# Antioxidant Compounds of the Edible Mushroom *Pleurotus ostreatus*

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**Abstract:** Mushrooms have been used since centuries in many ancient cultures as source of food and medicine. However, until now the therapeutic values of mushrooms position this group of macrofungi as one of the major component in traditional medicine practice especially in South East Asia and China. Of different species of known mushrooms, *Pleurotus spp.* is widely known as part of food chain based on its high nutritional value. However, of the more than 70 species known, only few species are cultivated in mass production and used such as *P. ostreatus, P. florida,* and *P. ajor-caju*. However, *P. ostreatus* (widely known as oyster mushroom) received more attention in food industries based on its high growth rate and ease of cultivation using different substrates. This mushroom is rich of wide range of bioactive molecules of proven medicinal values with many therapeutic activities as anticancer, immunomodulatory, antiapoptotic, anti hypocholesterolemic, anti hyperglycemic, antimicrobial, anti-inflammatory, anti-osteoporetic, and many others. This work focuses on reviewing on the different classes of oyster mushroom bioactive compounds of antioxidant activities such as phenolics, beta carotene, lycopene, ascorbic acid, tocopherols, and ergosterols. This review provides also comprehensive information on the recent research to enhance the antioxidant properties through alteration of the cultivation strategy and addition of some compounds during the cultivation of *P. ostreatus.*

**Keywords:** *Pleurotus ostreatus*, antioxidants, fruiting bodies, mycelium, mushroom bioactives.

## 1. INTRODUCTION

Oxidation is essential to human for the energy production providing the biological processes. However, the excessive production of reactive oxygen species (ROS) can cause damage to tissues, proteins, DNA, lipids carbohydrates and results in huge number of diseases including chronic diseases that may cause fatal [1]. Antioxidants are the molecules playing role in inhibiting or delaying the oxidation process in human body and their removal from the system. They aid to prevent the radical chain reactions of oxidation [2]. They might also be functioning as immune modulators and can be used for treatment of certain diseases along with the conventional therapy [3]. Antioxidants deter the progress of many chronic diseases including cancer [4,5]. Apart from free radial scavenging property, antioxidants play another role by altering the cellular signalling transcription factor [1]. Recent reports suggest that some endogenous and exogenous antioxidants are utilized to offset free radicals and sustaining redox balance in human body [6,7]. Due to these reasons, new interests have been established to identify antioxidants that are safe and effective, from the natural sources. Concern regarding toxicity and carcinogenic effect of synthetic antioxidants make it seems necessary for the search of natural antioxidants [8]. Some of the natural sources of antioxidants were including fruits [9], vegetables [10], mushrooms [11], tea [12] and coffee [13] on human health has been recognized to originate from their antioxidant activity. These antioxidant compounds were identified with abilities to remove reactive oxygen species (ROS) based on their structural properties. Numerous epidemiological studies also had highlighted their antioxidative properties that possibly could prevent various diseases by inhibiting or delaying oxidative reactions [14].

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Mushrooms have been known since centuries in different cultures as precious food and source of wide range of bioactive compounds of many medicinal applications [11,15,16]. However, based on the limited sources of naturally grown mushrooms, they were cultivated in green house for sustainable supply. However, many wild mushrooms are cultivable in green house this cultivation process take long time up to several months. Therefore, recently many research have been focused on production of mushrooms in closed submerged cultivation system to increase the yield of bioactive compounds, shorten the production time, and full production under sterile conditions [17,18]. *Pleurotus ostreatus* have long history as precious food based on its high nutritional value as source of protein, carbohydrates, minerals, vitamins, fatty acids, and volatile compounds [19]. In addition, this widely used mushrooms have many compounds of different groups of molecular weights of high medicinal values and act as antitumor, immunomodulator, antimicrobial, anti hypocholesterolemic, anti-inflammatory, and many other therapaeutic properties [11,20,21].

## 2. ANTIOXIDANT COMPOUNDS OF *P. OSTREATUS*

Secondary metabolites from macro fungi such as *Pleurotus* spp. have long history as functional food and health promoting nutrients. Most of these mushroom bioactive components have great ability to enhance oxidative stress defences, including inhibition of lipid peroxidation, reduction of human low-density lipoproteins, scavenging of free radicals and others. Various antioxidant compounds reported in *P. ostreatus* fruiting bodies and mycelium has been illustrated in Figure **1**. Phenolic compounds and polysaccharides are the major antioxidants reported in *P. ostreatus*. Presence of vitamins and their respective precursors are lower in *P. ostreatus* but, they have notable antioxidative effect [22].

### 2.1. Phenolic Compounds

The phenolic compounds are the most abundant antioxidative components found in all *Pleurotus* spp. and other mushrooms. These compounds possess one or more hydroxyl group (–OH), in the aromatic system. Besides mushrooms, phenolic compounds are also commonly found in other vegetables, fruits and other foods that form a significant portion in our daily meal. These phenolic compounds can be divided into four major groups which are phenolic acids, phenolic diterpenes, flavonoids and volatile oils [23,24].

The antioxidant activity of phenolic-rich extracts is commonly correlated with the total phenolic content. The methods of Folin–Ciocalteau assay described by Singleton and Rossi in 1965, is widely used (with slight modifications) to evaluate total phenolic compounds in the extract. The assay is a colorimetric analysis based

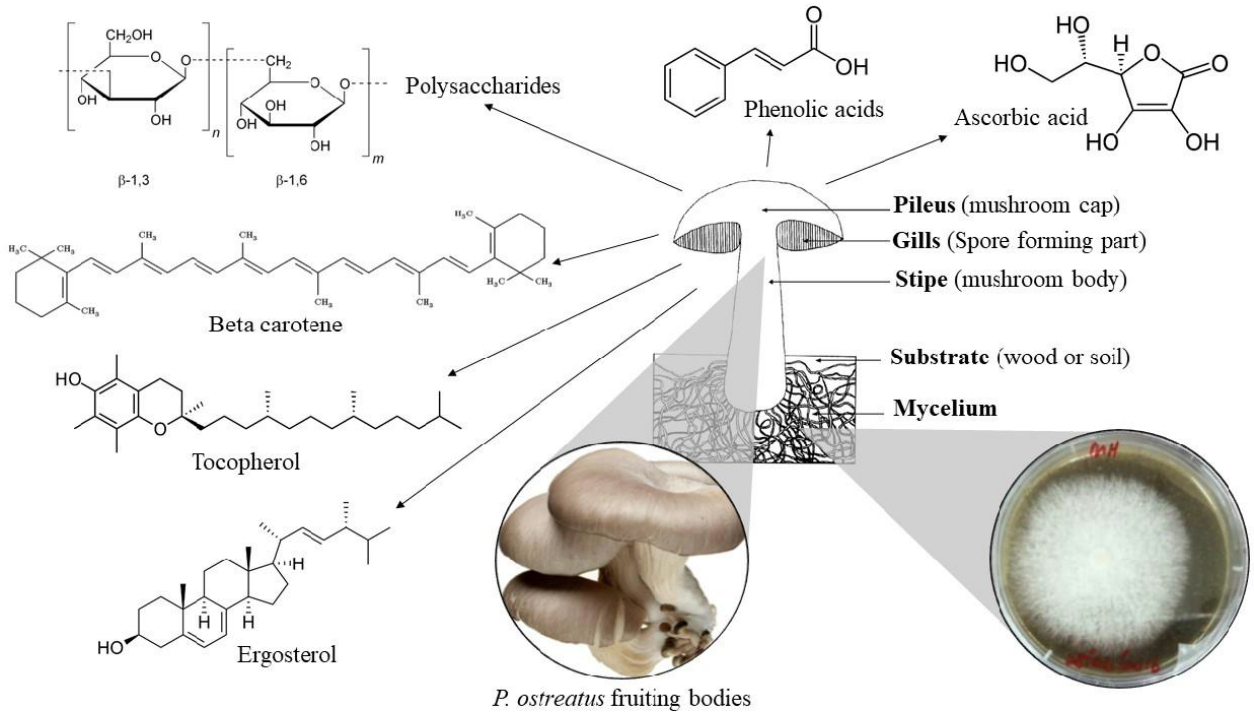


**Figure 1:** Antioxidant compounds in *P. ostreatus*.

on the transfer of electrons in alkaline medium from phenolic compounds and forming blue complexes that monitored spectrophotometrically at 750–765 nm [25,26]. The Folin–Ciocalteu assay is highly sensitive for monohydric phenols, polyphenols, flavonoids and tannins. However, this method also has its limitation. Non-phenolic but other readily oxidised substances such as sugars, ascorbic acid or amino acids (tyrosine, trytophan) could interfere with the colorimetric reagent (a mixture of phosphotungstic and phosphomolibdic acids). This could overestimate the total phenolic content. Furthermore, phenolics with more than one hydroxyl group are expected to double the molar colour yield; however, steric effects or substitutions in the aromatic ring could modify the expected response since the hydroxyl groups are not accessible for the chromophore reagent [25]. The total flavonoid content is determined by colorimetric assay employing catechin [27,28].

Phenolic compounds displaying a large diversity of structures where some of the compounds may escape the usual methodologies of analysis. The presence of isomers, difficulties in separation and lacking of standards could be the reasons for that. Thus, phenolic profiling is carried out to identify and quantify active phenolic compounds in the mushroom extract. It is commonly carried out by HPLC (high-performance liquid chromatography) coupled to distinct detection devices [25,27,28].

Among these, phenolic acids are the major phenolic compounds found in many mushrooms including all *Pleurotus* spp. Phenolic acids are classified into two groups: hydroxybenzoic acid (HBA) and hydrocinnamic acids (HCA) (Figure **2**). HBA derivatives are the complex and in bound structures linked to lignin, hydrolysable tannins and some of the plant sugars or organic acids. Meanwhile, HCA are mainly attached to the cell-wall structures such as cellulose, lignin and proteins. Some HCA are also attached to organic acids such tartaric and quinic acids. Both HBA and HCA compounds are derived from non-phenolic molecules of benzoic and cinnamic acid, respectively [23,24]. The 4-hydroxybenzoic; 2,5-dihydroxybenzoic; protocatechuic; gallic; veratric; syringic and vanillic acids are the examples of HBAs. The derivatives of HCA compounds are the p-coumaric; caffeic; ferulic; and t-cinnamic acids.

Gasecka *et al*. [28] have detected 4-hydroxybenzoic, ferulic, p-coumaric, protocatechuic, t-cinnamic and vanillic acids in the methanollic extract of *P. ostreatus* fruiting bodies, grown on Selenium-Zinc enriched mushroom substrates. In the previous study of this research group [26], only 4-hydroxybenzoic, p-coumaric and ferulic acids were detected in the Selenium enriched substrate. The study of Woldegiorgis *et al*. [29] has also confirmed presence of caffeic, gallic and p-hydroxybenzoic acids in the methanollic extract of cultivated *P. ostreatus* in Ethiopia. Caffeic acid was found in highest concentration among these phenolic acids determined in this study. However, Palacious *et al*. [25] did not detect any appreciable amount of caffeic acid in *P. ostreatus*. Instead, they had confirmed maximum concentration of homogentisic acid among other phenolic acids detected in *P. ostreatus* and also a higher content of gentisic acid as compared with other mushrooms analysed in this study. Besides, Palacious *et al*. [25] also detected the p-coumaric, ferulic, gallic, p-hydroxybenzoic, and protocatechuic acids. Reis *et al*. [30] founded difference in antioxidant concentrations of *P. ostreatus* fruiting bodies and mycelia. This study shown that p-hydroxybenzoic acid in higher in fruiting bodies while, mycelia contains higher content of cinnamic acid. Kim *et al*. [31] reported higher concentration of chlorogenic acids in the methanollic

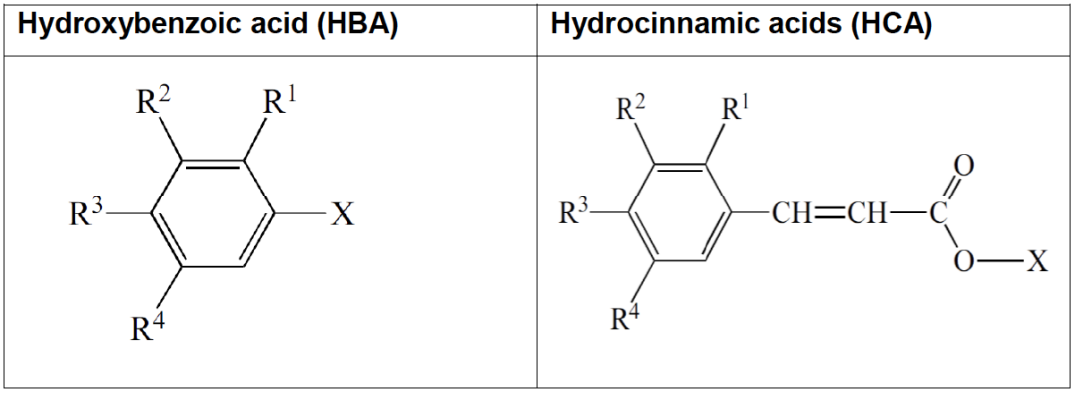


**Figure 2:** Chemical structure of main groups of phenolic acid.

extract of *P. ostreatus*. The study also quantified another two HBA derivatives of homogentisic and protocatechuic acids [31].

Accumulation of secondary metabolites such as flavonoids and phenolics is one of the common responses of fungi and plants to many biotic and abiotic stresses. Phenolic acids are produced by fungi and plants for the protection against ultra-violet light, insects, viral and bacterial infections. Dong *et al*. [32] has proven a short-term and a low concentration fumigation of nitrogen dioxide (NO) could stimulate and accumulate phenolic acid contents in mushrooms. However, adequate evidence does not exist to ascertain the mode of action of NO [33]. The HBA and HCA compounds are synthesized through shikimate pathway (Figure **3**), from L-phenylalanine or L-tyrosine, in mushrooms. Phenylalanine and tyrosine being the crucial amino acids in this pathway to induce biosynthesis of HBA and HCA derivatives. Primarily, the enzyme phenylalanine ammonia-lyase will deaminate the phenylalanine and forms the precursor molecule, cinnamic acid. Similarly, deamination of the tyrosine forms p-coumaric acids. Cinnamic and p-coumaric acid undergoes hydroxylation and methylation steps to form their HCA derivatives such as ferulic and caffeic acids. Benzoic acid is derived through beta-oxidation of cinnamic acid. Then, hydroxylation and methylation occurs in benzoic acid ring to derive other HBA compounds such as protocatechuic and p-hydroxybenzoic acids [23,34].

Flavonoids are another large group under polyphenolic type antioxidants naturally occurring in plants and mushrooms. Flavonoids are naturally occurring phenolic compounds having a benzo-γ-pyrone structure and are ubiquitously present in plants synthesized by phenylpropanoid pathway [36]. Flavonoids are polyphenols of diverse structure that can be found as aglycones or glycosides. The antioxidant action of flavonoids depends upon the arrangement of functional groups about the nuclear structure. Flavonoids have been recognized to have strong antioxidant properties in order to prevent lipid peroxidation, to scavenge free radicals and to chelate ferrous ions [35]. They are characterized for the carbon skeleton C6–C3–C6 [24]. The basic structure of these compounds consists of two aromatic rings linked by a three carbon chain that is usually in an oxygenated heterocycle ring, or C-ring (Figure **3**). Several classes of flavonoids are delineated on the basis of differences in the generic structure of the heterocycle C ring and can be classified into flavonols, flavones, flavanols, flavanones, anthocyanins and isoflavonoids. Numerous studies have reported total flavonoid content in *P. ostreatus* via colorimetric assay [27,28].

Multiple mechanisms have been identified as involved in the health-promoting effects of flavonoids, including antioxidant, anti-inflammatory and anti-proliferative activities, inhibition of bio-activating enzymes, or induction of detoxifying enzymes. The antioxidant property of flavonoids was the first

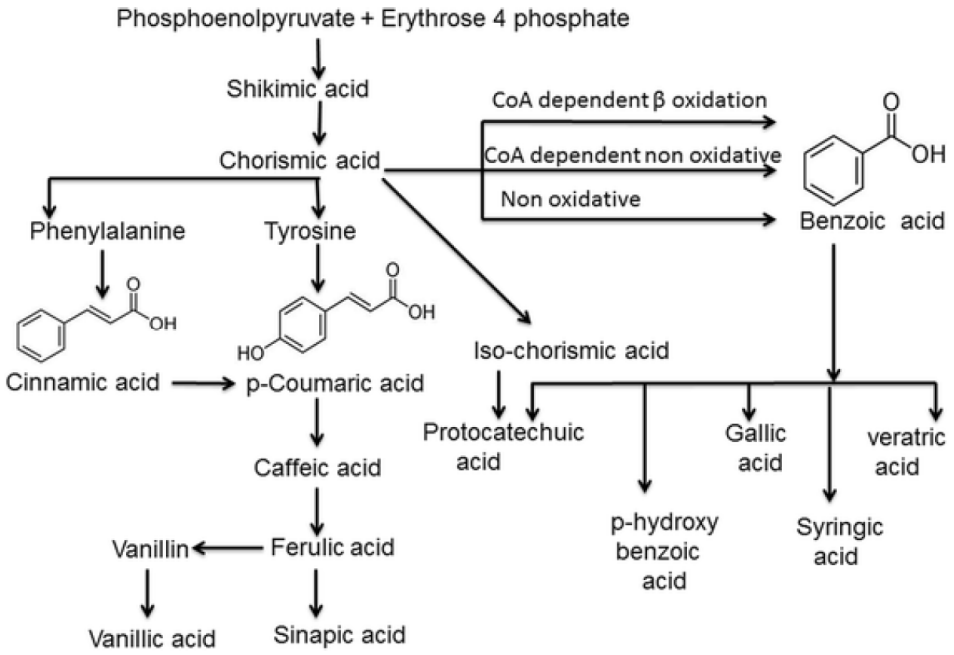


**Figure 3:** Biosynthetic pathway of phenolic acids in *P. ostreatus* [34].

mechanism of action studied, in particular with regard to their protective effect against cardiovascular diseases. Flavonoids have been shown to be highly effective scavengers of most types of oxidizing molecules, including singlet oxygen and various free radicals, which are possibly involved in DNA damage and tumour promotion [23,35]. Arbaayah and Umi [27] have reported a high content of flavonoids from ethanol extracts of various species of *Pleurotus* including *P. ostreatus* varieties. Jeena *et al*. [36] has also proven that the methanolic extract of matured *P. ostreatus* fruiting bodies exhibited the strongest antioxidant activities with highest flavonoid contents. However, González-Palma *et al*. [37] founded no flavonoid content in any growth stages of p. ostreatus, including the matured fruiting bodies and their primordium.

In addition, Gil-Ramírez *et al*. [38] reported that the mushrooms do not contain and synthesize flavonoids, based on whole genome analysis of *Pleurotus* spp.. The study shown that *P. ostreatus* do not have the main enzymes catalysing the biosynthesis of flavonoids. Flavonoid biosynthesis was described to start by condensation of 4-coumaroyl-CoA with three molecules of malonylCoA, yielding naringenin chalcone. This reaction is catalysed by chalcone synthase (CHS) enzyme [34,38]. However, sequences encoding for this enzyme in mushrooms was not found when searched using BLAST tool. There were no significant similarities were found in other edible mushrooms. Besides, there was no significant absorption was noticed from fruiting bodies cultivated in flavonoid-enriched substrates or from mycelia grown of flavonoid-supplemented lab media.

The study had questioned the validity of the spectrophotometric methods performed in the previous studies, to determine the total flavonoid content. Identification of these flavonoids in some of the previous studies using advanced identification devices such as DAD and MS was said to be contaminations or other pitfalls within the utilized protocols [35,38]. Flavonoids are reported as antifungal compounds produced by plants for protection against fungal infections and then, these compounds might negatively affect fungal growth. Therefore, presence of flavonoids in the *P. ostreatus* is still doubtful [38].

Overall, the levels of phenolic compounds depend on several factors such as cultivation techniques, cultivar, growing conditions, ripening process, processing and storage conditions, as well as stress conditions such as UV radiation, infection by pathogens and parasites, wounding air pollution and exposure to extreme temperatures [39].

### 2.2. Beta-Carotene and Lycopene

Beta-carotene can be defined as a red-orange pigment which can be found in fruits and plants. To be more precise it can be found in colourful vegetables like carrot [40]. Although *Agaricus bisporus* and *P. ostreatus* do not possess the carotene colour, but previous reports had shown that they may contain a very low amount of carotenoids in their fruiting bodies [22]. There are two types of beta-carotene which are α-carotene and β-cryptoxanthin [41]. Other than being as an attractive natural colorant, comparing with other carotenoids, beta-carotene contains the highest vitamin A and the effective source of vitamin which acts as a precursor of vitamin A and it will be converted into vitamin when consumed by human. There are around 500 different types of compound have been identified and among them beta-carotene is the most prominent that have good antioxidant property [42].

Mushrooms do not contain the any pigments that dominated in higher plants such as chlorophylls and anthocyanins. Many of the pigments of higher fungi are quinones or similar conjugated structures that are mostly derived from shikimate biosynthetic pathways (Figure **2**). The shikimate pathway provides a route to the essential amino acids phenylalanine, tyrosine and tryptophan that are involved the biosynthesis of phenolic compounds in mushrooms [34]. Carotenoids are also biosynthesised in mushrooms by a combined shikimate and polyketide pathway. The polyketide pathway yields either aromatic ketides or fatty acids. Combination of this two pathways raise to the long conjugated double bond skeleton of polyisoprenoid structure (Figure **4**) [34]. Higher concentration of carotenoids was reported in *Russula* spp., *Lentinula edodes* and *Cantharellus cibariusare* [40].

Carotenoids in *Pleurotus* mushrooms especially β-carotene were reported in vestigial amounts, either using HPLC coupled to UV or fluorescence detector, or by spectrophotometry. Jayakumar *et al*. [43] prepared dried mushroom extracts with a solution of 1% pyrogallol in 10 ml of methanol/dichloromethane and quantified β-carotene by UV detection by HPLC. The study reported was 3.10 mg per 100 g dried *P. ostreatus* fruiting bodies. Robaszkiewicz *et al*. [44] reported that methanolic extract of dried Polish grey oyster mushrooms contained much higher content of β-carotene than lycopene. In another research by

Jaworska *et al*. [45] had reported the changes in the carotenoid amounts of fresh and prepared *P. ostreatus* fruiting bodies. Reductions in level of both carotenoids were larger in mushrooms blanched prior to culinary treatment. The study also reported that analysed fresh *P. ostreatus* fruiting bodies contained typical amounts of β-carotene and slightly larger amounts of lycopene [45]. This finding was also similar to works done by Mishra and colleagues [46]. The methanolic and aqueous extracts of *Pleurotus sajur-caju* contained significantly higher amounts of lycopene compared with β-carotene. In addition, this study also reported that the methanolic extracts of mushrooms caps have higher total antioxidant activities than their stipes, proven by principal component analysis (PCA) [46].

### 2.3. Ascorbic Acid

Ascorbic acid or also known as vitamin C is a water soluble compound that mostly can be found in its deprotonated state under the physiologic conditions. Ascorbic acid includes two compounds with antioxidant activity: L-ascorbic acid and L-dehydroascorbic acid which are both absorbed through the gastrointestinal tract and can be interchanged enzymatically *in vivo* [47]. These compounds have great scavenging activity against superoxide, hydrogen peroxide, peroxyl radicals, hydroxyl radical and hypochlorite [48]. Moreover, it can react effectively in human plasma lipids by inhibiting the lipid peroxidation which initiated by peroxyl radical initiator. As it is water soluble, it can function both inside and outside the cells to fight free radical damages [48]. Ascorbic acid shows better scavenging activity when compare to other plasma components such as urate, thiols, bilirubin and as well as proteins. So by trapping the free moving radicals that present in the aqueous phase right before it starts to initiate lipid peroxidation, this ascorbic acid can provide protection to the membranes to prevent the occurring of peroxide damage [47,48]. The capacity of the ascorbic acid to carry out the antioxidant activity can be influenced by factor like the presence of other compounds. For example, according to Yang *et al*. [49], it was found that ascorbyl palmitate was thermally less stable when compared to other mixed tocopherols and propyl gallate when the induction period was used to measure the antioxidant activity of the oxidised sunflower oil. In addition, the antioxidant ascorbic acid (vitamin C) has been demonstrated to play an important role in stimulating the immune system via attenuating chronic inflammatory responses, the persistence of which is implicated in the etiology of various diseases, including cancer [50].

Human, unlike other animals or plant is unable to produce their own vitamin C due to lack of gulonolactone oxidase enzyme. Therefore, an intake of vitamin C from external sources is required by the body for its various biological and physiological processes. It can be found mostly from vegetables and fruits. Ascorbic acid is one of the simplest vitamins, was found in several mushroom species, including *P. ostretus* [43]; *A. bisporus*, and *L. edodes* [51]. Ascorbic acid contents were determined either by spectrophotometer [43] or by HPLC methods [51]. Jayakumar *et al*. [43] had reported that the ascorbic acid content of the *P. ostreatus* extract was comparatively higher (25 mg/100 g) than the values reported in other mushroom species such as white (17 mg/100 g) and brown button mushrooms (21 mg/100 g). Jonathan *et al*. [52] found vitamin C values of range 3.27 ± 0.47 to 3.65 ± 0.17 mg per 100 g of dried fruiting bodies of *P. ostreatus* grown on different substrates. Similarily, Gasecka *et al*. [28] had shown that addition of trace elements in growth substrates had increased ascorbic acid content in *P. ostreatus* fruiting bodies.

### 2.4. Tocopherols

Tocopherol or also known as vitamin E is a fat-soluble carotenoid which composed of eight different chemical compound which are alpha- and beta-tocopherols and also four other corresponding tocotrienols. Among the different forms of tocopherol, α-tocopherol is the most biologically active form of vitamin E. Due to its important role as antioxidant in foods, especially food that high in polyunsaturated fatty acids, this carotenoid has been studied extensively. This compound also capable of product human from degenerative diseases like cardiovascular diseases or cancer. The α-tocopherol functions as main vitamin E in plant leaves. It can be found in the chloroplast envelope and thylakoid membranes which help to deactivate the reactive oxygen species which has been photosynthesised to prevent the occurring of lipid peroxidation. It will scavenge the lipid peroxyl radicals that can be found in the envelope and thylakoid membranes [41,52,53].

The α-tocopherol molecule and the peroxy radical start to get attract and this will lead to their electrons being overlap to each other. When the overlapping happens, the proton tunneling starts to take place where the chromanol molecule starts to lose its hydrogen atom to the lipid peroxy radical. This process leads to the formation of chromanoxyl radical [53,54]. Once the chromanoxyl radical form, it will start to

radical couple with other radicals form adducts. The chromanoxyl radical reacts in a different way to the carbon centred and the oxygen centred lipid radicals under anaerobic radicals. The carbon-centred radicals which forms in the anaerobic conditions end up being added to the chromanoxyl oxygen forming 6-O-lipid alkyl-chromanol adducts. When it comes to the oxygen-centred peroxyl radicals, it tends to add at the 8a positions which lead to the formation of 8aalkyldioxy-tocopherones [51,54]. In addition to that, the dietary supplements of this α-tocopherol can increase the production of the antioxidant into the phospholipid membrane where the polyunsaturated fatty acids are placed [53].

As mentioned earlier, mushrooms including *P. ostreatus* are well known for high nutritional value. Many reports have been published on the tocopherols content of mushrooms but, tocopherols in the *P. ostreatus* are less [30,43,45,54]. Tocopherol determination in mushrooms were performed by the same methodology including saponification in the extraction process and analysis by HPLC coupled to UV detector. Jayakumar *et al*. [43] reported presence of α-tocopherol in the amount of 30.1 mg per 100 g dry weight of *P. ostretus* fruiting bodies, by sprectrophometer analysis. Jaworska *et al*., [45] had performed HPLC analysis to determine tocopherol content in *P. ostreatus*. The study extracted tocopherols with hexane mixed with butylhydroxytoluene, prior to elucidation using HPLC attached to fluorescence detector [45]. Highest tocopherol content was also reported in this study compared to previous findings. With similar detection method, Fernandes *et al*. [55] had determined different isoforms of tocopherol contents in *P. ostreatus* cultivated on different paper scraps (blank or printed paper) as substrates. Similarly, Reis *et al*. [30] also described the presence of α-, c- and d-tocopherols in a commercial sample of *P. ostreatus*, with a profile more similar to that of the control.

### 2.5. Polysaccharides

*P. ostreatus* mushrooms are important source of different types of polysaccharides with immunomodulating and anticancer activities. *P. ostreatus* is an oyster mushroom which belongs to basidiomycete phylum. It is one of the most widely cultivated mushrooms. Aside from that, it has also been recognized as the third most important cultivated mushroom for food purposes and it constitutes an integral part of the normal human diet. This mushroom is rich in protein, fiber, carbohydrates, vitamins, and minerals [52]. Several bioactive compounds have been identified in *P. ostreatus* showing strong anti-cancer activities such as polysaccharides, mainly β-D-glucans, proteoglycans, lectins, fibers, terpenoids, steroids, nucleic acids and others [56,57].

Exopolysaccharides (EPS) are high-molecular-weight polymers that are constituted of sugar remain generated by numerous microorganisms especially mushrooms due to their various biological and pharmacological activities [58]. On normal condition, exopolysaccharide was found secreted by the cells to its surrounding medium. In mushroom, the roles of EPS have been described as an adhesion to the substrate, immobilization of exocellular enzyme, prevention of hyphal dehydration and storage of extra nutrients [58,59]. Glucan, in general polysaccharides, is polymeric carbohydrate with different active unit linkage such as (1→3), (1→6)-β-glucan and (1→3)-α-glucans establish mushroom which implements immunomodulatory activity as they are biological response modifiers (BRMs). BRM categorized into two groups corresponding to their outcome on cytokine and immunomodulatory [53-55].

In general, the polysaccharide is polymeric carbohydrate which is formed by the glycosidic linkage between monosaccharide residues. This mush-room incorporates distinctive kinds of polysaccharides that belong to the b-glucan, heteroglycan, and proteoglycan families. Homoglycans by definition were confirmed as polysaccharides that include residues of only one type of monosaccharide molecules. For heteroglycans, a polysaccharide that involve residues of two or more types of monosaccharide molecules [17,22].

The beta-glucan has immune-modulating properties such as resistance to infection and anti-neoplasm of both a benign and malignant tumor. The bioactive polysaccharide produced by *P. ostreatus* can often be extracted from mycelia of the species without the need to wait for the fruit body to reach maturation. This, in turn, made the mycelia cultivation received great attention as an efficient method for industrial production of the valuable bioactive compound and various agro-industrial by-products. This cultivation also has been tried as an alternative cheaper growth medium for this mushroom cultivation [16-18]. A previous experiment was done by Bobek *et al.* [60] regarding the pleuran properties extracted from *P. ostreatus.* The result showed that pleuran has a positive effect on anti-oxidant activity and at the same time reduced the pre-

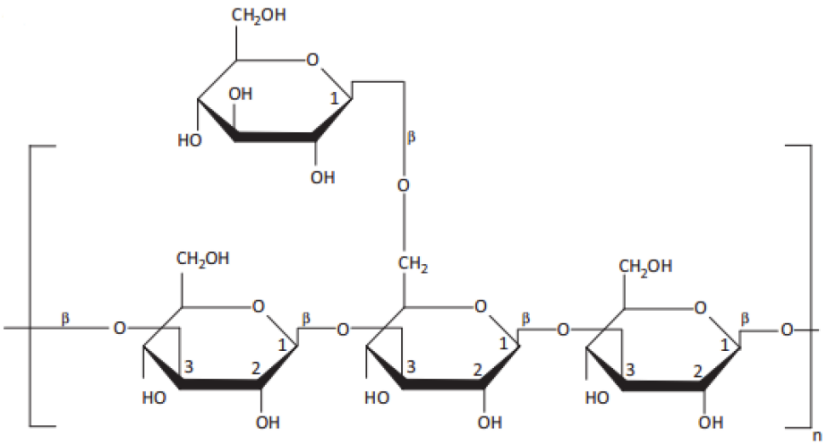

**Figure 4:** Molecular structure of Pleuran (CAS No. 159940-37) is an insoluble polysaccharide [17].

cancerous lesion in rat colon. It is known that pleuran can be found in various types of fungal species. Figure **4** shows the molecular structure of pleuran [17,60].

Pleuran (CAS No. 159940-37) is an insoluble polysaccharide (β-(1,3/1,6)-D-glucan), isolated form *P. ostreatus* species [56]. However, pleuran from *P. ostreatus* alongside lentinan from *L. edodes* are of the most industrially utilized polysaccharides [61]. Pleuran is a carbohydrate which contains a large number of β-glucose with a molecular formula of $(C_6H_{12}O_6)n$ and molecular weight of 762 kD. The molecular weight of 2.4 x $10^4$ Da was identified by Sun and Liu [62], for this water-soluble polysaccharide. Pleuran total carbohydrate composition was determined to be 95% and 95.6 %, respectively. The compositions of carbohydrate are of D-galactose and D-glucose as identified by gas chromatography in the proportion of 2:1 [61-63]. As in Figure **4**, the main chain of Pleuran consisting of backbone (1→3)- linked β-D glucose polymers unit. A β (1,6) or β (1,4) bonds link a β-D-glucosyl side at the -0-6 position of ever fourth anhydroglucose unit. The main chains of pleuran involve of triple helix coiled which joined with single or double filaments of glucopyranoses [16-18,63]. From this research also was stated, a triple helix conformation will appear when C2-position bonded with 3-H. The side chains contribution the stabilization of confirmation. The triple helix can be established in beta-glucans with molecular weight more than 90kDa [63].

β -glucans from *P. ostreatus* is most frequently used as an anti-tumor adjuvant. These fungi have been increasingly consumed by the majority of cancer patients during their treatment as dietary supplements. Larger molecular weight β-glucans such as pleuran appear to activate leucocytes directly. The phagocytic, cytotoxic and anti-microbial activities of the immune system are mediated by production of reactive intermediates, pro-inflammatory mediators, cytokines, and chemokines which are activated through the pleuran molecule recognition by the cell surface receptors. β -glucans is the key role in activation of helper lymphocytes known as T- helper lymphocyte 1 (Th1) and T-helper lymphocyte 2 (Th2). While Th1 lymphocytes direct intracellular immune system, the Th2 deal the immune system against extracellular pathogens. In one example, water-soluble polysaccharides extracted from *Pleurotus citrinopileatus* tested to mice resulted in significantly increased the number of T helper cells [60,63]. It is known that large spectrum of cytokines been secreted by a lymphocyte. The interferon gamma (IFN-γ) and interleukin 2 (IL-2) were synthesized by Th1 while the Th2 lymphocyte secreted the interleukin 4, interleukin 5 and interleukin 6.

A research was done by Jesenak *et al.* [64] to evaluate the pleuran effect in the prevention of recurrent respiratory tract infections (RRTIs). The treatment resulted in complex immunomodulatory activity on innate and adaptive immunity. The increase in all three immunoglobulin isotypes demonstrated that the pleuran supplement does support the physiological maturation of the humoral immune response. It is also documented that the pleuran supplementation to patients has slowed down the decline of T-cytotoxic lymphocytes, and also caused the increase in the NK cell number. The results also showed that the β-glucans pre-treated intestinal mucosa has high resistance towards inflammation and intestinal ulcer development. However, the mechanisms on how this exopolysaccharide protect the intestinal mucosa were still unknown [60-64].

### 2.6. Ergosterol

Phyto-sterols such as Vitamin D is a bioactive compound predominantly found in similar structure to cholesterol, with no toxicity effects to human and animal [51]. There are two major forms of vitamin D that normally found in nature, which are vitamin $D_2$ (ergocalciferol) and vitamin $D_3$ (cholecalciferol). Vitamin $D_3$ is photosynthesized from 7-dehydrocholesterol through UV irradiation in skin even though a small amount can also be acquired from the diet. Fungi, yeast and phytoplankton synthesize vitamin $D_2$ from ergosterol through UV irradiation [65]. This precursor molecule of ergosterol was reported as the most plentiful sterol by a greater extent in various types of mushrooms. Ergosterol is the major product of sterol biosynthesis in fungi, whereas mammalian systems synthesize cholesterol as the major membrane lipid [54].

In general, the cultivated mushroom species demonstrated higher substance, mainly *A. bisporus* and *L. edodes*. Among wild species, *Boletus edulis* was the mushroom with the highest substance in ergosterol [66]. Mattila *et al*. [51] demonstrated that ergosterol was the most plenteous sterol found in mushrooms, and its substance was higher in cultivated mushrooms (6.02 – 6.79 mg/g) than in wild mushrooms (2.96–4.89 mg/g). Varieties were significantly different for the ergosterol content. As the fungal growth increases, ergosterol also increased significantly in different types mushrooms. Cultivated of *P. ostreatus* mushrooms are higher content of ergosterol. Irradiation of mushrooms with UV-B light had been proven to initiate ergosterol photo- degradation for vitamin $D_2$ formation [67] at a level of several mg 100 $g^{-1}$ DM. Similarly, Chen *et al*. [65] shown that pulsed light could increase vitamin $D_2$ and ergosterol content in *Pleurotus* fruiting bodies (2.78 ug/g). Taofiq *et al*. [68] reported that ethanolic extract of dried *P. ostreatus* fruiting bodies contained higher ergosterol (78.20 ± 0.54 mg/g) content than *A. bisporus* and *L. edodes*. The study proposed the development of cosmeceutical potential in *P. ostreatus* antioxidants.

## 3. ENRICHMENT OF ANTIOXIDANT CONTENT IN CULTIVATED *P. OSTREATUS*

Numerous studies had been conducted to evaluate factors increasing the antioxidant contents in the *P. ostreatus* mushrooms. Besides the type of extraction solvents, time, and other physical factors of both extraction as well post-harvest processes that could have affected the antioxidant composition, cultivation practices still being a major factor. *P. ostreatus* is commercially cultivated mushroom species, at global level. Almost three quarters of world population consume *P. ostreatus* mushroom in their daily meals. Thus, numerous studies had been performed to improve over the cultivation techniques in order to enhance nutrient contents of this mushroom [2,35,58].

Trace element enrichment in the cultivation substrates of mushrooms was conducted as one of the way to overcome micronutrient deficiency in the foods [28,69]. Mushroom fruiting bodies able to accumulate trace elements essential for human health. Under permitted concentration of trace elements, recent studies shown that trace element enrichments also induced biosynthesis of valuable antioxidants. Vierra *et al*. [69] had shown the accumulation of iron in the *P. ostreatus* cultivated grown on coffee husk, enriched with trace elements. Enrichment of iron are usually lower in mushrooms as their presence in their growth substrates. This study had shown that with enriched husk of coffee presented content of iron approximately 10 times greater previous practices [69]. Additionally, these trace element treatments also had an impact on antioxidant activities and their contents, especially in *P. ostreatus*. Gasecka and colleagues [28] reported that enrichment of selenium and zinc in the pasteurized wheat straw substrates had improved over the antioxidant properties and their contents such as phenolic, flavonoid and ascorbic acid in *P. ostreatus* and *Pleurotus eryngii*. Their fruiting bodies were shown to accumulate these trace elements and also increase their antioxidant compositions of total phenolic, total flavonoid and total ascorbic acid contents [28]. Trace elements usually affects cellular metabolisms and their enzyme activities. In the case of zinc, it was proven to induce oxidative stress in mushrooms which in consequence stimulate synthesis of antioxidants such as ascorbic acid and phenolic compounds. Additionally, these enriched mushrooms also yield more metabolites as the result of detoxification mechanisms caused by elevated levels of the elements [70].

Substrates used in mushrooms cultivation have effect on chemical, functional and sensorial characteristics of mushrooms. As their saprophyte nature, mushrooms including *P. ostreatus* extract nutrients from cultivation substrates which are mainly from agricultural residues or wood. Mycelia of mushroom obtain substances necessary for its development, such as carbon, nitrogen, vitamins and minerals. Agro-industrial waste is produced in huge amounts, and it becomes an interesting substrate, due

its commercial exploitation as well as associated environmental problems [35,58]. Numerous studies have been reported the ability of *P. ostreatus* to utilize different growth substrates such as rice straw, wheat straw, cotton wastes sawdust, banana leaves, papers and others [52,55,56,59,71]. Mixtures of agricultural residues also proven to enhanced growth of *P. ostreatus*, improving over yield, biological efficiency and proximate compositions. The use of different types of substrate by fungus will depend on its capacity to secrete enzymes such as oxidative (ligninase, laccase, manganese peroxidase) and hydrolytic (cellulase, xylanase and tannase) enzymes which are involved in utilizing lignocellulosic substrates. These enzymes degrade the agricultural residues to increase nutritional content essential for their growth. Eventually, utilization of agricultural residues with the release of various extracellular enzymes had also increased nutritional value of this mushroom [52,55,56,59,71]. Li *et al*. [71] reported that utilization of perilla stalks content on the substrate had promoted higher antioxidant activity in *P. ostreatus*. Fernandaes *et al*. [55] shown that paper wastes could be utilized by *P. ostreatus* and this had enhanced production of tocopherols in the fruiting bodies.

## 4. IMPACT OF CULINARY TECHNIQUES ON ANTIOXIDANT CONTENT OF MUSHROOM

Food processing techniques had been proven to induce significant changes in the texture and chemical composition. The thermal treatments could reduce the food quality; as most of the bioactive compounds are relatively unstable to heating. For example, boiling of vegetables could cause leaching of soluble substances, vitamins and antioxidants [72]. Yet, different cooking techniques on different food samples were reported to either increase, decrease or induce no significant change in the antioxidant activity of foods. Most of the mushrooms are commonly cooked before being consumed. They are usually cooked by different methods such as boiling, microwaving, steaming, stir-frying and pressure cooking. However, information on the changes in the nutritional quality after culinary treatments on mushrooms is scarce. Alteration in the total antioxidant values due to different cooking methods is scientifically important, especially as it has great impact on human dietary nutrition. Radzki *et al*. [73] investigated the impact of some processing methods in *P. ostreatus*, confirming that the content and the antioxidant activity of polysaccharides decreased due to the culinary processing, where the temperature and heating time being key factors.

Jaworska *et al*. [45] has reported a reduction in the level of nutraceuticals and antioxidant activity in blanched *P. ostreatus* fruiting bodies compared with raw ones. After prepared for consumption, the β-carotene and lycopene content were significantly reduced ($P < 0.05$) at 7 – 44% and 31–47%, respectively for both carotenoids. The reported reduction of antioxidants such as total phenols, flavonoids, ascorbic acid and carotenoids in *P. ostreatus* were also correlated with reductions in antioxidant activity. Although, carotenoids are well-known for thermal stable plant antioxidant, but further storage of blanched mushrooms had led to further decrease of carotenoid content in mushroom [45]. This is contrary to a previous study by Abdullah *et al*. [74] The study had reported that 30 minutes of boiling treatment did not affect the total phenolic contents of hot water extract of five *Pleurotus* spp. (*P. cystidiosus, P. eryngii, P. flabellatus, P. floridanus and P. pulmonarius*). This was also supported by other studies [75,76]. The prolonged boiling process might help the release of bound-polyphenols from the *Pleurotus* fruiting bodies into the boiling water. The antioxidant activities in boiled water of oyster mushroom is higher, especially the percentage of scavenging activity in the heated sample compared to the control. This could be attributed to the antioxidants which largely leach from mushroom tissue into the boiling water with increase in cooking time. Thus, boiling mushrooms, especially *P. ostreatus* to prepare soup and gravies would be a good choice of cooking method to optimize antioxidant intake [74-76].

Kim *et al*. [77] reported that the texture and shape of the mushroom varieties also could impact the antioxidant content and activities, before or after culinary treatments. This could be observed clearly on the impact of microwave treatment on *Pleurotus* varieties. The antioxidant activity of microwaved *P. floridanus*, *P. flabellatus* and *P. pulmonarius* were significantly higher than the uncooked samples [78]. This study also reported that microwaved *P. cystidiosus* and *P. eryngii* exhibited lower antioxidant activity compared to the corresponding uncooked sample. It was described that the thick and firm fruiting bodies of *P. eryngii* may not be penetrated by the microwave heat. However, a study by Sun *et al*. [62] reported that microwave cooking had shown better retention of certain phenolic acids in *Boletus* mushrooms than pressure-cooking, steaming, boiling and frying.

Tan and colleagues [78] had shown that pressure based cooking methods could increase the antioxidant

activity among *Pleurotus* varieties. Pressure cooking at low moisture and high temperature might cause the release of active antioxidants from the fibrous complexes. The application of high temperature in pressure cooking was described to promote the release of higher concentration of phenolic compounds from disruption of the thinner and softer tissues of *P. ostreatus* [78].

Recent study by Radzki *et al*. [73] had reported that boiling technique improved the total glucan content by enhancing the amount of b-glucans in the *Pleurotus* extracts. This increase could be due to the leaching of soluble substances during boiling, which could result in concentrating the fraction of insoluble carbohydrates. However, frying technique caused more severe losses in protein, ash, and carbohydrates content but increased the fat and energy of mushroom [73]. Similar finding was also reported by Roncero-Ramos *et al*. [79]. The proximate composition was affected by the cooking method and the mushrooms species. Roncero-Ramos *et al*. [79] suggested that microwaving and grilling were the best culinary technique to maintain the nutritional profile of *P. ostreatus*. Grilling is the best treatment to cook *Pleurotus* mushrooms, since this treatment induced the major values for antioxidant activity and polyphenols content, which is consistent with previous finding [80]. Therefore, this was clearly known that the cooking technique highly influences the nutritional value and the antioxidant activity of mushrooms. The proper selection of treatments is a key factor to prevent or reduce the nutritional losses. These findings suggested that customized cooking method might increase the health beneficial effects associated with increase of antioxidant activities in the *P. ostreatus* mushrooms.

## 5. CONCLUSION

As shown, *Pleurotus ostreatus* is considered as one of the important mushrooms not only because of high nutritional value but also it include wide range of bioactive metabolites of diverse biological activities. The antioxidant activity of *P. ostreatus* is not only because of one compound but it based on the presence of wide range of bioactive molecules. This increases the attractiveness of this type of mushroom. However, further research is still needed to understand the potential complementary antioxidant activity in human body of these compounds. In addition, the biosynthesis of these bioactive compounds under different cultivation still need further investigation. The role of cultivation medium composition in the expression of some regulatory genes during the production of antioxidant bioactive metabolites of *P. ostreatus* is also one of the topics which need further studies in proteomic and metabolomics levels.

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