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# FREE RADICAL SCAVENGING ABILITY, MECHANISMS OF ACTION AND HEALTH IMPLICATIONS OF OYSTER MUSHROOMS (*Pleurotus* species)

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**ABSTRACT**

Due to the importance of Oyster mushrooms (*Pleurotus* species) as a source of food and medicine to man, they have been cultivated both on large and small scales or collected in the wild. The present study evaluated the different antioxidant activities, mechanisms of action and various health implications on human of Oyster mushrooms. Since a single study is not effective in determining the antioxidant property of mushroom, several *in vitro* assays were reviewed including scavenging activities of DPPH, superoxide, nitric oxide, hydroxyl and ABTS as well as Oxygen radical absorbance capacity (ORAC) activities among others. *In vivo* assays like enzymatic (catalase (CAT), superoxide dismutase (SOD), glutathione *S*-transferase (GST), glutathione peroxidase (GPx), glutathione reductase (GR), and ascorbate peroxidase (APx) and non-enzymatic (Ascorbic acid, Vitamin E and Glutathione (GSH)); using mice, rats and porcine brain were also reviewed. Oyster mushrooms extract scavenged free radicals and prevented oxidative stress both *in vitro* and *in vivo*, and can influence their activities as natural immune boosters and thereby affect human health positively. The review has revealed oyster mushrooms as functional foods enriched with antioxidants and a good potential to oppose the formation of reactive oxygen and nitrogen species, preventing oxidative stress and scavenging free radicals.

**Keywords:** Oyster mushrooms; antioxidant property; human health; biomedical activities; medicinal mushroom

## INTRODUCTION

## OYSTER MUSHROOMS; *PLEUROTUS* SPECIES

Over the years, mushrooms have been considered as a valuable and nutritional delicacy with dual functions; serving as both foods and medicine to man **(Chang and Buswell, 2003; Adebayo *et al*. 2012b; Bamigboye and Oloke, 2016b)**. The relationship between man and mushrooms can be traced far back to the ancient past, mainly due to richness in protein, essential amino acids, minerals and polysaccharide metabolites such as beta-glucan **(Bamigboye *et al*., 2013; Bamigboye and Oloke, 2016b).** In recent times, however, the oyster mushroom is significant in the mushroom market worldwide, and several of its species are developed in both large and small scales all over the world **(Adebayo *et al*., 2012b)**. *Pleurotus* species are cultivated on diverse agricultural wastes since they are more preeminent wood decomposers than other mushroom species **(Bamigboye *et al*., 2019a)**. The nutritional content of *Pleurotus* species is, therefore, a function of the genetic make-up, differences in the physical and chemical composition of the growth medium **(Akyuz and Kirbag, 2010; Bamigboye *et al*., 2019a)**, substrate type, pileus size and harvest time. *Pleurotus* species reported to be beneficial medicinally are often rich in protein, minerals (P, Ca, Fe, Na and K) and vitamins (Niacin, Folic acid Riboflavin, and Thiamine) **(Caglarimak, 2007; Khan and Tania, 2012)**, proximate composition (protein, fat, ash, carbohydrates and energy) **(Adebayo *et al*., 2014a; Carneiro *et al*., 2013; Kalac, 2013)**. Some sugars, including fructose, mannitol, sucrose, trehalose and fatty acids mainly palmitic, oleic, stearic, linoleic and linolenic acids were found in *Pleurotus***(Carneiro *et al*., 2013; Varverde *et al*., 2015)**. Oyster mushrooms have high potassium to sodium ratio, making them an ideal food for hypertensive patients and those with heart diseases **(Patil *et al*., 2010)**. *Pleurotus* is rich in nutraceutical substances including lectins, which are glycoproteins with antitumor, immunomodulatory and antiproliferative activities. Also, polysaccharides (β-glucan, polysaccharopeptides and polysaccharide proteins) have found applications as pharmaceuticals with hepatoprotective **(Bamigboye *et al*., 2019b)**, anti-inflammatory, immune-enhancing and anticancer properties **(Varverde *et al*., 2015)**. Heteroglycans of *Pleurotus* sp. is known to stimulate macrophages and they have antiproliferative and proapoptotic effects on cancer cells **(Lavi *et al*., 2006; Tong *et al*., 2009)**. Besides, their phytochemical and phenolic contents such as saponins, alkaloids, steroids, anthraquinones phlobatannins, and flavonoids **(Adebayo *et al*., 2012a)**, alkaloids, tannins, saponins and flavonoids **(Dandapat and Sinha, 2015)** antioxidants, and anti-inflammatory activities for *Pleurotus* species have been reported. Phenolic compounds of different species of *Pleurotus* are secondary metabolites, which enhance physiological properties of mushrooms like anti-inflammatory, antiallergenic, cardioprotective, antiatherogenic, antimicrobial, antithrombotic, antioxidants and can also provide protection against several degenerative disorders **(Varverde *et al*., 2015)**. Free radicals are released from ultra-violet light, cellular metabolism and polluted air, this then result into inflammation, damage to immune cells and other body cells. Free radicals require intense attention by researchers because they are involved in some disease disorders such as diabetics, heart diseases, respiratory diseases, neurodegenerative disorders, cancers, cataract development and rheumatoid arthritis **(Phaniendra *et al*., 2015).** In earlier studies, the therapeutic, biotechnological and nutritional benefits of *Pleurotus* spp were documented in a review **(Correa *et al*., 2016)**, likewise, some factors that may affect production of *Pleurotus* spp have also been discussed (Belletini *et al*., 2019). More recently, Barbosa *et al*., (2020) elaborated on the bioactivities of polysaccharides from *Pleurotus* spp and the development of new extraction methods. Till date, there are very few or no reviews that have collated the promising potency of extracts from *Pleurotus* spp in scavenging free radicals. Therefore, this present review discussed the antioxidant activities of *Pleurotus* extracts, their mechanisms of action and implications on human health.

## FREE RADICALS AND THEIR SOURCES

Free radicals are molecular species existing autonomously with an unpaired electron in an atomic orbital. They behave as reductants or oxidants through the donation of an electron to or by accepting an electron from other molecules; they are highly reactive and unstable **(Lobo *et al*., 2010)**.In a broad sense, free radicals are a type of reactive oxygen species (ROS) and Reactive nitrogen species (RNS) which associate with the oxygen atom (O) and react strongly with other molecules, but not with oxygen **(Kurutas *et al*., 2016)**. They can react with

nucleic acid, membrane lipids, enzymes and proteins among others, thereby leading to cellular damage **(Adebayo *et al*., 2014b)**. Free radicals commonly encountered include hydroxyl radicals, highly reactive oxygen-containing molecules, superoxide anion radicals, nitric oxide radicals singlet oxygen, and various lipid peroxides; lipid hydroperoxide (ROOH), lipid peroxyl radical (ROO), and lipid alkoxyl radical (RO) **(Adebayo *et al*., 2014b; Kurutas *et al*., 2016)**. Free radicals are generated both endogenously and exogenously. Humans generate low levels of free radicals during oxidation processes, representing the major endogenous source of free radicals. Metabolic processes such as aerobic respiration and some active organelles including stimulated polymorphonuclear leukocytes, macrophages, mitochondria, peroxisomes, and endoplasmic reticulum and can generate ROS/RNS in living organisms. On the other hand, free radicals that are frequently generated exogenously include ionizing radiations, pesticides and organic solvents, certain pollutants, industrial solvents, cigarette smoke, heavy metal, transition metals, radiation medications such as paracetamol and halothane **(Pham-Huy*et al*., 2008; Adebayo *et al*., 2012a Adebayo *et al*., 2014b; Phaniendra *et al*., 2015; Sanchez, 2017).**

## FREE RADICAL SCAVENGERS

The free radicals scavengers are majorly antioxidant compounds that prevent oxidative damages which could result in ageing and life-threatening diseases **(Khan *et al*., 2010).** Therefore, antioxidants are defined as chemicals, whether synthetic or natural, capable of preventing the oxidative reactions of free radicals by exchanging its electrons with that of the free radicals for stabilization **(Sanchez, 2017).** Interestingly, when free radicals occur at low levels in the body system, the innate antioxidant defence systems (glutathione peroxidase, superoxide dismutase, lipase, catalase and other enzymes) can effectively scavenge these free radicals. At higher levels, an antioxidant must be obtained from an external source; mainly dietary. Diets rich in antioxidants include fruits (rich in carotenoids), vitamin C, nuts rich in vitamin E, beta-glucans and most especially mushrooms (Figure 1). Apart from preserving life via the protection of the human body, antioxidants can also serve as food preservatives **(Shahidi and Ambigaipalan, 2015)**. Most of the commonly used synthetic antioxidants such as butylated hydroxyanizole (BHA) and butylated hydroxyltoluene (BHT) are regulated in foods, and other chemicals like ascorbic acids, glutathione, carotenoids, α-tocopherol and polyphenol compounds are suspected to be carcinogenic **(Jayakumar *et al*., 2011)**.

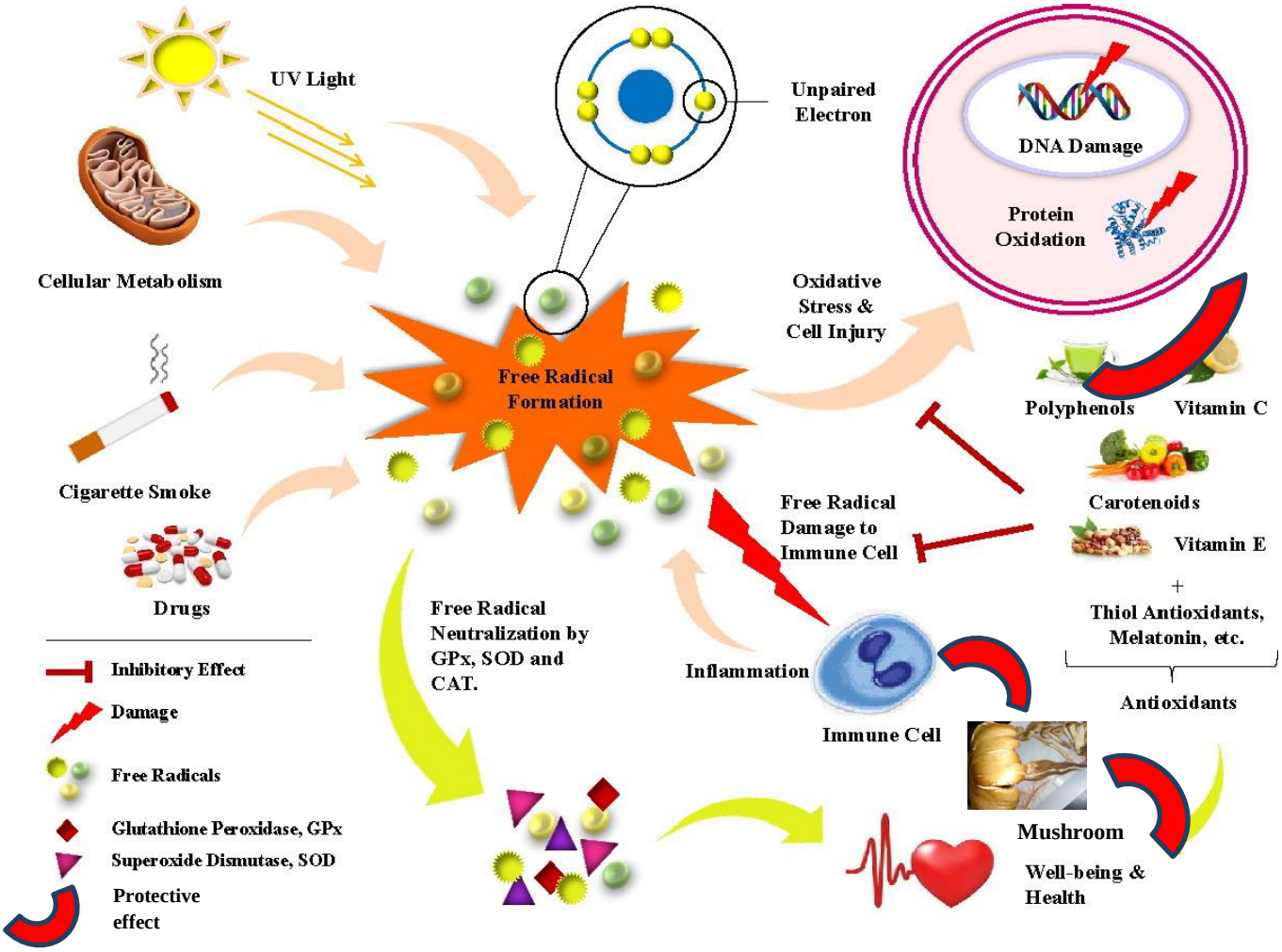


**Figure 1** A schematic representation on the sources of free radicals, damages involved and counter-actions by different antioxidants (polyphenols, mushrooms, carotenoids and vitamin C) to maintain human health and well-being.

Thus, the need for research on natural antioxidant compounds, which may be enzymatic and non-enzymatic antioxidant groups cannot be overemphasized. Traditionally, some *Pleurotus* species or their extracts have promising reports in the treatment of some diseases in different parts of Nigeria **(Idu *et al*., 2007; Osemwegie *et al*., 2010)** Japan, China and other Asian countries **(Xu *et al*., 2011)**. In the past three decades, many polysaccharides and polysaccharide-protein complexes have been extracted from fungi, this includes glycans, heterogeneous and homogeneous polysaccharides and glycan-protein complexes which have been shown to improve the general wellbeing of consumers **(Jong *et al*., 2002)**. A good number of these bioactive polysaccharides has demonstrated haematological, antitumor, antibiotic, antioxidant, immunomodulatory, anti-inflammatory, Hepatoprotective, and antimicrobial effects **(Cohen *et al*., 2002; Hamzah *et al*., 2014; Adebayo *et al*., 2014b; Bamigboye *et al*., 2016a; 2019a)**. Mushrooms can amass a variety of secondary metabolites and phytochemicals, such as flavonoids, phenolic compounds, polyketides, steroids, terpenes, alkaloids, tannins and saponins **(Adebayo *et al*., 2012b; Dandapat and Sinha, 2015)**, which are excellent antioxidants. They are not mutagenic and can work synergistically with synthetic and natural antioxidants **(Ishikawa *et al*., 2001)**. Some non-enzymatic antioxidants, such as vitamin C (Ascorbate), phenolic compounds, vitamin E (-tocopherol), flavonoids, carotenoids, polyphenols etc. **(Fereshteh *et al*., 2017)**, can act directly as anti-oxidative agents and can be obtained from mushrooms **(Adebayo *et al*., 2012a; Ren *et al*., 2014; Woldegiorgis *et al*., 2014; Adebayo *et al*., 2018)**. The sufficient intake of mushrooms or mushrooms materials can enhance adequate antioxidant defence, fight against diseases and positively contribute to a good healthy life.

## FREE RADICAL SCAVENGING CAPACITY OF OYSTER MUSHROOMS

### PRINCIPLES OF FREE RADICAL SCAVENGING ACTIVITIES OF *PLEUROTUS* SPECIES

The need for exogenous means of antioxidants has prompted many studies to investigate the antioxidant potentials of several natural compounds. In this regard, ergothioneine (ERG) contain mainly the amino acid is a natural thiol which is found abundantly in mushroom and is a good source of antioxidant. On this basis, oyster mushroom free radical scavenging principle has been documented **(Jayakumar *et al*., 2011; Dubost *et al*., 2007).***Pleurotus* species are a rich source of vitamin A, C and E, carotenoid, flavonoids and other bioactive phenolic compounds **(Ren *et al*., 2014; Woldegiorgis *et al*., 2014)**, thereby enhancing their free radical scavenging capacity. There are four proposed antioxidants principle mechanisms such as; Preventive antioxidants mechanism: in this case, antioxidants suppress the formation of free radicals by reducing hydrogen peroxide and hydroperoxides to water and alcohols, respectively before they form radicals **(Lobo *et al*., 2010)**. Catalase and Glutathione peroxidase is implicated in this reaction mechanism and is always *in vivo* in nature. Another mechanism is through the initiation of chain suppression and breaking of chain propagation reactions. The endogenous antioxidants implicated are vitamin E, vitamin C, phenolic compounds, flavonoids, carotenoids and polyphenols. Repair and *de novo* antioxidant is another principle mechanism for antioxidants defence. Proteolytic enzymes such as proteases, peptidases and proteinases present in the mitochondria and cytosol in a mammalian cell, detect, destroy and evacuate oxidized proteins and prevent its accumulation **(Salehi *et al*., 2018)**. The last principle is an adaptation, in this mechanism the endogenous antioxidants empower the cell to design a mechanism to maintain, regulate and adapt itself to the inflow of free radicals not to exert oxidative reactions. For instance, polysaccharide–peptide complex (F22) from the sporophore of *Pleurotus abalones* was reported to act as an excellent free radical scavenger **(Li *et al*., 2007)**. In another experiment, *in vitro* demonstration of extract from *P. ostreatus* showed good compelling antioxidant effect by scavenging superoxide and hydroxyl radicals, chelating ferrous ions, impeding lipid peroxidation and decreasing the power of ferric ions **(Nau *et al*., 1998)**. Also, extracts of *P. ostreatus* exhibited notable *in-vivo* free radical scavenging action by decreasing the intensity of lipid peroxidation and by improving the non-enzymatic and enzymatic antioxidants action. Polyphenolic compounds in mushroom have been heavily linked to lipid oxidation stabilization and are connected with antioxidant activity **(Gulcin-Buyukokuroglu *et al*., 2003)**. Extracts of *P. porrigensat* and *P. florida* had interesting scavenging activity against DPPH in contrast to butylhydroxytoluene (BHT), butylated hydroxyanisole (BHA) and tert-butylhydroquinone (TBHQ) **(Wong and Chye, 2009)** and the reproducible antioxidant activities of *Pleurotus* species were believed to be owed to the high content of flavonoid, phenol and some antioxidant phytoconstituents of these extracts **(Patel *et al*., 2012).** The free radical scavenging properties of oyster mushrooms have been heavily linked to the presence of polysaccharides. Mushrooms polysaccharides have been identified as an effective reducing agent, ferrous chelator and free radical scavenger. **Wang e*t al*. (2016)** proposed a likely mechanism for polysaccharide antioxidants to involve polysaccharide conjugations. This implies the combination of polysaccharides with other components such as phytochemicals which together make up the crude polysaccharide. Presence of metal-ion enriched polysaccharide such as Selenium (Se), enhances the polysaccharide antioxidant potentials. Polysaccharide chelating metals bind ions such as copper and ferrous which will inhibit the free radical generations. Another mechanism may occur via the chemical modification of polysaccharides. This may be in the form of sulfation, acetylation, phosphorylation, carboxymethylation and benzoylation which is believed to increase the antioxidant potentials of polysaccharides. Besides, chemical and structural features of polysaccharides such as glycosidic linkage, conformation, molecular mass and chemical compositions have profound effects on their antioxidant potentials. Mushroom polysaccharides can as well utilize any of the above strategies in scavenging the free radicals.

### ANTIOXIDANT MODELS: *IN VITRO* ASSAYS OF FREE RADICAL SCAVENGING CAPACITY OF *PLEUROTUS* SPECIES

Many antioxidant properties of biological materials have been detected using *in vitro* experimental models. However, the methods can be divided into two main methods namely: hydrogen atom transfer (HAT) and single electron transfer (SET), which was determined based on their reaction mechanisms **(Gulcin, 2012; Tan and Lim, 2015)**. The quenching of free radicals by hydrogen donation is the principle used by HAT, making use of total radical-trapping antioxidant parameter (TRAP), oxygen radical absorbance capacity (ORAC), inhibition of induced low-density lipoprotein (LDL) oxidation, and total oxyradical scavenging capacity assay **(Wang *etal*. 2016)**. The SET method reduces certain compounds such as metals, carbonyls and radicals by transferring one electron which then results in colour change as evidenced in the reduced compound. This method is utilized in 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) assay, 2,2-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS) assay, Trolox equivalence antioxidant capacity (TEAC) assay, hydrogen peroxide scavenging, ferric ion reducing antioxidant power (FRAP) assay, superoxide radical scavenging **(Wang *et al*., 2016)** and many others. High antioxidant activities reported from *Pleurotus* by different researchers are due to bioactive components such as phytochemicals, enzymes, vitamins, proteins and polysaccharides. The potential of *Pleurotus* species to show activities in different arrays of antioxidant assays (DPPH, ABTS, ORAC, β-carotene radical scavenging, superoxide, nitric oxide and hydroxyl scavenging etc.) have been examined and reported.

#### DPPH Antioxidant assays of Oyster Mushrooms

DPPH receives an electron or hydrogen radical to develop a stable diamagnetic molecule. Hydrogen donating ability of DPPH is responsible for its high antioxidants potential. The reduction in absorbance of DPPH radical at 517 nm triggered by antioxidants is said to be the result of the reaction between the radical and antioxidant molecules and thereby leading to the scavenging of the radical via hydrogen donation. Thus, DPPH is typically used as a substrate to estimate scavenging activities of antioxidants **(Duh *et al*., 1999)**. Several studies have reported the DPPH scavenging activity of oyster mushroom. High scavenging activities of 81 % in both solid state and submerged fermentation was reported **(Adebayo *et al*., 2014b)** for *Pleurotus pulmonarius* at 10 mg/mL. Sporophore Extract of *P. djamor* grown on rice straw and sawdust mixture inhibited DPPH radicals by 32.8 and 32% respectively at concentration of 0.5 mg/ml **(Rich *et al*., 2016)**. Also, intracellular and extracellular polysaccharide fractions of mutants strains of *P. tuber-regium* effectively scavenged DPPH radicals with an $EC_{50}$ of less than 0.1 µg **(Bamigboye *et al*., 2016)**. The DPPH scavenging and $EC_{50}$ of the following *Pleurotus* species: *pulmonarius, levis, ostreatus, tuber-regium, squarrosulus, sajor-cajor, euos, eryngii* and *cystidiosus* have been reported by several studies (Tab 1). **Arbaayah and Kalsom (2013)** reported the antioxidant potentials of oyster mushrooms in the DPPH free radical scavenging activity test with $IC_{50}$ that ranged between 4.20- 12.0 mg/ml for *P. pulmonarius*,*P. djamor* var. roseus, *P. djamor*var. djamor,and *P. ostreatus.* Moreover,the Polysaccharide of *Pleurotus ostreatus* (Jacq.) P. Kumm demonstrated a concentration dependent DPPH free radical scavenging activity (within 0.2-5.0 mg/ml), with highest activity obtained at higher concentrations which tallied with that of ascorbic acid at 1.2 mg/ml equivalent to 72.23 % **(Pk *et al*., 2019)**.The scavenging capacities of intracellular mycelia polysaccharide of *P. florida*, *P. sajor-caju*, *P. eryngii*and *P. ostreatus* ranged from 16- 71.29 % within the concentrations of 1- 10 mg/ml**(Dundar*et al*., 2013)**. The highest scavenging potential of 81.3 % at 200 µg/ml was displayed by methanolic extract from *P. ostreatus***(Elmastas *et al*., 2007)**. In another study, up to 92.2 and 74.4% of DPPH radicals were scavenged by 5 mg/ml of ethanolic extracts of *P. eryngii* and *P. ostreatus* respectively **(Lo, 2005)**. Additionally, **Talkad *et al*. (2015)** described that Lovastatin extract of *Pleurotus ostreatus* displayed noteworthy antioxidant action when compared with Quercetin (a typical antioxidant), with $IC_{50}$ values of 3.5 and 7.5 µg/ml respectively in a DPPH free radical scavenging assay. **Puttaraju *et al*. (2006)**, reported the antioxidant potentials of aqueous and methanolic extracts of *P. sajor-caju*, and the $IC_{50}$values attained in DPPH free radical scavenging assay were 1.80 and 2.50 mg/ml respectively. Also, the $EC_{50}$ values of DPPH free radical scavenging of *Pleurotus eryngii* and methanol extract of *P. eous* were 9.21 and 4.2 mg/ml respectively **(Reis *et al*., 2014; Sudha *et al*., 2012)**. Also, **Kalyoncu *et al*. (2010)** opined that ethanolic extract of *Pleurotusostreatus* and *P. eryngii*had effective DPPH free radical scavenging activity. **Jeena *et al*. (2016)** studied the DPPH radical scavenging activity of methanolic extract from three *Pleurotus* sp. viz *P. sapidus*, *P. ostreatus*, and *P. sajor-caju*. The authors specified that *P. sajor-caju*had the least $EC_{50}$ which was 2 mg/ml concentration.**Debnath*et al*. (2017)** reported the antioxidant potentials of methanolic extracts of mycelia of some*Pleurotus*species which are *P. sapidus* (DMRP-4), *P. membranaceus* Massee. (DMRP-189),*P. sajor-caju* (Fr.) Singer (DMRP-112), *P.ostreatus* (DMRP-262), *P. florida* (Mont.) Singer (DMRP-88), *P. flabellatus* (Berk. & Broome) Sacc (DMRP-5), *P. hypsizygus* ulmarius (Bull.) Redhead (DMRP-115), *P. eryngii* (DMRP-135), *P. djamor* (DMRP-205) and *P. citrinopileatus* Singer (DMRP-10). In the study, the DPPH free radical scavenging experiment revealed that the mycelia methanolic extracts of the ten *Pleurotus* species displayed antioxidant activities within the range of 62.97-92.05 % at 8.0 mg/ml, and the strongest $EC_{50}$ was displayed by *P. sapidus* at a concentration of 0.352 mg/ml compared to the other nine *Pleurotus* species. Also, **Acharya *et al*. (2017)** reported that *Pleurotus djamor*,had an $EC_{50}$ value of 0.653 mg/ml when evaluated using DPPHantioxidant assay.The DPPH antioxidants assay by extracts of *Pleurotus* species has revealed its potentials as dominant reducing agents by using single electron transfer strategy, which confirmed *Pleurotus* extract as powerful antioxidant agents. This prospect make *Pleurotus* mushrooms play essential roles in scavenging radicals, act as immune boosters, control of some diseases as well as taken as functional foods.

**Superoxide, Nitric Oxide and Hydroxyl Scavenging Activities of Oyster Mushrooms**

The superoxide radical is very dangerous to some cell components because of its ability to produce more reactive oxygen species **(Dundar *et al.*, 2013)**. The superoxide radical-scavenging activity of the cultured mycelia extracts of *Pleurotus eryngii*, *P. ostreatus*, *P. florida*, and *P.sajor-caju* reported as percentage inhibitions which ranged from 67.92 – 76.14 % at 10 mg/ml. This can result from the interrelations ofthediverse flavonoids in the extracts **(Zhishen *et al.*, 1999)**. Also, **Jayakumar *et al.* (2009)** studied and reported that the superoxide anion radical scavenging action of ethanolic extract of *P. ostreatus* basidiocarp was 60.02%. The extracts of *P. squarrosulus* had high superoxide and nitric oxide scavenging activities with $EC_{50}$ of 8.63 mg/ml and 4.46 mg/ml respectively **(Pal *et al.*, 2010)**. Extracts of *P. ostreatus* inhibited hydroxyl compound by 50% at 8 mg/ml **(Jayakumar *et al.*, 2009)**. Various extracts of *P. florida*and *P. pulmonarius* had varying hydroxyl scavenging activities as reported by **(Ajith and Janadhanan, 2007)**. The superoxide, nitric oxide and hydroxyl scavenging activities of oyster mushrooms are also presented in Table 2.

**ABTS Radicals Scavenging Potentials of Oyster Mushroom**

A couple of studies have reported the prospect of Oyster mushrooms and their metabolites in mopping up or scavenging ABTS radicals which further emphasize their usefulness as natural antioxidants. Extracts of *P. euos, P. ostreatus, P. eryngii,*and *P. cystidiosus* were reported to possess potentials to scavenge ABTS radicals although at low concentrations **(Kongkla and Poeaim, 2016)**, while higher scavenging activities were reported by **Adebayo *et al.* (2018)** for extracts of *P. levis, P. ostreatus, P. tuber-regium, and P. pulmonarius* . Also, **Pk *et al.* (2019)** reported that polysaccharide of *P. ostreatus* displayed significant ABTS radicals scavenging activity - 81.02 %, which equalled the activities of ascorbic acid at 1.4 mg/ml. The antioxidant potentials of various *Pleurotus* mushroom species against ABTS radicals are also reported in Table 3.

**Table 1** DPPH Scavenging Activity of Oyster Mushrooms along with solvent, $EC_{50}$ values, highest scavenging activity and cited literatures

| Mushroom | Extractant | Concentration (mg/mL) | DPPH Scavenging assay (%) | Reference |
|---|---|---|---|---|
| *P. pulmonarius* LAU 09 | - | 5.0 | 78.4 | Adebayo *et al*. (2012b) |
| | | 10.0 | 81.0 | |
| | Methanol | 5.0 | 78.0 | Adebayo *et al*. (2014b) |
| | | 10.0 | 81.0 | |
| *P. pulmonarius* CP-16 | Hydro-alcohol | 0.0007 | 50.0 ($EC_{50}$) | Adebayo *et al*. (2018) |
| *P. pulmonarius* CP-799 | Hydro-alcohol | 0.00049 | 50.0 ($EC_{50}$) | Adebayo *et al*. (2018) |
| *P. sapidus*NE 07 | Methanol | 5.0 | 40.0 | Adebayo *et al*. (2014b) |
| | | 10.0 | 76.0 | |
| *P. cornucopiae*NE 02 | Methanol | 5.0 | 35.0 | Adebayo *et al*. (2014b) |
| | | 10.0 | 68.0 | |
| *P. ostreatus*LAU 10 | Methanol | 5.0 | 60.0 | Adebayo *et al*. (2014b) |
| | | 10.0 | 71.0 | |
| *P. levis* CP-30 | Hydro-alcohol | 0.00051 | 50.0 ($EC_{50}$) | Adebayo *et al*. (2018) |
| *P. ostreatus* CP-50 | Hydro-alcohol | 0.00105 | 50.0 ($EC_{50}$) | Adebayo *et al*. (2018) |
| *P. ostreatus* CP-800 | Hydro-alcohol | 0.00063 | 50.0 ($EC_{50}$) | Adebayo *et al*. (2018) |
| *P. ostreatus* | Methanol | 5.0 | 50.0 ($EC_{50}$) | Kongkla & Poeaim (2016) |
| *P. tuber-regium* CP-182 | Hydro-alcohol | 0.00168 | 50.0 ($EC_{50}$) | Adebayo *et al*. (2018) |
| *P. squarrosulus* | Cold water | 0.465 | 50.0 ($EC_{50}$) | Pal *et al.* (2010) |
| | Hot water | 0.304 | 50.0 ($EC_{50}$) | |
| | Methanol | 1.5 | 50.0 ($EC_{50}$) | |
| *P. sajor-caju* | Methanol | 5.2 | 50.0 ($EC_{50}$) | Kongkla & Poeaim (2016) |
| *P. sajor-caju* | Methanol | 6.0 | 61.0 | Ramkumar *et al*. (2010) |
| *P. euos* | Methanol | 5.4 | 50.0 ($EC_{50}$) | Kongkla & Poeaim (2016) |
| | Hexane | 0.5 | 32.0 | |
| *P. eryngii* | Methanol | 8.4 | 50.0 ($EC_{50}$) | Kongkla & Poeaim (2016) |
| *P. cystidiosus* | Methanol | 9.1 | 50.0 ($EC_{50}$) | Kongkla & Poeaim (2016) |

**$EC_{50}$: 50 % Effective Concentration; Fermentation: solid state**

**Table 2** Superoxide (SO), Nitric oxide (NO) and Hydroxyl (OH) Scavenging Assay of Oyster Mushroom

| | | | | | | |
|---|---|---|---|---|---|---|
| ***P. squarrosulus*** | Cold water | 1.830 | 0.360 | 0.364 | 50.0 ($EC_{50}$) | |
| | Hot water | 1.473 | 0.320 | 0.268 | 50.0 ($EC_{50}$) | Pal *et al.* (2010) |
| | Methanol | 8.630 | 4.460 | 0.706 | 50.0 ($EC_{50}$) | |
| ***P. ostreatus*** | Ethanol | 8.000 | NR | 8.000 | 50.0 ($EC_{50}$) | Jayakumar *et al*. (2009) |
| ***P. florida*** | Ethyl acetate | NR | NR | 0.530 | | |
| | Methanol | NR | NR | 0.263 | 50.0 ($EC_{50}$) | Ajith and Janardhanan (2007) |
| | Aqueous | NR | NR | 0.263 | | |
| ***P. pulmonarius*** | Methanol | NR | NR | 0.476 | 50.0 ($EC_{50}$) | Ajith and Janardhanan (2007) |

**$EC_{50}$: 50 % Effective Concentration; Fermentation: solid state; NR: Not Recorded**

**Table 3** ABTS Scavenging Activity of Oyster Mushrooms along with solvent, $EC_{50}$ values, highest scavenging activity and cited literatures

| Mushroom | Extractant | Concentration (mg/mL) | ABTS Scavenging assay (%) | Reference |
|---|---|---|---|---|
| *P. levis* CP-30 | Hydro-alcohol | 0.00050 | 50.0 ($EC_{50}$) | Rich *et al*. (2016) |
| *P. ostreatus* CP-50 | Hydro-alcohol | 0.00042 | 50.0 ($EC_{50}$) | Rich *et al*. (2016) |
| *P. ostreatus* CP-800 | Hydro-alcohol | 0.00024 | 50.0 ($EC_{50}$) | Rich *et al*. (2016) |
| *P. pulmonarius* CP-16 | Hydro-alcohol | 0.00035 | 50.0 ($EC_{50}$) | Rich *et al*. (2016) |
| *P. pulmonarius* CP-799 | Hydro-alcohol | 0.00037 | 50.0 ($EC_{50}$) | Rich *et al*. (2016) |
| *P. tuber-regium* CP-182 | Hydro-alcohol | 0.00050 | 50.0 ($EC_{50}$) | Rich *et al*. (2016) |
| | Methanol | 5.10 | 50.0 ($EC_{50}$) | Kongkla & Poeaim (2016) |
| *P. sajor-caju* | Methanol | 4.51 | 50.0 ($EC_{50}$) | Kongkla & Poeaim (2016) |
| *P. eous* | Methanol | 3.92 | 50.0 ($EC_{50}$) | Kongkla & Poeaim (2016) |
| *P. eryngii* | Methanol | 6.98 | 50.0 ($EC_{50}$) | Kongkla & Poeaim (2016) |
| *P. cystidiosus* | Methanol | 7.18 | 50.0 ($EC_{50}$) | Kongkla & Poeaim (2016) |

**$EC_{50}$: 50 % Effective Concentration**

**Metal Chelating ability of Oyster Mushrooms**

The initial development of radicals can be catalyzed by transition metals. Transition metals can be made steady by chelating agents in living systems to restrain free radicals generation, as a result limiting the induction of free radical damage **(Dundar *et al*., 2013)**. The chelating potential of an extract provides a means of preventing the generation of free-radical and iron-overload via the chelation of metal ions. Also, metal ion chelating ability played an important action in antioxidant mechanism, since ferrous ions are the most efficient pro-oxidants in food structures. The effectiveness of extracts to impede the development of ferrozine and ferrous complex will suggest their capacity for chelating activities **(Debnath *et al*., 2017).**The oxidative loss and iron-overload in the systems may be prevented by chelating agents (Lai *et al*., 2001). Chelating activities of *P. pulmonarius*, *P. citrinopileatus* and *P. florida* at 1.0 mg/ml were reported to range from 78.14-82.07 % which were significantly higher than EDTA (50.66%) and citric acid (46.84%) **(Khatun *et al*., 2015)**. According to **Lee *et al*. (2007)**, the chelating abilities of *P. citrinopileatus* extracted in ethanol, cold and hot water at 5 mg/ml were 46, 67 and, 82% respectively. **Jayakumar *et al*. (2009)** reported that ethanolic extracts of *P.ostreatus* fruiting body had 60.68% scavenging activity at 10 mg/ml concentration. Also, the chelating activity of ethanolic extracts of fruiting bodies of *P. ostreatus* and *P. eryngii* at 5 mg/ml was described to be 41.4 and 64.0 % **(Lo, 2005).Dundar *et al*. (2013)** reported that at all of the concentrations ranging from 2.0- 10 mg/ml, ethanolic extracts of mycelia of *P. florida*, *P. eryngii*, *P. sajor-caju*, and *P. ostreatus* showed higher chelating activities than the standard antioxidants (BHT and α-tocopherol) with percentage inhibition of 57.3- 69.5 % at 10 mg/ml. **Mau *et al*. (2001)** reported that methanolic extracts of ear mushroom was able to chelate 85.1 – 96.5% of ferrous ions at a concentration of 5 mg/mL, thereby they are excellent chelators. An ethanolic extract of *P. eryngii* had a high chelating activity of approximately 75 % at an $IC_{50}$ of 1.00 mg/ml **(Yildirim *et al*., 2012)**.

**Ferric Reducing Ability and Power of Oyster Mushroom**

The potential antioxidant activity of a compound can be determined by its reducing capacity. The various mechanisms such as chain initiation prevention, peroxides decompositions, transition metal ion binding catalysts, hydrogen abstraction discontinued, reductive capacity and scavenging of free radicalhave been attributed toputative antioxidant activity of various antioxidants substances**(Gulcin *et al*., 2003)**. Antioxidant compounds can transform the oxidized form of iron in ferricyanide ($Fe^{3+}$) to ferrous ($Fe^{2+}$) ion. The electron transfer ability is associated with its reducing power and may serve as animportantpointer tointeresting antioxidant activity **(Debnath*et al*., 2017).***P. sajor-caju* and *P. ostreatus* were reported to have high scavenging power, 1.980 and 1.780 respectively at 10 mg/ml concentration**(Jeena *et al*., 2016).** According to **Sudha *et al*. (2012)**, the scavenging powers of hot water, methanolic, and ethyl acetate fractions of *P. eous*at 10 mg/ml was quite high, being1.632, 1.132, and 1.950 respectively. Also, *P. pulmonarius*, *P.djamor* var. djamor, *P. ostreatus* and *P. djamor* var. roseus were reported to display substantial reducing activities at concentrations between 2 and 10 mg/ml depending on their capacity to reduce ferricyanide complex to ferrous form which varied from 0.29 to 1.23. Although the values obtained for synthetic antioxidants, Quercetin and BHA serving as the standards were appreciably higher by two folds than those of the mushroom extracts **(Arbaayah and Kalsom, 2013)**. **Khatun *et al*. (2015)** reported that the optical density at 700 nm in evaluating the reducing power of *P. citrinopileatus*, *P. pulmonarius*, and *P. florida* showed values which range from 0.073 - 0.217 at 1.0 mg/ml concentration which was appreciably higher than ascorbic acid. **Debnath*et al*. (2017)** showed that the reducing powers of methanolic extracts of *Pleurotus* species mycelia tested (which are *P. sajor-caju* (DMRP-112), *P. sapidus* (DMRP-4), *P. ostreatus* (DMRP-262), *P. membranaceus*(DMRP-189), *P. florida* (DMRP-88), *P. flabellatus* (DMRP-5), *P. hypsizygus* ulmarius (DMRP-115), *P. eryngii* (DMRP-135), *P. djamor* (DMRP-205) and *P. citrinopileatus* (DMRP-10)) were excellent, increasing steadily with increasing concentrations (0.5-8 mg/ml). Also, it was shown that *P. sajor-caju* displayed the highest ferric reducing power of 0.17 – 1.08 at 8 mg/ml. The scavenging activity of ethanol, hot, and cold water extracts from the sporophore of*P. citrinopileatus*at 5 mg/ml were in the range of 1.03–1.10 **(Lee *et al*. 2007)** and these were higher than those of the mycelia and filtrate. **Dundar *et al*. (2013)** reported that reducing power within 0.74 – 0.86 were recorded for four *Pleurotus* sp. (*P. florida*, *P. sajor-caju*, *P. ostreatus* and *P. eryngii*) at 10 mg/ml and these were higher compared to results for both BHA and BHT excluding α-tocopherol with reducing powers of 0.99. The extracts of *P. squarrosulus* isolated from laterite region in the wild had high chelating and reducing power at 1.225 mg/mL and 13 mg/mL respectively **(Pal *et al*., 2012).***P. eous* also reportedly had reducing power of 0.4% while *P. sajor-caju* and *P. platypus* had 0.2% at 6 mg/ml **(Rankumar *et al*., 2010)**.It has been earlier proven that chemically synthesized antioxidants, among which are, BHA, BHT and TBHQ, are mutagenic. Thus, alternative antioxidants that are derived from natural resources are quite interesting and easily acceptable by consumers **(Lo, 2005; Elmastas *et al*., 2007)**. The ferrous ion chelating potential and reducing power of oyster mushrooms are presented in Table 4.

**β-Carotene Radical Scavenging and Antioxidant Capacity by Phosphomolybdenum Method**

Few studies have examined the antioxidant prospect of Oyster mushrooms and their metabolites by the Phosphomolybdenum method and as well reported their potentials in scavenging β-carotene radical. *In vitro* analysis of the radical-producing systems have acknowledged the ability of β-carotene to reduce free radicals viacertain processes which include abstraction of hydrogen, addition of the radical to the carotenoid and/or transfer of electron**(Krinsky, 2001)**. Generally, mushrooms are known to have an appreciable quantity of β-carotene, vitamins A and C, all of which protect against cellular damage due to their excellent antioxidant power**(Murcia *et al*., 2002)**.The methanolic extract of *P. cornucopiae* and *P. sapidus* scavenged β-carotene radicals by 98% while *P. pulmonarius* and *P. ostreatus* scavenged radical β-carotene by 62% **(Adebayo *et al*., 2014b)**. Submerged fermentation of *P. pulmonarius* showed high antioxidant activity of 87 µmoles/100g as determined by phosphomolybdenum method while *P. sapidus* and *P. ostreatus* had 28 µmoles/100g. The $EC_{50}$ for β-carotene radical scavenging activities for extracts from *P. pulmonarius*, *P. ostreatus*, *P. levis*, *P. tuber-regium* and *P. squarrosulus* have also been reported (Adebayo *et al*. 2018). Also, according to **Dundar *et al*. (2013)**, the ethanolic mycelial extract of of *P. eryngii*, *P. ostreatus*, *P. sajor-caju* and *P. florida*, exhibited inhibition of peroxidation in a β-carotene–linoleic acid system at a concentration of 0.5 – 10 mg/mL within the range of 34.04 – 62.12 %.The β-carotene radical scavenging and antioxidant capacity measured by phosphomolybdenum methods are presented in Table 5.

**Table 4** Chelating Ability of Ferrous Ions and Reducing Power Assay of Oyster Mushroom

| Mushroom | Extractant | Chelating ability (mg/mL) | Reducing power (mg/mL) | Reducing power (%) | Reference |
|---|---|---|---|---|---|
| | Cold water | 0.090 | 1.33 | 50.0 ($EC_{50}$) | |
| *P. squarrosulus* | Hot water | 0.075 | 1.14 | 50.0 ($EC_{50}$) | Pal *et al*. (2010) |
| | Methanol | 1.225 | 13.00 | 50.0 ($EC_{50}$) | |
| *P. ostreatus* | Ethanol | 6.000 | ND | 50.0 ($EC_{50}$) | Jayakumar *et al*. (2009) |
| *P. sajor-caju* | Methanol | NR | 6.00 | 0.20 | Ramkumar *et al*. (2010) |
| *P. euos* | Methanol | NR | 6.00 | 0.40 | Ramkumar *et al*. (2010) |
| *P. florida* | Methanol | NR | 6.00 | 0.22 | Ramkumar *et al*. (2010) |
| *P. platypus* | Methanol | NR | 6.00 | 0.20 | Ramkumar *et al*. (2010) |
| *P. djamor* | Methanol | NR | 6.00 | 0.30 | Ramkumar *et al*. (2010) |

**$EC_{50}$: 50 % Effective Concentration; Fermentation: solid state; NR: Not Recorded**

**Table 5** β - Carotenes Radical Scavenging and Antioxidant Capacity (as Equivalent to Ascorbic Acid) by Phosphomolybdenum Method of Oyster Mushroom

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | 1.0 | NR | 25.6 | |
| | Submerge | - | 2.0 | 62.1 | NR | Adebayo *et al*. (2012b) |
| *P. pulmonarius*LAU 09 | | | 5.0 | 78.4 | 87.1 | |
| | | | 1.0 | NR | 25.5 | |
| | Solid state | Methanol | 2.0 | 62.0 | NR | Adebayo *et al*. (2014b) |
| | | | 5.0 | 78.0 | 87.0 | |
| *P. pulmonarius*CP-16 | Solid state | Hydro-alcohol | >0.005 | 50.0 ($EC_{50}$) | NR | Adebayo *et al*. (2018) |
| *P. pulmonarius*CP-799 | Solid state | Hydro-alcohol | 0.00022 | 50.0 ($EC_{50}$) | NR | Adebayo *et al*. (2018) |
| *P. sapidus*NE 07 | Solid state | Methanol | 1.0 | NR | 28.0 | Adebayo *et al*. (2014b) |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | 2.0 | 98.0 | NR | |
| | | | 5.0 | 95.0 | 57.0 | |
| | | | 1.0 | NR | 34.0 | |
| *P. cornucopiae*NE 02 | Solid state | Methanol | 2.0 | 70.0 | NR | Adebayo *et al*. (2014) |
| | | | 5.0 | 98.0 | 69.0 | |
| | | | 1.0 | NR | 28.0 | |
| *P. ostreatus*LAU 10 | Solid state | Methanol | 2.0 | 62.0 | NR | Adebayo *et al*. (2014b) |
| | | | 5.0 | 88.0 | 72.0 | |
| *P. ostreatus*CP-50 | Solid state | Methanol | 0.00034 | 50.0 ($EC_{50}$) | NR | Adebayo *et al*. (2018) |
| *P. ostreatus*CP-800 | Solid state | Methanol | 0.00017 | 50.0 ($EC_{50}$) | NR | Adebayo *et al*. (2018) |
| *P. levis* CP-30 | Solid state | Methanol | 0.00059 | 50.0 ($EC_{50}$) | NR | Adebayo *et al*. (2018) |
| *P. tuber-regium* CP-182 | Solid state | Methanol | 0.00016 | 50.0 ($EC_{50}$) | NR | Adebayo *et al*. (2018) |
| | | Cold water | 0.554 | 50.0 ($EC_{50}$) | NR | |
| *P. squarrosulus* | Solid state | Hot water | 0.550 | 50.0 ($EC_{50}$) | NR | Pal *et al*. (2010) |
| | | Methanol | 3.793 | 50.0 ($EC_{50}$) | NR | |

**$EC_{50}$: 50 % Effective Concentration. NR: Not Recorded**

**Total Phenolic Content of Oyster Mushroom**

Phenolic compounds are aromatic hydroxylated compounds which have one or more aromatic rings and one or more hydroxyl groups. They include hydroxybenzoic acids, phenolic acids, flavonoids, hydroxycinnamic acids, tannins, lignans, oxidized polyphenols and stilbenes **(Sanchez, 2017).** It has been established that phenolic compounds demonstrate free radical scavenging effect in the body, functioning as peroxide decomposers, free radical inhibitors, oxygen scavengers or metal in activators. Mushrooms in general contain an appreciable quantity of polyphenols at different concentrations ranged from 3.62 to 6.25 mg/ml (Ramesh and Pattar, 2010; Barros *et al*., 2008). Phenolic compounds can be found and extracted from different mushroom, both mycelia and fruiting body, for instance, the mycelium of *Pleurotus albidus***(Gambato *et al*., 2016)** and the fruit bodies of *Pleurotus cornucopiae***(Kolayli *et al*., 2012)**. Phenolic substances, including phenolic acids and flavonoids, are reported to have antioxidant activity. Also, phenolic compounds in mushrooms are known as exceptional synergists and antioxidants which are not mutagenic **(Jayakumar *et al*., 2011)**. Phenols are vital plant components owing to the presence of hydroxyl groups, hence having scavenging potential **(Dundar *et al*., 2013)**. Some studies have convincingly shown that eating phenol-rich foods can lower the menace of cardiovascular diseases by reducing the development of atherosclerosis since they function as antioxidants **(Singla *et al*., 2010; Halliwell and Gutteridge, 2015)**. Dietary phenolic compounds have been said to have the ability to trigger some biological mechanisms, such as chelation of metals, free-radical scavenging, and regulation of enzymatic activity; they likewise affect the initiation of transcription factors, signal transduction and gene expression **(Srinivasan *et al*., 2005)**. They have received unique attention due to their purported function in the inhibition of some human illnesses**(Nardini and Ghiselli, 2004)**. The presence of phenols in mushrooms has contributed significantly to theirefficiency at scavenging peroxy radicals **(Murcia *et al*., 2002)**. The total phenolic content of *Pleurotus* sp has been described in several studies as either tallic acid, gallic acid or ascorbic acid equivalent or percentage (Tab 6). Extracts of *P. djamor* had a reportedly high phenolic content of 982 ascorbic acid equivalents **(Rankumar *et al*., 2010)**, while extracts of *P. tuber-regium* had 0.05 gallic acid equivalents. This great difference in the phenolic content could be linked to the variation in the standard used in reporting the phenolic content equivalence. **Arbaayah and Kalsom (2013)** reported that the number of total phenolics in *P. djamor* var. djamor, *P. pulmonarius*,*P. ostreatus*and *P. djamor* var. roseus, ranged from 39.36 to 51.94 mg TAE/g of dry weight of the crude extract. Also, the total phenolic content of *Pleurotus ostreatus* was 0.09 mg/g, while that of *Pleurotus eryngii* was 0.03 mg/g of dry weight **(Kim *et al*., 2008)**.The total phenol content in*P. sajor-caju* and *P. djamor*was 14.43 and 13.22 mg/g of extract respectively **(Puttaraju *et al*., 2006)** while *P. ostreatus was* estimated to havea total phenolic content of 0.71 mg/g dry weight **(Jayakumar, 2008).Khatun *et al*. (2015)** showed that the total phenol content of *P. pulmonarius*, *P. florida*, *P. citrinopileatus* observed ranged from 64- 119 µg catechol $g^{-1}$dw. The phenolic content in ethanolic extract of *P. aureovillosus*,*P. sajor-caju*, and *P. florida* were reported to range from 6.001- 7.501 µg/mg **(Loganathan *et al*., 2008)**. The research conducted by **Dundar *et al*. (2013)** revealed that the total phenolic compounds found in the ethanolic mycelia extracts of *P. sajor-caju*, *P. florida*, *P. eryngii*, and *P. ostreatus* ranged from 3.97- 4.56 mg/g (dry weight). **Debnath *et al*. (2017)** examined ten *Pleurotus* species and reportedthat their total phenol content ranged between 0.50 – 4.33 mg/g with *P. flabellatus* and *P. ostreatus* have the lowest and highest total phenol contents respectively. The total flavonoid content assay is carried out to extract and estimate flavonoids, neoflavonoids and isoflavonoids or all together called as bioflavonoids. Previous studies had proved that flavonoids function as an antioxidant by breaking the radical chains and converting them into more stable products in liver microsomal membranes, with the capacity to protect low density lipoprotein from being destroyed by macrophages and heavy metals which also play a central task of providing inherent fortification to counter oxidative stress and side effects by the presence of vitamins **(Arbaayah and Kalsom, 2013).** The total flavonoid was highest in *P. pulmonarius* with 12.2 µg/mg as reported by **Adebayo *et al*. (2012b; 2014)** and lowest in *P. squarrosulus* with 3.07 µg/mg **(Pal *et al*., 2010). Arbaayah and Kalsom (2013)** reported that the amount of total flavonoid content in *P. pulmonarius*, *P. djamor* var. djamor, *P. ostreatus*, and *P. djamor* var. roseus, from 3.02 to 14.88 mg QE/g of dry weight of crude extract.

**Oxygen Radical Absorbance Capacity (ORAC) and Lipid Peroxidation of Oyster Mushrooms**

The oxygen radical absorbance capacity (ORAC) and lipid peroxidation of several oyster mushrooms have been reported as presented in Table 7. *P. ostreatus* had the highest ORAC of 8199.2 trolox equivalents while *P. tuber-regium* had the lowest with 3316.0 trolox equivalent as reported by **Adebayo *et al*. (2018)**. Extract of *P. pulmonarius* inhibited lipid peroxidation by 0.960 mg/ml with *P. florida* inhibiting with 0.320 mg/ml **(Ajith and Janadhanan, 2007)**. Extracts of *P. populinus* inhibited lipid peroxidation by 26.64 µM **(Okafor *et al*., 2017)**. **Lakshmi et al. (2004)** presented ORAC values for *P. florida*, *P. sajour-caju* and *P. rimosus* extracts using spectrofluorimetric assay as follows; (45.65 ± 5.85), (43.94 ± 8.66), and Pr (30.59 ± 1.59) as micromoles of Trolox equivalent per gram of fresh weight. From the above, it was clearly shown that *P. florida recorded* most potent scavenging peroxyl radicals in the ORAC assay.

***IN VIVO* ASSAY OF FREE RADICAL SCAVENGING PROPERTY OF *PLEUROTUS* SPECIES**

The *in vivo*assay encompasses both enzymatic and non-enzymatic processes for scavenging or neutralizing free radicals **(Govindan *et al*., 2016)**. In the enzymatic antioxidant assay, biomarker enzymes includes; catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD), glutathione reductase(GR), glutathione *S*-transferase (GST), and ascorbate peroxidase (APx)**(Govindan *et al*., 2016; Islam *et al*., 2019)**. The non-enzymatic assays mainly utilize Vitamin E (α-tocopherol), Glutathione (GSH), and vitamin C or ascorbic acid **(Kim *et al*., 2010; Bhattacharyya *et al*., 2014; Islam *et al*., 2019)**. Enzymatic antioxidant activity of extracts of *Pleurotus abalones*, *P. djamor*,*P. ostreatus*, and *P. eryngii* var. *tuoliensis* increased the activity of SOD and inactivating superoxide radicals **(Li *et al*., 2007; Anandhi*et al*., 2013; Zhang*et al*., 2015; Krishnamoorthy and Sankaran, 2016; Xu*et al*., 2017)**. CAT activity was increased by up regulating gene expression with subsequent prevention of toxicity resulting from the generation of hydrogen peroxide **(Anandhi*et al*., 2013; Xu*et al*., 2017; Krishnamoorthy and Sankaran, 2016).**

**Table 6** Total Phenolic and Total Flavonoid Content

| Mushroom | Fermentation | Extractant | Concentration (mg/mL) | Total phenolic content | Total flavonoid content (µg/mg) | Reference |
|---|---|---|---|---|---|---|
| *P. pulmonarius* LAU 09 | Submerge | - | 1.0 | NR | 12.178 | Adebayo *et al*. (2012b) |
| | | | 2.0 | 42.6 TAE | NR | |
| | | | 5.0 | 38.4 TAE | NR | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Solid state | Methanol | 1.0 | NR | 12.2 | Adebayo *et al*. (2014b) |
| | | | 2.0 | 39.0 TAE | NR | |
| | | | 5.0 | 43.0 TAE | NR | |
| *P. pulmonarius* CP-16 | Solid state | Hydro-alcohol | - | 0.080 GAE | NR | Adebayo *et al*. (2018) |
| *P. pulmonarius* CP-799 | Solid state | Hydro-alcohol | - | 0.098 GAE | NR | Adebayo *et al*. (2018) |
| *P. sapidus* NE 07 | Solid state | Methanol | 1.0 | NR | 10.5 | Adebayo *et al*. (2014b) |
| | | | 2.0 | 30.0 % | NR | |
| | | | 5.0 | 57.0 % | NR | |
| *P. cornucopiae* NE 02 | Solid state | Methanol | 1.0 | NR | 11.0 | Adebayo *et al*. (2014b) |
| | | | 2.0 | 34.0 % | NR | |
| | | | 5.0 | 69.0 % | NR | |
| *P. ostreatus* LAU 10 | Solid state | Methanol | 1.0 | NR | 10.4 | Adebayo *et al*. (2014b) |
| | | | 2.0 | 29.0 % | NR | |
| | | | 5.0 | 43.0 % | NR | |
| *P. levis* CP-30 | Solid state | Hydro-alcohol | - | 0.057 GAE | NR | Adebayo *et al*. (2014b) |
| *P. pulmonarius* LAU 09 | Submerge | - | 1.0 | NR | 12.178 | Adebayo *et al*. (2014b) |
| *P. ostreatus* CP-50 | Solid state | Hydro-alcohol | | 0.066 GAE | NR | Adebayo *et al*. (2018) |
| *P. ostreatus* CP-800 | Solid state | Hydro-alcohol | | 0.129 GAE | NR | Adebayo *et al*. (2018) |
| *P. ostreatus* | Solid state | Methanol | | 17.6 GAE | NR | KongklaPoeaim (2016) |
| *P. tuber-regium* CP-182 | Solid state | Hydro-alcohol | | 0.05 GAE | NR | Adebayo *et al*. (2018) |
| *P. squarrosulus* | Solid state | Cold water | | 0.08 GAE | 3.62 | Pal *et al*. (2010) |
| | | Hot water | | 0.82 GAE | 5.45 | |
| | | Methanol | | 0.18 GAE | 3.07 | |
| *P. djamor* | Solid state | Acentonitrile | | 982.0 AAE | NR | Rich *et al*. 2016 |
| | | Hexane | | 35.0 AAE | NR | |
| *P. sajor-caju* | Solid state | Methanol | | 15.75 GAE | NR | Kongkla and Poeaim (2016) |
| *P. euos* | Solid state | Methanol | | 11.02 GAE | NR | Kongkla and Poeaim (2016) |
| *P. eryngii* | Solid state | Methanol | | 8.77 GAE | NR | Kongkla and Poeaim (2016) |
| *P. cystidiosus* | Solid state | Methanol | | 07.17 GAE | NR | Kongkla and Poeaim (2016) |

**$EC_{50}$: 50 % Effective Concentration; NR: Not Recorded; TAE: Tallic Acid Equivalent; GAE: Gallic Acid Equivalent; AAE: Ascorbic Acid Equivalent.**

**Table 7** Oxygen Radical Absorbance Capacity (ORAC) and Lipid Peroxidation Inhibition

| Mushroom | Extractant | Oxygen radical absorbance capacity (TE) | Lipid peroxidation inhibition | Reference |
|---|---|---|---|---|
| *P. pulmonarius* CP-16 | Hydro-alcohol | 6496.8 | NR | Adebayo *et al*. (2018) |
| *P. pulmonarius* CP-799 | Hydro-alcohol | 4455.2 | NR | Adebayo *et al*.( 2018) |
| *P. pulmonarius* | Methanol | NR | 0.960mg/mL | Ajith and Janardhanan (2007) |
| | Hexane | NR | 28.86 µM | Okafor *et al*. (2017) |
| *P. levis* CP-30 | Hydro-alcohol | 5080.8 | NR | Adebayo *et al*. (2018) |
| *P. ostreatus* CP-50 | Hydro-alcohol | 8199.2 | NR | Adebayo *et al*. (2018) |
| *P. ostreatus* CP-800 | Hydro-alcohol | 34646.0 | NR | Adebayo *et al*. (2018) |
| *P. ostreatus* | Hexane | NR | 35.36 µM | Okafor *et al*. (2017) |
| *P. tuber-regium*CP-182 | Hydro-alcohol | 3316.0 | NR | Adebayo *et al*. (2018) |
| *P. florida* | Ethyl acetate | NR | 0.496mg/mL | Ajith and Janardhanan (2007) |
| | Methanol | NR | 0.320mg/mL | |
| *P. sajor-caju* | Hexane | NR | 32.26 µM | Okafor *et al*. (2017) |
| *P. populinus* | Hexane | NR | 26.64 µM | Adebayo *et al*. (2018) |

**NR: Not Recorded. TE: Trolox Equivalent; Fermentation: solid state**

Metabolites from *Pleurotus abalones*, *P. eryngii P. ostreatus*,and *P. djamor* impacted positively on GPx activity by reducing hydrogen peroxide to water and scavenging endogenous peroxides species. It also decreased oxidative stress and prevent many complex diseases **(Anandhi*et al*., 2013; Ma*et al*., 2015; Zhang *et al*., 2015; Krishnamoorthy and Sankaran, 2016; Xu *et al*., 2017)**. Activity of GST improved with administration of *P. ostreatus* extract in rodent cell protected against free radicals induced cell damage **(Jayakumar *et al*., 2010; Thomas *et al*., 2014).***P. ostreatus* extract improved the function of APx which catalysed theconversion of hydrogen peroxide to water **(Thomas *et al*., 2014)**. Non-enzymatic antioxidant assays using ascorbic acid components of *P. ostreatus* reduced ascorbate radicals and enhanced better antioxidant performance **(Anandhi*et al*., 2013; Krishnamoorthy and Sankaran, 2016)**. Vitamin E or α-tocopherol from *P. ostreatus* inhibited lipid peroxidation in cell membranes **(Anandhi*et al*., 2013; Krishnamoorthy and Sankaran, 2016),** and activity of GSH prevented oxidation of GSH and safeguard the redox enzymes **(Jayakumar *et al*., 2010; Anandhi*et al*., 2013; Krishnamoorthy and Sankaran, 2016)**. A number of studies have reported the free radical scavenging property of oyster mushroom in experimental animals such as mice, rat, porcine etc. as summarized in Table 8. Remarkable improvement of thiobarbituric acid reactive substances was noted in porcine brains after administering fruiting body and mycelium of *P. ostreatus* and *P. eryngii* **(Reis *et al*., 2012)**. In addition, both methanolic extract and powdery form of *P. ostreatus* were found effective in the treatment of streptozotocin induced diabetes in mice **(Mircea *et al*., 2018)**. Likewise, enzyme assisted extraction of mycelia zinc polysaccharides of *P. eryngii* effectively treated hyperlipidemia in mice **(Xu *et al*., 2017)**. Also, the fruiting body of *P. ferulae* induced an increased insulin level in streptozotocin induced diabetic rats **(Wang *et al*., 2014),** likewise pooled fruiting body of *P. ostreatus* and *P. cystidiosus* increased the utilization of glucose in Type 2 diabetic patients **(Jayasuriya *et al*., 2015).**

## HEALTH IMPLICATIONS OF *Pleurotus* SPECIES

*Pleurotus* are exotic and healthy foods, which are low in calories and fat, high in chitin, vitamins, protein and mineral content **(Mattila *et al*., 2002; Akindahunsi and Oyetayo, 2006)**. They also have elevated quantities of ornithine and γ-aminobutyric acid (GABA). GABA is a non-essential amino acid that acts as neurotransmitter while ornithine is one of the building blocks required for producing arginine **(Jayakumar *et al*., 2006)**. Oyster mushrooms have been reported to be moderately high in polysaccharides and other antioxidant compounds (Tab 9).

The multidirectional therapeutic and health-promoting effects of mushrooms of the genus *Pleurotus* result from the occurrence of secondary metabolites, that were extracted from both fruiting bodies and mycelia of oyster mushrooms **(Morris *et al*., 2017).** The bioactive compounds found in *Pleurotus* mushrooms can be distributed into low and high molecular weight compounds. High-molecular weight bioactive components primarily are polysaccharides, including β-glucans, peptides and proteins while low-molecular weight bioactive compounds consist of terpenes, fatty acid esters and polyphenols **(Patel and Goyal, 2012)**. In addition to free radical scavenging activities, oyster mushrooms are nutritional in nature and thus, could improve the general human health **(Khan and Tonia, 2012; Kalac, 2013; Adebayo *et al*., 2014a).**

The diverse health benefits of *Pleurotus* species are associated to their high nutritive value. For instance, **Khatun *et al*. (2015)** studied three *Pleurotus* species and reported that the lowest protein content was found in *P. pulmonarius* (15–18% dw); trailed by *P. citrinopileatus* (20–22% dw) and *P. florida* (22–25% dw). Also, the cholesterol content of the three *Pleurotus* species studied ranged from 0.6 to 0.8% dw, therefore they are low in cholesterol, but rich in protein. In general, mushroom polysaccharides remain the most acknowledged and potent mushroom-derived bioactives. Precisely, natural mushroom polysaccharides, principally obtained from the culture media, fruiting body, and mycelia during mushroom cultivation, have received enormous attentions due to their richnessinbiopharmaceuticals and nutraceutical activities as well as anti-inflammatory, antioxidant, **(Im *et al*., 2014)**, antihypertensive **(Miyazawa *et al*., 2008)**, anti-aging **(Zhang *et al*., 2014)** and immune-boosting properties **(Cui *et al*., 2015; Oloke and Adebayo, 2015)**. Oyster mushrooms are characteristically high in homopolysaccharides as reported by **Lakhanpal and Thakur (2016)**. Free radicals are generated in pathological and/or normal cellular metabolism **(Elmastas *et al*., 2007)**. Nonetheless, the unrestrained generation of oxygen-based free radicals is involved at the inception of some ailments, including rheumatoid arthritis, cancer, atherosclerosis, and cirrhosis, likewise in deteriorating processes connected with ageing **(Gulcin *et al*., 2008)**. Oxidative enzymes, including catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidise (GSHPx)**(Tadhani *et al*., 2007),** or chemicals, includingtocopherol, carotenoids, ascorbic acid and polyphenolic compounds are accountable for protecting organisms against the damages caused by free radicals**(Gulcin *et al*., 2008)**.The enzymatic antioxidant activity of *P.pulmonarius*, *P. florida* and *P. citrinopileatus* were evaluated and it was observed that catalase activities ranged from 1128- 2264 g/dry weight, peroxidase activities ranged from 14.14 – 18.76 $g^{-1}min^{-1}$ and superoxide dismutase (SOD) activities ranged from 203.7- 345.7 g/dry weight**(Khatun *et al*., 2015)**. Moreover, the presence of the diverse types of polysaccharides, alkaloids, flavonoids and other compounds in the oyster mushroom could be accountable for their high scavenging activities against the free radicals. This is believed to confer definitive defence power against the development of some diseases and general enhancement of human fitness. Reactive oxygen species (ROS), free radicals and oxidative stress are produced in the body through endogenous reactions, exposure to some physicochemical conditions and pathophysiological states **(Devasagayam *et al*., 2004)**. These activities can result into changes in DNA, proteins, lipids and other biomolecules, consequently leading to disease development in the central nervous system, liver, heart, lungs, blood, kidneys, reproductive organs, skin and joints, which can later develop to systemic diseases and may spread to other multiple organs **(Hepel and Andreescu, 2015)**. Antioxidants are capable of preventing and opposing the formation of reactive nitrogen and oxygen species (RNS/ROS) and oxidative stress, thereby preventing the damage or change in biomolecules in the body system **(Halliwell, 1996)**. This is referred to as antioxidant defence mechanism, which aids in the maintenance of the overall health of an organism. Apart from the direct prevention of free radicals, *Pleurotus* species and their metabolites have been widely studied for various health benefits. According to **Jayakumar *et al*. (2007)**, *P.ostreatus*extracts were reported to ease the hepatotoxic effect of description

Carbon tetrachloride($CCl_4$) in rats and this notably protected some organs, including the heart, brain, and liver of matured rats against oxidative damage. **Pk *et al*. (2019)** reported an *in vitro* cytotoxic test using MTT assay, that the polysaccharide of *Pleurotus ostreatus* (Jacq.) P. Kumm extracted at $10^{-3}$ to $10^{3}$ µg/ml displayed time and dose-dependent inhibitory results on the mitotic division of EAC cells, with suppression above 90% at the highest concentration and at 72h. Also, **Selegean *et al*. (2009)** stated that both intracellular and extracellular proteins extract of *P. ostreatus* contained polysaccharides that have immunomodulating potentials. Percutaneous or oral administration of extract of *P. eryngii* in mice was reported to repress inflammation in delayed-type hypersensitivity allergic response **(Jedinak *et al*., 2011)**. Moreover, *P. ostreatus* is one of the natural producers of lovastatin (including some higher fungi),

**Table 8** *In vivo* Antioxidant Study of Oyster Mushroom

| Mushroom | Sample | Animal Used | Antioxidant Study | Result of the Study | Reference |
|---|---|---|---|---|---|
| *P. ostreatus* and *P. eryngii* | Fruiting Body and Mycelium | Porcine (*Susscrofa*) Brains | Lipid Peroxidation Inhibition (TBARS Assay) | Although, *P. eryngii* showed lower TBARS assays, both species showed significant improvement of the TBARS. | Reis *et al*. (2012) |
| *P. ostreatus* | Methanol Extract and Powder | Mice with streptozotocin-induced diabetes (50 mg/kg, intraperitoneal) | SOD and Catalase Enzyme Determination | Improved the diabetic condition as detected via the SOD and Catalase enzyme | Mircea *et al*. (2018) |
| *P. eryngii* | Enzymatic-extractable mycelia zinc polysaccharides | Hyperlipidemic mice induced by high-fat-high-cholesterol emulsion (HFHCM). | SOD, GSH-Px, CAT, T-AOC, MDA and LPO | The species showed potential of preventing the HFHCM-induced hyperlipidemia and non-alcoholic fatty liver | Xu *et al*. (2017) |
| *P. ferulae* | Fruiting Body | Streptozotocin-induced diabetic rats | Insulin and HbA1c Levels | Insulin levels increased and HbA1c levels decreased | Wang *et al*. (2015) |
| *P. ostreatus and P. cystidiosus* | Fruiting Body | Type 2 diabetic patients | utilization of glucose by peripheral tissues | Increased utilization of glucose by peripheral tissues, reduced glycogen synthase kinase with improved glycogen synthesis after 2 weeks | Jayasuriya *et al*. (2015) |

**Table 9** Type of Polysaccharides in Oyster Mushroom (% of fresh mushroom)

| *Pleurotus species* | Type of Polysaccharides | References |
|---|---|---|
| *P. eryngii* | Homopolysaccharides | Lakhanpal and Thakur (2016) |
| *P. florida* | Homopolysaccharides | Lakhanpal and Thakur (2016) |
| *P. ostreatus* | Homopolysaccharides | Lakhanpal and Thakur (2016) |
| *P. .sajor-caju* | Lovastatin polysaccharide | Lakhanpal and Rana (2005) |

and it is primarily used for the prevention of cardiovascular diseases and for the treatment of dyslipidemia. Lovastatin, which is used as a drug for reducing the level of blood cholesterol, was created using*P. ostreatus* andappropriated for use in 1987 by the Federal Development Agency **(Talkad *et al*., 2015)**.

In other related studies, α-glucan obtained from *P. ostreatus* and proteins extracted from *P. nebrodensis* have also been reported for anticancer activities **(Wu *et al*., 2011; Lv *et al*., 2009)** while polysaccharides obtained from *P. ostreatus* have also been reported for its antitumor potentials **(Tong *et al*., 2009).** Also, heteroglycan obtained from *P. ostreatus* have been reported for immunomodulatory potentials **(Devi *et al*., 2013). Khan *et al*. (2011)** reported that lovastatin extracted from *P. sajor-caju* and *P. florida* possessed potentials to impact antihypercholesterolemic activities while the antihypertensive potentials of peptides obtained from *P. cornucopiae* have been reported **(Jang *et al*., 2011).** Hypoglycemic potentials of β-glucans from *P. sajor-caju* and polysaccharide-peptide from *P. abalonus* have been reported **(Kanagasabapathy *et al*., 2012; Chen *et al*., 2015)**. Polysaccharides from *P. ostreatoroseus* and *P. ostreatus* were reported to possess anti-inflammatory activities **(Gunawardena *et al*., 2014; Correa *et al*., 2015)**. Also, β-glucans extracted from *P. ostreatus* and*P. sajor-caju*has been reported for anti-arthritic **(Patel *et al*., 2012; Rovensky *et al*., 2011)**.

Furthermore, the antibacterial, antifungal and antiviral potentials of oyster mushrooms have also been aptly reported. The therapeutic potentials of *Pleurotus* have been exploitedfor combating simple and multiple drug-resistantt isolates of *S. aureus* and *E. coli* according to report by **Akyuz *et al*. (2010).** The antibacterial and antifungal activity of *Pleurotus* species were reported to be dependent on the type of the extractant solvent used, for instance, acetone extract were less potent against Gram-negative bacteria compared to ether extract **(Iwalokun *et al*., 2007)**. Acetone and ether extracts of *Pleurotus*

mushrooms effectively inhibited the growth of*S. cerevisiae*,*B. subtilis*, and *E. coli*. β-glucans extracted from *P. ostreatus*was reported to show antibacterial activities **(Karaman *et al*., 2010; Mirunalini *et al*., 2012)**. Also, **Suseem and Saral (2013)** reported that fatty acids esters obtained from *P. eous* displayed antimicrobial activities. Moreover, **Erjavec *et al*. (2012)** reported that proteins extracted from *P. eryngii* and *P. ostreatus* displayed antifungal activities while ergosterol extracted from *P. cystidiosus* also showed antifungal potentials **(Menikpurage *et al*., 2009)**.**Wang *et al*. (2007)** showed that hot water extracts of *P. pulmonarius* and *P. sajor-caju* inhibited the activity of HIV-1 reverse transcriptase by SU2 molecule, with a molecular weight of 4.5 kDa. Also, lectin which was obtained from fresh sporophores of *P. citrinopileatus* reportedly hindered the activities of HIV-1 reverse transcriptase **(Li *et al*., 2008)**. According to a report by **Lv *et al*. (2009)**, proteins obtained from *P. nebrodensis* were observed to possess antiviral activities, while polysaccharides and lectins extracted from *P. abalones* and *P. citrinopileatus* respectively were also reported to possess antiviral potentials **(Wang *et al*., 2011; Hassan *et al*., 2015)**.

## CONCLUSION

The current review has established different species of oyster mushrooms as good sources of natural antioxidant through *in vitro* assays (including DPPH, superoxide, nitric oxide, hydroxyl, ABTS scavenging and Oxygen radical absorbance capacity (ORAC) activities) and *in vivo* assays (enzymatic and non-enzymatic) using mice, rats and porcine brain. The potentials of antioxidants to oppose the formation of reactive oxygen and nitrogen species, prevent oxidative stress and scavenge free radicals which may be responsible for some human diseases have been established. However, future work should be focused on detailed structure-activity study of identified antioxidant molecules obtained from different *Pleurotus* spp. Moreover, the potential of oyster mushrooms as good antioxidants can positively influence their activities as natural immune boosters, anti-ageing and anti-cancer effects which can significantly improve human health. Hence, oyster mushroom, a portion of natural functional food could be recommended as a excellent source of antioxidants.

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