. (2013). A review of chemical composition and nutritional value of wild-growing and cultivated mushrooms. *Journal of the Science of Food and Agriculture*, *93*(2), 209-218.

Kalač, P. (2013). A review of chemical composition and nutritional value of wild-growing and cultivated

mushrooms. *Journal of the Science of Food and Agriculture*, *93*(2), 209-218.

Kaliyaperumal, M., Kezo, K., &Gunaseelan, S. (2018). A global overview of edible mushrooms. *Biology of Macrofungi*, 15-56.

Kallithraka, S., Bakker, J., & Clifford, M. N. (2001). Correlations between saliva protein composition and some T–I parameters of astringency. *Food Quality and Preference*, 12, 145-152.

Khan, A. A., Gani, A., Khanday, F. A., &Masoodi, F. A. (2018). Biological and pharmaceutical activities of mushroom β-glucan discussed as a potential functional food ingredient. *Bioactive Carbohydrates and Dietary Fibre*, *16*, 1-13.

Liang, X. X., Gao, Y. Y., Pan, Y., Zou, Y. F., He, M., He, C. L., &Lv, C. (2019). Purification, chemical characterization and antioxidant activities of polysaccharides isolated from Mycenadendrobii. *Carbohydrate Polymers*, *203*, 45-51.

Lin, S.-Y., Chen, Y.-K., Yu, H.-T., Barseghyan, G. S., Asatiani, M. D., Wasser, S. P., & Mau, J.-L. (2013). Comparative study of contents of several bioactive components in fruiting bodies and mycelia of culinary-medicinal mushrooms. *International Journal of Medicinal Mushrooms*, 15, 315-323.

Liu, Y., Hu, C., Feng, X., Cheng, L., Ibrahim, S., & Wang, C. (2020a). Isolation, characterization and antioxidant of polysaccharides from Stropharia rugosoannulata. *International Journal of Biological Macromolecules*, 155, 883-889.

Ma, L., Chen, H., Zhu, W., & Wang, Z. (2013). Effect of different drying methods on physicochemical properties and antioxidant activities of polysaccharides extracted from mushroom Inonotus obliquus. *Food Research International*, *50*(2), 633-640.

Milano, S., Dieli, M., Millott, S., Miceli, M. D., Maltese, E., &Cillari, E. (1991). Effect of isoprinosine on IL-2, IFN-γ and IL-4 production in vivo and in vitro. *International Journal of Immunopharmacology*, *13*(7), 1013-1018.

Nasrollahzadeh, M., Sajjadi, M., Nezafat, Z., &Shafiei, N. (2021). Polysaccharide biopolymer chemistry. *Biopolymer-Based Metal Nanoparticle Chemistry for Sustainable Applications: Volume 1: Classification, Properties and Synthesis*, *1*, 45.

Nie, C.; Zhu, P.; Ma, S.; Wang, M.; Hu, Y. (2018), Purification, characterization and immunomodulatory activity of polysaccharides from stem lettuce. *Carbohydr. Polym.* 188, 236-242.

Patel, Y., Naraian, R., & Singh, V. K. (2012). Medicinal properties of Pleurotus species (oyster mushroom): a review. *World Journal of Fungal and Plant Biology*, *3*(1), 1-12.

Qian, J. Y., Chen, W., Zhang, W. M., & Zhang, H. (2009). Adulteration identification of some fungal polysaccharides with SEM, XRD, IR and optical rotation: A primary approach. *Carbohydrate Polymers*, *78*(3), 620-625.

Qian, J. Y., Chen, W., Zhang, W. M., & Zhang, H. (2009). Adulteration identification of some fungal polysaccharides with SEM, XRD, IR and optical rotation: A primary approach. *Carbohydrate Polymers*, *78*(3), 620-625.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical biology and Medicine*, *26*(9-10), 1231-1237.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free radical Biology and Medicine*, *26*(9-10), 1231-1237.

Reddy, S. M. (2016). Diversity and applications of mushrooms. In B. Bahadur, M. Venkat Rajam, L. Sahijram, & K. Krishnamurthy (Eds.), Plant biology and biotechnology (pp. 231-261). Springer.17

Reddy, S. M. (2016). Diversity and applications of mushrooms. In B. Bahadur, M. Venkat Rajam, L. Sahijram, & K. Krishnamurthy (Eds.), *Plant biology and Biotechnology*,18 pp. 231-261.

Sanchez, ´ C. (2017b). Bioactives from mushroom and their application. In M. Puri (Ed.), Food bioactives (pp. 23–57). Springer International Publishing AG.

Sánchez, C. (2004). Modern aspects of mushroom culture technology. *Applied Microbiology and Biotechnology*, *64*(6), 756-762.

Sánchez, C. (2017). Reactive oxygen species and antioxidant properties from mushrooms. *Synthetic and Systems Biotechnology*, *2*(1), 13-22.

Shimada, K., Fujikawa, K., Yahara, K., & Nakamura, T. (1992). Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. *Journal of Agricultural and Food Chemistry*, *40*(6), 945-948.

Singh, B. P., & Passari, A. K. (2018). Biology of Macrofungi. Cham: Springer.

Subramanian, S. K., & Ramani, P. (2020). Antioxidant and cytotoxic activities of Indian caper (*Capparis brevispina* DC (Capparaceae)) leaf extracts. *European Journal of Integrative Medicine*, *33*, 101038.

Synytsya, A., Míčková, K., Synytsya, A., Jablonský, I., Spěváček, J., Erban, V., &Čopíková, J. (2009). Glucans from fruit bodies of cultivated mushrooms Pleurotusostreatus and Pleurotuseryngii: Structure and

potential prebiotic activity. *Carbohydrate polymers*, *76*(4), 548-556.

Thimmaraju, A., & Govindan, S. (2022). Novel studies of characterization, antioxidant, anticoagulant and anticancer activity of purified polysaccharide from Hypsizygusulmarius mushroom. *Bioactive Carbohydrates and Dietary Fibre*, *27*, 100308.

Wang, N., Wu, Y., Jia, G., Wang, C., Xiao, D., Goff, H. D., & Guo, Q. (2021). Structural characterization and immunomodulatory activity of mycelium polysaccharide from liquid fermentation of Monascuspurpureus (Hong Qu). *Carbohydrate Polymers*, *262*, 117945.

Wang, W.; Li, X.; Chen, K.; Yang, H.; Jialengbieke, B.; Hu, X. Extraction optimization, characterization and the antioxidant activities in vitro and in vivo of polysaccharide from Pleurotus ferulae. *International Journal Biological Macromolecules.* 2020, 160, 380-389.

Wasser, S. P. (2011). Current findings, future trends, and unsolved problems in studies of medicinal mushrooms. *Applied Microbiology and Biotechnology*, *89*(5), 1323-1332

Xu, X., Yan, H., Chen, J., & Zhang, X. (2011). Bioactive proteins from mushrooms. *Biotechnology Advances*, 29, 667-674.

Yan, J. K., Ding, Z. C., Gao, X., Wang, Y. Y., Yang, Y., Wu, D., & Zhang, H. N. (2018). Comparative study of physicochemical properties and bioactivity of Hericiumerinaceus polysaccharides at different solvent extractions. *Carbohydrate Polymers*, *193*, 373-382.

Zhang, J., Wen, C., Gu, J., Ji, C., Duan, Y., & Zhang, H. (2019). Effects of subcritical water extraction microenvironment on the structure and biological activities of polysaccharides from Lentinusedodes. *International Journal of Biological Macromolecules*, *123*, 1002-1011.

Zor, T., & Selinger, Z. (1996). Linearization of the Bradford protein assay increases its sensitivity: theoretical and experimental studies. *Analytical Biochemistry*, *236*(2), 302-308.