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Mushrooms as a Phenomenal Functional Food – A Comparative Analysis on its Health Benefits and *In-vitro* Antioxidant Capacity

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ABSTRACT
Antioxidants are substances that counteract the deterioration caused by free radical scavengers. This property makes them the most anticipated source that have role in functional foods and pharmaceutical products. One among are mushrooms, the prime source of numerous therapeutic compounds and constitute to substantial antioxidant capacity. To manifest this, four variegated mushrooms namely Agaricus bisporus, Lentinula edodes, Grifola frondosa, Volvariella Volvacea were took and prepared as extracts with methanol and examined their In-vitro antioxidant capacity incorporated with varying scavenging assays. The results from the study indicate that the methanolic extract of Agaricus bisporus mushroom was found to be higher due to tremendous bioactive compounds that comprise lectins, polyphenols when compared to other above mentioned mushroom species. In denouement, mushrooms could might act as a readily available component of natural rich antioxidant food which might strengthen the body's defence against oxidative damage.
Keywords- *Antioxidant capacity, Agaricus bisporus, Functional foods, Free radicals, Grifola frondosa, Lentinula edodes, Volvariella volvacea.*

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INTRODUCTION

Oxidative stress is caused by imbalanced metabolism in the body that lead to many health disorders. The dietary intake of antioxidants and natural antioxidant defense systems may control our oxidative homeostasis. As a result, there is a need for natural, alternative fountainhead of antioxidant foods and the predominant source are mushrooms. [1,2] Typically, in healthy cells during oxidative phosphorylation, NADPH oxidase generates reactive oxygen species (ROS). The initial defensive responses of cells are also mediated by ROS and are engaged in detoxification, apoptosis, and phagocytosis. In recent years, mounting evidence has revealed that excessive ROS and oxygen-derived free radical production may accord to a number of clinical effects and cause a number of ailments such as cancer, atherosclerosis, diabetes, and rheumatoid arthritis. Antioxidants, both synthetic as well as natural, are found to lessen oxidative damage brought on by ROS. However, synthetic antioxidants thought to be the cause of liver damage and carcinogenesis [1]. To shield the human body from free radicals and halt the progression of many chronic diseases, it is crucial to produce natural, nontoxic antioxidants and several mushrooms that are both palatable have been shown to have powerful antioxidant activity [2]. The higher-class fungi's flora family called mushrooms are commonly consumed by humans. There are currently about 14,000 existing varieties of mushrooms, of which 2,200 have been determined to be edible. Of these, roughly 650 species have been extensively researched, grown, and used for medical and health reasons. For instance, there are 850 native mushroom species in India alone, and these species have been studied for their potential applications in the food and drug sectors as well as for their nutritional and ethnomedical benefits. [1] The most economically significant edible mushrooms are the white button mushroom, or *Agaricus bisporus* (*A. bisporus*) which incorporates sky-scraping levels of polyphenols, ergothioneine, vitamins, minerals, and polysaccharides and is regarded as an excellent nutritious food. It is also regarded as an antioxidant rich food as they are the prime source of various nutrients [3]. It is a species of edible Basidiomycota that is widely grown in Europe and North America, making up 35–45% of the total global production of edible mushrooms. [20]. Fruiting bodies of *Agaricus bisporus* have shown to have antibacterial, anti-inflammatory,

antitumor, and immune modulatory activity [16]. Food-grade fungi include the shiitake mushroom (*Lentinus edodes*). It is indigenous to China and Japan and contains lentinan. These are high in macro- and micronutrients, as well as a variety of bio-active substances such as polysaccharides, antioxidants, dietary fibre, and ergosterol. These mushrooms are well-known edible ones with a high nutritional content and are renowned for their therapeutic capabilities in raw or dried forms in Chinese medicine. They are the second most widely consumed mushrooms in the world. Shiitake accelerates vital energy, prevents hunger, treats colds, and outperforms body fluid energy, Later research revealed that the fungus contained a number of significant nutrients. Additionally, modern scientific studies have isolated several chemicals and discovered proof of their health-promoting properties. [3]. A useful, edible and therapeutic fungus called Maitake mushrooms (*Grifola frondosa*) contains a variety of biological properties, such as antioxidant activity, immunological modulation, and hypoglycaemic effects [16]. These mushrooms are supposed to have gained their name from the fact that when people discovered them in the wild and their amazing healing abilities, they danced with joy. This mushroom is a form of adaptogen that helps the body fight against any kind of physical or mental challenge. [5]. These mushrooms typically grow at the bases of Oak, Elm, and Maple trees in the natural environment. Beta-glucans, vitamins B and C, copper, potassium, fibre, minerals, and amino acids are all abundant in maitake mushrooms. Additionally, the mushrooms have zero cholesterol, no fat, low salt, and few calories. [8]. *Volvariella volvacea* contains a variety of biological properties, such as immunological modulation, and hypoglycemic effects. Paddy straw mushrooms, often referred to as warm mushrooms, are able to grow at temperatures that are substantially higher. It was first grown in China in 1822 and is an edible fungus. Under ideal growth circumstances, it is a mushroom that grows quickly. [20]. In contempt of the rising therapeutic impact of antioxidants, the vital need to optimize medicinal importance has sparked us to design the current research on the assessment of antioxidant potential of four different mushrooms namely *Agaricus bisporus, Lentinus edodes, Grifola frondosa* and *Volvariella volvacea* and were subjected to various antioxidant assays namely, 1,1-diphenyl-2-picrylhydrazyl [DPPH], 2,2-azino-bis (ethylbenzothiazoline-6-sulfonic acid) [ABTS], Nitric Oxide, Superoxide anion, Hydroxyl scavenging assays. These assays were incorporated with the methanolic extract of various mushrooms selected and the antioxidant capacity were determined.

MATERIAL AND METHODS

Chemicals

Methanol, glutathione, reduced nicotinamide adenine dinucleotide (NADH), 1,1',3,3'- tetramethoxypropane were acquired from Sigma-Aldrich Chemicals Pvt. Ltd., Bangalore, India. 2,2'-Azinobis (3-ethylbeczothiazoline-6-sulfonic acid) (ABTS), thiobarbituric acid (TBA),2,2-Diphenyl-1-picryl hydrazyl (DPPH), Trichloroacetic acid (TCA), Nitro blue tetrazolium (NBT), phenazinemethosulphate (PMS), Griess reagent and Phosphate buffer saline (PBS), were purchased from Hi- media Laboratories Mumbai, India. All other chemicals and solvents used were of analytical grade.

Methanolic Extraction of Mushrooms

Fresh mushrooms *Agaricus bisporus, Lentinula edodes, Grifola frondosa* and *Volvoriella volvaceae* was purchased from Chidambaram. Then they were swiftly moved to the lab and properly cleaned with distilled water. Mushroom was cut into small pieces and immediately placed for drying at room temperature in order to block tyrosinase activity. After one week drying, the mushrooms were finely grinded and powdered. Here, Methanol was used as a solvent. The powdered *Agaricus bisporus* [Extract 1], *Lentinula edodes* [Extract 2], *Grifola frondosa* [Extract 3] and *Volvoriella volvaceae* [Extract 4] were soaked in methanol separately for overnight. The extract powders and methanol were added in 1:2 ratio. After that, the solution was kept in magnetic stirrer at 540 rpm for 6 hours. Then the stirred extract powder and methanol was then filtered with Whattmann filter paper and the supernatant was discarded and the solution was analysed using several antioxidant tests.

In vitro antioxidants assays

1,1-diphenyl-2-picrylhydrazyl [DPPH] radical scavenging activity

The extreme scavenger behaviour of methanolic extract of *Agaricus bisporus, Lentinula edodes, Grifola frondosa, Volvariella volvaceae* was determined spectrophotometrically in a dark environment against DPPH by the method suggested Brand [10]. 1ml of DPPH (90.25 mM) and equal volume of methanolic extraction of *Agaricus bisporus, Lentinula edodes, Grifola frondosa, Volvoriella volvacea* sample (10, 20, 30, 40, 50 µg/ml) was poured into each test tube to a total of 2.0 ml, and the remaining 3 ml was filled with water. At 517 nm, the blue colour developed was read. The standard used was ergosterol.

2,2-azinobis-(3-ethylbenzothiazoline-6sulfonic acid) (ABTS) assay

The radical scavenging activity of the *Agaricus bisporus, Lentinula edodes, Grifola frondosa Volvoriella volvacea* sample was determined [11]. The improved method for producing ABTS incorporates a reaction between ABTS and potassium persulfate that results in the direct creation of the blue/green ABTS

chromophore. Addition of *Agaricus bisporus, Lentinula edodes, Volvoriella volvacea, Grifola frondosa* sample and other antioxidants compete with ABTS and reduce the production of colour. To create a 7 mM ABTS radical cation solution, 2.5 mM potassium persulfate was added. In a total volume of 3 mL, the reaction mixture included *Agaricus bisporus, Lentinula edodes, Grifola frondosa, Volvoriella volvacea* sample solution at different concentrations (10-50 µg/mL) was mixed with ABTS solution (2.7 mL). Then the reaction mixture was left to sit for 30 minutes and absorbance was recorded using spectrophotometer at 734 nm. Ergosterol was used as reference.

Nitric Oxide Scavenging Activity

Nitric oxide scavenging activity of methanolic extract of *Agaricus bisporus, Lentinula edodes, Grifola frondosa, Volvariella Volvacea* sample were determined using the approach propounded [12]. In brief, 2.0 ml of 10 mM sodium nitroprusside in phosphate buffered saline was combined with various doses of the extract and incubated for 150 minutes at 25° C. 1ml of sulphanilic acid was combined with 0.5 ml of the incubated solution. At 540 nm, the absorbance of the chromophore produced following nitrite diazotization with sulphanilic acid and subsequent coupling with NEDD (Naphthy Ethylene DiamineDihydrochloride) was determined. Ergosterol was used as a standard.

Superoxide Anion Radical Scavenging assay

The superoxide scavenging activity was analyzed based on the method described by [13]. 1ml of the sample solution was combined with 1ml of the Nitro blue tetrazolium solution and 1ml of the Nicotinamide adenine dinucleotide solution. This combination was thoroughly mixed before being added to Phenazine methosulfate and incubated at 30°C for 15 minutes. At 560nm, absorbance was determined spectrophotometrically. Ergosterol was used as standard.

Hydroxyl radical scavenging assay

The hydroxyl radical scavenging activity was determined by [14]. In this assay, hydroxyl radicals are produced by the reduction of H_2O_2. The generation of hydroxyl radical is detected by its ability to degrade deoxyribose to form products, which on heating with Tertiary butyl alcohol forms a pink color chromogen. Addition of Fiestin battle with deoxyribose for hydroxyl radicals and color formation. The following reagents were added in the order stated below. The reaction mixture in a total volume of 1 ml contained 0.1 ml of potassium dihydrogen phosphate, potassium hydroxide buffer, different concentration of methanolic extraction of *Agaricus bisporus, Lentinula edodes, Grifola frondosa, Volvariella Volvacea* (10, 20, 30, 40, 50 µg/ml). 0.1 ml of ergosterol, 0.2 ml of ferric chloride, 0.1 ml of H_2O_2, 0.1 ml of EDTA, and 0.2 ml of 2-deoxy ribose. All reagent contents were mixed thoroughly and incubated at room temperature for 60 min following addition of 1ml of Tertiary butyl alcohol and 1ml of Trichloro acetic acid . Further, all the tubes were kept in a boiling water bath for 30 min. The absorbance was read at 532 nm in spectrophotometer. The efficiency of extract was compared with standard ergosterol. Decreased absorbance of the reaction mixture indicated hydroxyl radical scavenging activity.

Scavenging activity were measured using the following formula -

$$\textbf{Scavenging activity (\%)} = [(A_0 - A_1) / A_0] \times 100$$

Where A_0 is the absorbance of the control, and A_1 is the absorbance of the sample.

RESULTS AND DISCUSSION

1,1-diphenyl-2-picrylhydrazyl [DPPH] assay

DPPH is a relatively stable free radical that is commonly utilised as a substrate in antioxidant assays. This assay determines the ability of extracts from the *Agaricus bisporus, Lentinula edodes, Grifola frondosa Volvariella volvacea* by transferring unpaired electrons to paired ones. The inhibitory concentration 50 [IC50] of ergosterol was 44.04 µg/ml and that of Extract 1 was 48.79µg/ml which was higher when compared to other mushroom extracts.

2,2-azino-bis (ethylbenzothiazoline-6-sulfonic acid) ABTS assay

The ABTS assay works by inhibiting the absorbance of the radical cation ABTS+, which has a distinctive long wavelength absorption spectrum. ABTS, a protonated radical, exhibits a maximum absorbance at 734nm that declines with proton radical scavenging. The result obtained imply the activity of the methonolic extract from *Agaricus bisporus, Lentinula edodes, Grifola frondosa, Volvariella Volvacea* either by inhibiting or scavenging the $ABTS^+$ radicals. The inhibitory concentration 50 [IC50] of ergosterol was 47.7 µg/ml and that of Extract 1 [*Agaricus bisporus*] was 61.07 µg/ml which was higher when compared to other mushroom extracts.

Nitric Oxide Scavenging assay

Endothelial cells, macrophages, and neurons all produce nitric oxide, which is an important chemical mediator. Upon conducting the assay, the results showed that, inhibitory concentration 50 [IC50] of ergosterol was 36.87 µg/ml and that of Extract 1 [Agaricusbisporus] was 42.94 µg/ml which was higher

when compared to other mushroom extracts that included the methanolic extract of *Lentinula edodes*, *Grifola frondosa* and *Volvariella volvacea*.

Superoxide anion scavenging assay

Superoxide anion is a reduced version of molecular oxygen that is formed after receiving one electron. The superoxide scavenging activity of the selected mushrooms highlighted that inhibitory concentration 50 [IC50] of ergosterol was 41.36 µg/ml and that of Extract 1 [*Agaricus bisporus*] was 45.45 µg/ml which was higher when compared to other mushroom extracts.

Hydroxyl radical scavenging assay

The hydroxyl radical is a reactive free radical created in biological systems. The inhibitory concentration 50 [IC50] of ergosterol was 47.67 µg/ml and that of Extract 1 [*Agaricus bisporus*] was 67.51 µg/ml which was higher when compared to other mushroom extracts. Therefore, when comparing the antioxidant activity of all the different mushrooms, it is shown that the extract from *Agaricus bisporus* has a higher antioxidant activity. This may be because of the high nutritional content present, which may serve as a convenient source of naturally high antioxidant food and may help to improve the immune system against oxidative damage [15-20].

In the food and health industries, mushrooms are typically seen as valuable items with a multifariousness of uses. They are nutrient-dense and incorporate range of vitamins and minerals, appending provitamin D2, vitamin B1, B2, B6, B12, and niacin, making them a possible source of nutrition. The minerals and vitamins include potassium, manganese, magnesium, iron, phosphorus and are also rich in several bioactive substances, such as polysaccharides, antioxidants, dietary fiber, and ergosterol, into the bargain to critical macro- and micronutrients [21]. Bioactive substances may be useful in preserving users' health and shielding them from sickness. Because of their appealing culinary and sensory qualities, edible mushrooms are regarded delicacy food components. The practice of growing edible mushrooms is generally widespread due to its obvious benefits of minimal resource requirements and simplicity of cultivation. On the other hand, about 1-3% of all people are allergic to mushrooms, which may be brought on by spores or oral ingestion of allergens. The significant edible white button mushrooms are quite rich in high levels of polyphenols, ergothioneine, vitamins, minerals, and polysaccharides, it is regarded as an excellent nutritious food and our results also put forward that it has higher antioxidant activity due to the same. Hence through this debate, the white button mushrooms have found to have a greater nutritious content and higher antioxidant capacity as they are fat free, low sodium content intake, high calorie supplement and are cholesterol free [17, 6].

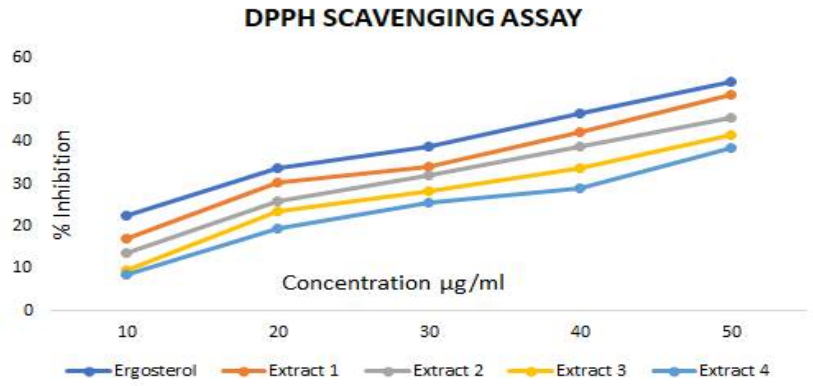

Figure 1- DPPH scavenging assay

Extract 1 - *Agaricus bisporus*, extract 2- *Lentinula edodes*, Extract 3- *Grifola frondosa* and Extract 4- *Volvariella volvacea*

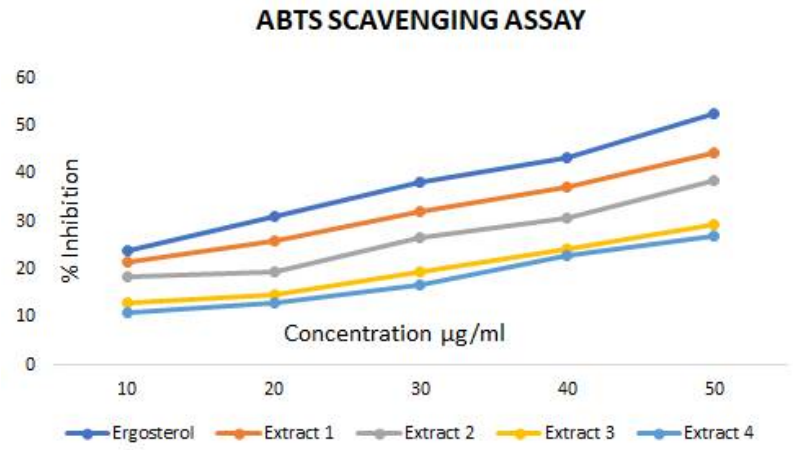

Figure 2 - ABTS scavenging assay

Extract 1 - *Agaricus bisporus*, Extract 2- *Lentinula edodes*, Extract 3- *Grifola frondosa* and Extract 4- *Volvariella volvacea*

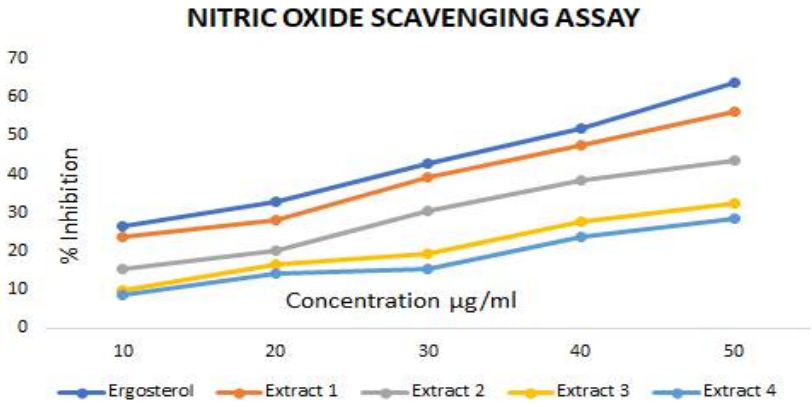

Figure 3 - Nitric oxide scavenging assay

Extract 1 - *Agaricus bisporus*, Extract 2- *Lentinula edodes*, Extract 3- *Grifola frondosa* and Extract 4- *Volvariella volvacea*

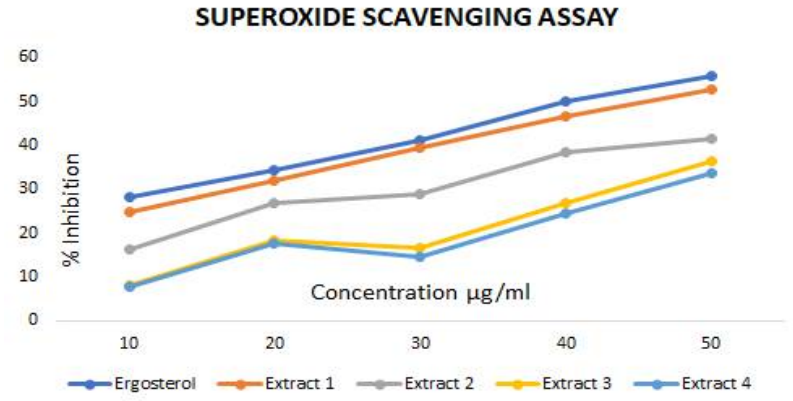

Figure 4- Superoxide anion scavenging assay

Extract 1 - *Agaricus bisporus*, Extract 2- *Lentinula edodes*, Extract 3- *Grifola frondosa* and Extract 4- *Volvariella volvacea*

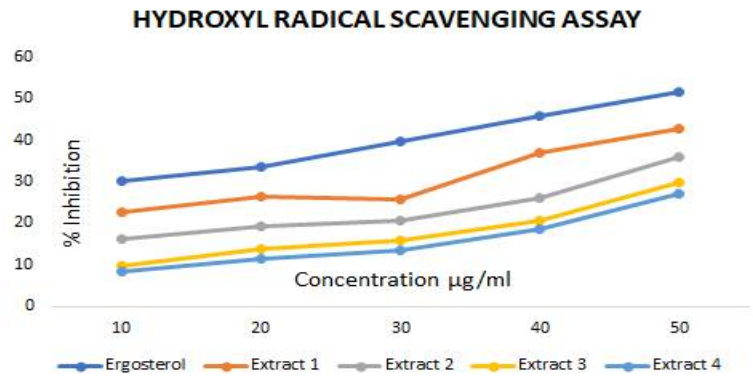

Figure 5 – Hydroxyl radical scavenging assay
Extract 1 - *Agaricus bisporus*, Extract 2- *Lentinula edodes*, Extract 3- *Grifola frondosa* and Extract 4- *Volvariella volvacea*

CONCLUSION

In denouement, mushrooms have tremendous medicinal and mineral values and are a vital contribution to human welfare and have indeed been exploited in herbal medicine for ages and are recognized primarily due to their potent properties. From our results, the antioxidant activity of the mushroom extracts - *Agaricus bisporus, Lentinula edodes, Grifola frondosa, Volvariella Volvacea* were concentration dependent that is with in most cases, greater extract quantities block lipid oxidation more effectively. The results obtained from this antioxidant study strongly suggest that these extracts have significant antioxidant activity, this may be due to **presence of** beta-glucans, ergosterol, ergothioneine, vitamin D and an antioxidant compound usually reported as flavonoids; with variable concentrations based on the species of mushroom, and the highest antioxidant activity was in extract 1 which may be due to the high nutritious content present that could be used as a reluctance source of natural high antioxidant food to boost the immune system's resistance to oxidative damage, or it could be used as a potential supply of therapeutic agent. Even though they are small plants, it treasures major qualities which act as a fundamental premise for many studies in pharmacology, pharmacognosy, microbiology, and biotechnology.

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ETHICS STATEMENT

No animals were used for the study.

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