Mushroom

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Abstract

Mushrooms have been consumed over years as a part of human diet. Due to the therapeutic value of mushroom, it is categorized as a functional food with the property of disease prevention in humans. The presence of biologically active compounds having different medicinal properties provides an opportunity to develop edible mushrooms into functional foods with enhanced nutritional value and numerous health benefits. Mushrooms have cardiovascular, antidiabetic, and immune-modulating properties in order to prevent the risk of cancer and control blood sugar level with substantive antioxidant activity, which are recorded in both wild as well as cultivated species. Various bioactive compounds in mushrooms like phenolics and alkaloid and organic acid contents have the ability to inhibit lipooxidase enzymes, scavenge free radicals, and capture metals and thus contribute to the antioxidant property of mushrooms. Due to the antioxidant properties of the phenolic compounds which are present in mushrooms, there is ample scope for the provision of a lot of health benefits. Flavonoids constitute the major proportion of phenolic compounds present in mushrooms. The bioactive compounds of mushrooms which are responsible for producing various therapeutic effects are commonly obtained from their fruiting bodies. The content and concentration of these bioactives depends upon the cultivation technique, extraction method, and the type of bioactive component.

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14.1 Introduction

Botanical name: *Agaricus bisporus.*

Common name: Mushroom.

Mushroom (*Agaricus bisporus)* is the fleshy spore-bearing body of a fungus, which is generally produced on the soil or its food source. It typically refers to the fruit bodies of members belonging to the order *Agaricales*, whose type genus is *Agaricus*. However, the extended definition of the term "mushroom" can also refer to either the entire fungus when in culture, the thallus (called a mycelium) of species forming the fruiting bodies called mushrooms, or the species itself.

Mushrooms are consumed as a part of diet across the world due to their potential nutritional properties. There is a wide range of compounds which are obtained from mushrooms, with their distinct properties providing different health benefits. This makes mushrooms an interesting field of study and a very useful source of bioactives for the prevention of various diseases (Gao et al. 2004; Mattila et al. 2002). Mushrooms have been consumed since ancient times across different cultures around the world. They are broadly classified into three groups, i.e., edible mushrooms, medicinal mushrooms, and poisonous mushrooms. In case of edible mushrooms, the fruiting body is consumed as such or in any other dried form, e.g., *Agaricus bisporus, Pleurotus ostreatus*, etc. Medicinal mushrooms such as *G. lucidum* are not essentially used directly as diet due to their undesirable organoleptic characteristics, but are consumed in the form of extracts or concentrates. Poisonous mushrooms are inedible due to the presence of certain poisonous substances in them, e.g., *Amanita phalloides* (Cheung 2010).The common mushroom cultivated worldwide is *Agaricus bisporus* followed by *Pleurotus ostreatus* and *Flammulina velutipes.*

Various studies have been carried out to explore the use of mushrooms for nutritional as well as therapeutic purposes. The different proposed effects on health include antitumor, immunomodulating, antioxidant, radical scavenging, cardiovascular, antihypercholesterolemia, antiviral, antibacterial, detoxification, hepatoprotective, and antidiabetic effects (Wasser 2010). The fundamental approach to study the different health benefits of mushrooms on the human body involves the isolation, characterization, and administration of pure bioactive compounds from the extracts of mushrooms. The different bioactives can also produce a synergic effect, thereby providing promising therapeutic effects. The use of different bioactives in combination has been shown to produce better health effects due to their concerted effect (Borchers et al. 2004; Vickers 2002).

The bioactive compounds of mushrooms which are responsible for producing various therapeutic effects are commonly obtained from their fruiting bodies. The content and concentration of these bioactives depends upon the cultivation technique, extraction method, and the type of bioactive component (Barros et al. 2007b; Mattila et al. 2001). The different bioactive compounds present in the mushrooms include polysaccharides, glycoproteins, proteins, lipids, and secondary metabolites (Lull et al. 2005).

Polysaccharides are the most potential bioactives present in mushrooms, which are usually the components of the cell wall, such as β-glucans, trehalose, chitin, etc. (Sánchez 2017). They have been demonstrated to provide health benefits such as anticancer, antioxidant characteristics, neuroprotection, and immunomodulation (Valverde et al. 2015). The bioactive proteins are essential functional components of mushrooms which possess ample applications in the development of pharmaceutical components. These include lectins, immunomodulatory proteins, ribonucleases, ribosome-inactivating enzymes, etc. (Xu et al. 2011). These proteins have a wide range of biological activities by possessing antitumor characteristics and immunomodulatory activities (Zhang et al. 2010). Mushrooms are generally low in their fat content, mostly dominated by unsaturated fatty acids such as palmitic acid, oleic acid, and linoleic acid (Carneiro et al. 2013). The secondary metabolites present in mushrooms include phenolic compounds, organic acids, terpenes, steroids, terpenoids, alkaloids, lactones, and vitamins, which offer a lot of health benefits (Sánchez 2017). The different terpenes found in mushrooms are shown to possess an inhibitory effect on cholesterol synthesis (Komoda et al. 1989) and angiotensin-converting enzymes (Morigiwa et al. 1986). The phenolic compounds in mushrooms provide a lot of health benefits by possessing antioxidant properties. They produce anti-inflammatory, antimicrobial, antithrombotic, cardioprotective, and vasodilator effects (Ferreira et al. 2009; Heleno et al. 2012).

14.1.1 History

Mushrooms have been used in the world since time immemorial not only because of their aromatic and nutritional properties but also for their medicinal values. Presently, they are well known for culinary properties due to the presence of high-quality proteins, vitamins, fibers, and other medicinal properties due to which they are called nutraceutical (Benzie and Strain 1996). The consumption of mushrooms and their medical application are deep rooted in Asian countries and their modern societies. However, they have been neglected for centuries in many European countries. The cultivation of mushrooms started with artificial inoculation of twigs with Auricularia auricular- judae in China 600 years AD. Also, Shiitake in China was first cultivated on logs around 1000 to 1100 AD (Lull et al. 2005). In Europe, around 300 years ago, mushroom cultivation came up in Paris, when gardeners first grew button mushrooms (*Agaricus bisporus*) on beds fertilized with dung (Zhishen et al. 1999). In different countries for different people, the word “mushroom” means different things. Mushrooms, called as the “Food of Gods,” have been liked by humans since ancient times. They provided strength for warriors in battles. Traditionally, mushrooms were harvested wild and were difficult to domesticate and cultivate (Mattila et al. 2002). Collection of edible mushrooms from wild lands is still common in southern Asia and developing countries. The cultivation of some species of mushrooms has a long history; they were grown on a small scale and only in particular areas and seasons. The consumption of mushrooms in the early days of civilization was due to their palatability and unique flavor. The consumption of mushrooms in the early days has been reviewed by various workers (Buller 1915; Rolfe and Rolfe 1925). The current use of mushrooms is different from traditional use as research on chemical composition has been done to a great extent, which showed that mushrooms can be utilized as a diet to battle various diseases in humans.

14.1.2 Production in India and the World

Mushroom production is increasing very fast throughout the world due to the greater awareness of its nutritional and medicinal value. Besides its unique flavor and texture, the consumption of this fancied item is also a natural corollary to the economic development of a country (Carneiro et al. 2013). The production of mushrooms in the world is estimated at about 12 million tons with more than an 8% annual growth rate. However, India, being a late starter, is rapidly catching up; the present production is at the lakh ton mark and the annual growth is still above 15% (Cheung et al. 2003). Currently, the highest production of mushrooms is from Punjab followed by Haryana and Maharashtra. The task is not confined to the seasonal growing in the northern area but it has spread widely in the country. Apart from seasonal farmers, various large environmentally controlled units have emerged as export-oriented units. The country has the largest mushroom unit of the world, which produces 200 tons of button mushrooms per day and its export estimate is about 25% of the US imports (Jeong et al. 2010). The production of edible mushrooms in the world has increased more than 30-fold from 1978 (1 billion kg) to 2013 (34 billion kg). This is a remarkable achievement considering the population of the world has increased only 1.7-fold from 1978 (4.2 billion) to 2013 (7.1 billion). Therefore, the mushroom per capita consumption has increased at a faster rate, mainly from 1997, which now exceeds 4.7 kg annually. In 2013, almost all consumption of mushrooms in China, the European Union, and India was supplied from domestic sources and consumption in the US, Japan, Canada, and Australia was supplied mainly by domestic sources but by significant amounts of imports. China is the largest producer of mushrooms. More than 30 billion kg of edible mushrooms were produced in China in 2013, which accounts for about 87% of the total production. The production of mushroom increases continuously, with China being the largest producer in the world. However, wild mushrooms have been found to be more important for their nutritional and sensory characteristics (Moro et al. 2012).

14.1.3 Botanical Description of Mushrooms

Mushrooms are a diversified group of macro fungi belonging to Ascomycetes and Basidiomycetes with a cell cycle including the formation of sexual spores (Wang et al. 2013). The spores are located in a special structure called the ascus (Ascomycetes) or the basidium (Basidiomycetes).

These mushrooms grow below the soil surface at a depth of 10–20 cm (hypogenous macro fungi or truffles) or grow above the soil surface (epigeous macro fungi) in an umbrella-like structure, which include basidiospores (Zhang et al. 2015). The former of these belong to ascomycetes and grow with a host plant as ectomycorrhizal mushrooms (EMM) in a symbiotic relationship. The members bear thin, bladelike gills on the undersurface of the cap from which spores are shed. The sporophore consists of a pileus and a stalk. The sporophore emerges out from an underground network of threadlike strands called as mycelium. The fruiting bodies of mushrooms occur in rings called fairy rings. The falling of spores in a favorable spot leads to the formation of mycelium and producing hyphae that grows in all directions, forming a circular mat of hyphal threads. Fruiting bodies, produced at the edge of this circular mat, may widen the ring for hundreds of years (Kozarski et al. 2015a, 2015b).

Edible mushrooms have been divided into five categories according to the derivation of their names, viz., those named according to the taste, morphology, growth habit, texture, and habitat (Oso 1975). Examples in each category are taste *(Volvariella volvacea, Volvariella esculenta)Termitomyces clypeatus*; morphology (*Termitomyces manniformis*) *Termitomyces robustus*, *Schizophyllum commue*, *Agrocyber broadway*; growth habit (*Termitomyces globulus*, *Pleurotus tuber-reguim*); texture *Pleurotus squarrousulus*, *Psathyrella atroumbonata*; habitat *(Francolinus bicalcaratus*). Furthermore, the natives have observed the growth of many fungi on different kinds of dead wood and have named each fungus after the wood on which it grows (Mattila et al. 2002).

14.2 Antioxidant Properties of Mushrooms

Mushrooms are nutritionally considered as functional foods that act as an integral part of our diet for years. They are recognized for their potential therapeutic properties, which are effective to treat and prevent varieties of disorders (Lim et al. 2007; Moro et al. 2012; Silveira et al. 2014). The common mushroom species produced in a suitable ecological environment are *Hericium erinaceus* (Lion's head or pompom), *Auricularia auricula-judae* (ear),*Grifola frondosa* (maitake), *Agaricus* spp., *Lentinula edodes* (shiitake), *Pleurotus* spp. (oyster), *Volvariella volvacea* (straw) *Ganoderma lucidum* (lingzhi), *Flammulina velutipes*, *Tremella fuciformis*, *Pholiota nameko*, *Lepista nuda* (blewit), and *Coprinus comatus*(shaggy mane). Those of highest financial value are usually fashioned under artificial circumstances, i.e., on a well distinct substrate and under full climatization. These are mostly *Agaricus bisporus* (button mushroom) *Flammulina velutipes, Lentinula edodes*, *Pleurotus* spp. Mushroom manufacturing is increasing continuously, with China being the biggest manufacturer around the world. Fruiting bodies of fungi (mushrooms) are valued for their chemical and nutritional properties apart from their texture and flavor. Mushrooms belonging to different taxa having diverse ecological niches show various degrees of antioxidant potentials. Insufficient nutrition due to current lifestyle and raise of average stability are the two main reasons for the growing incidence of disease all over the world. Imbalanced metabolism and an excess of reactive oxygen species (ROS) causing oxidative stress result in various disorders such as heart disease, metabolic disease, severe neural disorders such as Parkinson's and Alzheimer's, some cancers, and premature aging. However, wild edible mushrooms have high carbohydrate, protein, water, fiber content, rich source of trace elements, and low in fat/energy levels, thus making them an admirable source for making low caloric foods. Some species of edible mushroom species are highly nutritious and may evaluate satisfactorily with eggs, milk, and meat. Amino acid compositions of mushrooms are equivalent to animal proteins, which is predominantly imperative considering the cost of those proteins and the occurrence of diseases caused due to animal meat. These natural products act as a good resource for the development of therapeutic compounds with anti-inflammatory properties without any toxic effects (Yuan et al. 2006). Some bioactive compounds has been isolated from plants (Wang et al. 2013), rhizomes (Debnath et al. 2013), and marine algae (Kim et al. 2014) for their anti-inflammatory effects, studied by various mechanisms. Mushrooms (fruiting bodies, mycelia, or their submerged fermentation broth) are a rich source of bioactive compounds whether wild, edible, or cultivated species (Alves et al. 2013) and these bioactive metabolites comprise of phenolic compounds, polysaccharides, terpenoids, lectins, glycoproteins steroids, and several lipid components (Reis et al. 2012). Anti-inflammatory properties are related with a fall in the production of nitric oxide (NO) and other inflammatory mediators such as tumor necrosis factor (TNF-a), interleukins (IL 1b, IL-6, IL-8), and prostaglandin E2 (PGE2), which results in the reduction of inflammation (Choi et al. 2014). Mushrooms have cardiovascular, antidiabetic, and immune-modulating properties in order to prevent the risk of cancer and controlling blood sugar level with substantive antioxidant activity, which are recorded in both wild and cultivated species. Various bioactive compounds in mushrooms such as phenolics, alkaloids, and organic acid contents contribute to the antioxidant activity and free radical scavenging properties with their ability to inhibit lipooxidase scavenge free radicals and capture metals. Wild varieties of mushrooms are consumed by local and tribal communities and impact the health and nutrition as they contain significant amounts of flavonoids, protein, lycopene, β-carotene, etc., which are usually missing in a poor man's diet. The main bioactive compound found in mushroom is polysaccharide, which has an important antitumor role. Extraction of bioactive compounds mainly polysaccharides from Pleurotusostreatus reported high antitumor activity against the HeL tumor cell line (Tong et al. 2009). Crude polysaccharides from fruit bodies of Lentinus polychrous species of mushrooms have been reported to have about 45% of cytotoxicity against the human breast adenocarcinoma cell (Thetsrimuang et al. 2011). In recent times, it was discovered that methanol and/or water extracts from edible mushrooms contain important antioxidant activities (Mau et al. 2005; Ferreira et al. 2007; Ramırez-Anguiano et al. 2007; Gursoy et al. 2009; Vaz et al. 2011). Further investigation revealed that phenols contain the main antioxidant activity present in crude extracts of mushrooms (Barros et al. 2007a; Mau et al. 2005; Ramırez-Anguiano et al. 2007). Mushrooms containing phenolic compounds exhibit a wide range of biological effects such as anti-inflammatory, antibacterial, antiallergic, antithrombotic, hepatoprotective, anticarcinogenic, antiviral, and vasodilatory actions; these biological functions have been accredited to their antioxidant activity and free radical scavenging. Flavonoids are an extensively distributed group of plant phenolic and have been reported to be highly effective scavengers for most types of oxidizing molecules. However, singlet oxygen and various free radicals are possibly involved in tumor promotion and DNA damage. Various health benefits of tocopherol as a bioactive compound are well renowned. R-Tocopherol is the primary form of vitamin E; it is a lipid-soluble antioxidant, functions as a chain breaking antioxidant for lipid peroxidation (LP) in cell membranes, and also acts as a scavenger of ROS such as singlet oxygen. It acts as the first line of defense against LP; it also protects polyunsaturated fatty acids (PUFAs) in cell membranes from free radical attack through its scavenging activity in biomembranes at early stages of LP. The antioxidant properties of carotenoids exhibit various health benefits; it is inversely related with cancer risk in epidemiologic studies and promising results are reported in laboratory assays. Lycopene helps in the prevention of chronic diseases, which has been reported in epidemiological studies as well as in tissue culture experiments by using human cancer cell lines, human clinical trials, and animal studies. Oxidation is very necessary for every living organism for fabrication of energy to carry out various biological processes. However, on the other hand, the uncontrolled production of oxygen results in formation of free radicals, which form the bases of many diseases such as rheumatoid arthritis, cancer, and atherosclerosis as well as in degenerative processes linked with aging (Turkoglu et al. 2006). Organisms are protected against free radical damage by various enzymes such as catalase and superoxide dismutase or compounds such as tocopherols, ascorbic acid, and glutathione (Mau et al. 2002). Reactive oxygen species are not generated internally only in an organism, but is also caused through various external sources such as ionizing radiation, ultraviolet light chemotherapeutics, environmental toxins, and inflammatory cytokines. Inhalation of various toxic chemicals from the environment has become inevitable in modern civilization. Dietary intake is another very imperative source of antioxidants and may add to oxidative homeostasis apart from endogenous antioxidant defense mechanisms of an organism. Antioxidant-containing foods and antioxidant supplements may be used to protect food quality and reduce oxidative damage by preventing oxidative deterioration. In food packing and production, antioxidants are expansively used in anti-aging, health care, and cosmetics. The growing penchant for cosmetics and healthy food, health and wellness foodstuff is influencing the growth of the antioxidant markets. With increasing demand for cosmetics and nutrition, products obtained from natural sources are also pouring into the natural antioxidant market. Population growth and the rising healthcare spending levels have led to a reliable increase in the demand for antioxidant products. The antioxidant market by product type is categorized into natural antioxidants and synthetic antioxidants. Natural antioxidants are segmented into fungal extracts and plant spices (marjoram, oregano, sage, rosemary, thyme, clove, cinnamon, basil, pepper, and nutmeg), glutathione, zinc (Zn), selenium (Se), flavonoids, ubiquinol (fully reduced form of coenzyme Q10), vitamin A (including carotenoids), vitamin E (including tocopherols and tocotrienols), and vitamin C. Synthetic phenolic antioxidants comprise of butylated hydroxytoluene (BHT) butylated hydroxyanisole (BHA), and others, e.g., *tert*-butylhydroquinone (TBHQ), propyl gallate, and ethoxyquin (EQ), which all successfully inhibit oxidation. Synthetic antioxidants, as compared to the natural ones may lead to the production of toxic substances under certain circumstances. However, BHA, which is very often used as an additive in food commerce, can have negative effects on the guideline of the activity of nitrogen-activated protein kinase (MAPK), depending on the dosage. Various synthetic antioxidants are certified for use as feed additives in the European Union. According to Bhattacharyya et al. 2014, various factors are responsible for the generation of reactive oxygen species.

The nutritive value of mushrooms has suddenly become known and documented not only by mushroom farmers and researchers but also by the general consumers. Moreover, along with their good flavor, mushrooms have constructive chemical composition with high amounts of functional low total fat level, proteins, and a high proportion of polyunsaturated fatty acids (PUFAs), which helps to make low-calorie diets. Edible mushrooms provide a nutritionally significant content of vitamins (B_1, B_2, B_{12}, C, D, and E). Mushrooms have high contents of mannitol and low glycemic index, which is especially beneficial for diabetics. Mushrooms have very low content of sodium (Na) concentration, which is valuable for hypertensive patients and a high concentration of phosphorus (P) and potassium (K), which is an imperative orthomolecular feature. In Asian countries, mushrooms are used as a vital source of home remedies against various diseases caused due to oxidative stress. There is no significant difference between medicinal mushrooms and edible mushrooms because many of the edible species have therapeutic properties. Apart from antioxidant properties, mushrooms have received substantial consideration for their biological activities, such as antidiabetic, hypolipidemic, hepatoprotective, immune-stimulant antitumor, antiviral, anticomplementary, anticoagulant, and immunological activities, which made them suitable for use in cosmetics, agriculture, food, biomedicine, and wastewater environmental management. Numerous varieties of edible wild mushroom species, growing in various ecological surroundings, are known.

14.2.1 Polyphenols Including Flavonoids

Polyphenols comprise of most copious group of antioxidants in the diet. Various effects of dietary polyphenols on human health have developed considerably in the past 20 years. One of the major difficulties of elucidating the health effects of polyphenols is the huge amount of phenolic compounds present in food comprising of different biological properties. These components may be categorized into different groups on the basis of their phenolic rings and the structural elements which bind these rings to each other. Thus, a difference is made between the stilbenes, phenolic acids, flavonoids, and lignans. Most of the polyphenols present in food are in the form of polymers esters or glycosides. These substances are not able to absorb in their native form, so they must be hydrolyzed by the colonic microflora before absorption or intestinal enzymes. During absorption, polyphenols are conjugated and this system mostly includes glucuronidation methylation and sulfation conjugated derivatives of polyphenols are expansively bound to albumin. However, no conjugated metabolites are usually present in the blood or may be present in low concentrations. Major differences in bioavailability of polyphenols are now well-established facts and the influence of structural factors is better understood.

14.2.2 Regulation of Antioxidant Systems

Due to oxidative stress, an organism develops responses in order to prevent or neutralize negative ROS effects. These responses are mainly based on up-regulation of enzymes and antioxidants. Their mechanisms include transduction of signals through specific pathways' ROS sensing and up-regulation of target genes in order to boost the level of their products. Humans comprise of a complicated system of adaptive responses to ROS exposure, having several components. Due to low-intensity oxidative stress, the Kelch-like ECH-associated protein 1/NF-E2-related factor 2 (Keap1/Nrf2) system up-regulates genes encoding antioxidant enzymes. It is acknowledged to be activated by minute amounts of ROS. Transitional intensity oxidative stress up-regulates antioxidant enzymes and induces soreness proteins and heat shock proteins via the MAPK heat shock factor and NF-κB, activator protein-1 *(*AP1), (heat shock transcription factor, HSF). Due to high intensity oxidative stress, perturbations in mitochondrial permeability transition pores (MPTP) may take place. The oxidative stress also leads to the destruction of electron transporters and finally activates the apoptosis cascade (Lushchak 2014). Up-regulation of antioxidant systems raises their potential to abolish ROS, creating in this way auto-regulated negative feedback control loops.

14.3 Characterization of the Chemical Compounds Responsible for Antioxidant Properties and the Pathways Involved in the Biological Activities

The antioxidant activity of mushrooms is due to the presence of bioactive components found in both cultivated as well as wild varieties. The antioxidant activity of some members of *Agaricus* species is found to be higher than gallic acid and ascorbic acid. The type of bioactive component present in mushrooms depends on factors such as species, stage of development, and habitat (Pinto et al. 2013). These components can act as free radical scavengers at different stages of oxidation process as well as provide various health benefits to humans such as

Table 14.1 Bioactive compounds present in mushrooms

Bioactive component	Subclasses	Examples present in mushrooms	References
Phenolic compounds	Phenolic acids	*Hydroxybenzoic acids* such as p-hydroxyl benzoic acid, gallic acid, gentisic acid, homogentisic acid, syringic acid, vanillic acid, veratric acid, protocatechuic acid, and vanillic acid. *Hydroxycinnamic acids* such as p-Coumaric acid, o-coumaric acid, caffeic acid, ferulic acid, sinapic acid, and quinic acid.	Ferreira et al. (2009)
	Flavonoids	Myricetin, catechin, hesperetin, naringenin, naringin, formometin, biochanin, pyrogallol, resveratrol, quercetin, rutin, kaempferol	
Organic acids		Oxalic acid, malic acid, citric acid, and fumaric acid.	Stojkovi'c et al. (2014)
Poly Saccharides		β glycans	Batbayar et al. (2012)
Carotenoids		B-carotene, lutein, and lycopene	Kozarski et al. (2015a, 2015b)
Vitamins	Ascorbic acid (vitamin C)		
	Tocopherols	α, β, γ, and δ tocopherols	Ferreira et al. (2009)

prevention of heart attack and reduction in glucose and cholesterol levels in blood (Jeong et al. 2010).The bioactive compounds present in mushroom are shown in Table 14.1.

Before the process of characterization of antioxidant compounds in mushroom, steps such as sample preparation and extraction are necessary to be carried out. The steps involved in sample characterization are described below:

Preparation of the Sample The fruiting bodies of mushrooms after procurement are cleaned and washed usually by distilled water to remove the residual soil and dirt. The sample is then cut into pieces, dried, and ground before extraction. Drying of the sample can be done by a conventional oven at 40˚C for about 12 hours. Nowadays, freeze-drying is carried out for better retention of antioxidant properties of mushrooms. The ground samples are packed in plastic bags or hermatically sealed in airtight containers and stored at freezing temperature before extraction (Gan et al. 2013).

Extraction It is considered as the most important step for characterization of antioxidant compounds in any sample. For extraction of antioxidants from mushrooms, 5 grams of the ground sample is taken in a conical flask and mixed with about 200–250 ml of solvent (ethanol, methanol, distilled water, or acetone). The mixture is stirred or agitated between 160 and 200 rpm for 1 hour at ambient temperature. After the agitation period is over, the mixture is centrifuged and then

Table 14.2 Antioxidant characterization techniques used in the study

Chemical techniques/assays	Biochemical techniques/assays	Electrochemical techniques/assays
(i) Measurement of bioactive components (ii) Measurement of DPPH scavenging activity (iii) Measurement of reducing power	(i) Measurement of inhibitory effect on bleaching of β-carotene (ii) Measurement of inhibitory effect on hemolysis of erythrocytes (iii) Measurement of inhibitory effect on peroxidation of lipids	(i) cyclic voltammetry (ii) differential pulse voltammetry

filtered to remove the residual material from the extract. The extract is concentrated to dryness in a rotary vacuum evaporator at 40°C and reduced pressure to remove the solvent without affecting the antioxidant property of the extract. The dried extract is stored in the dark and then used for analysis (Tibuhwa 2014).

Characterization It has been studied that mushrooms are an important source of components with antioxidant activity. Barros et al. (2008) used a simple classification of techniques used for characterization of antioxidant activity of mushrooms. The antioxidant characterization techniques used in the study are shown in Table 14.2.

According to various studies, the most commonly used analytical methods for characterization of antioxidant components in mushroom are discussed below:

Estimation of Bioactive Components It involves estimation of total phenols, flavonoids, tocopherols, carotenoids, etc.

14.3.1 Total Phenolic Content (TPC)

Total phenolic content in mushrooms is determined according to a method given by Xu and Chang 2007. This method involves spectrophotometric determination of TPC by the Folin–Ciocalteau method using gallic acid as a standard. A standard curve is prepared between absorbance and gallic acid concentration values. Folin–Ciocalteau's Reagent (FCR) is diluted with water (about 10 times). The required quantities of sample extract, FCR (diluted form), and sodium carbonated (known concentration) are added to a test tube. The mixture is allowed to stand for about 2 hours in dark conditions and then the absorbance is taken at 765 nm. TPC values are expressed as mg GAE/gram of extract.

Gan et al. (2013) studied that different solvents show different extraction yields of phenols. It was shown that water resulted in higher extraction of phenols than aqueous ethanol and total phenolic content of Brazilian mushroom was found to be higher than button mushroom. According to a study conducted on few varieties of mushroom, the TPC of these varieties ranged from 5.39 to 9.24 mg GAE/gram of sample extract with minimum values present in *C. cornucopioides* and maximum value present in *C. ventricosum*.

14.3.2 Total Flavonoid Content (TFC)

Total flavonoid content of mushrooms is also determined spectrophotometrically using quercetin or catechin as a standard. According to a method given by Jagadish et al. (2009) used for studying the effect of boiling on antioxidant and other preventive properties of *Agaricus bisphorus*, the sample extract is mixed with a known amount of distilled water (deionized) and sodium nitrate solution (known concentration). The mixture is allowed to stand at room temperature for 5 minutes followed by addition of a required quantity of aluminum trichloride solution (known concentration). The mixture is again allowed to stand at room temperature for about 6 minutes followed by addition of a few milliliters of sodium hydroxide solution (1 M). Absorbance of the mixture is recorded at 510 nm. Total flavonoid content values are taken as mg CAE/gram or catechin equivalents per gram of extract from the sample. TFC values of few varieties vary from 1.69 to 3.47 mg/g, with minimum values present in *S. rugoso-annulata* and maximum values present in *L. amethystea*. Various studies revealed that the stage of development of mushrooms also affects the flavonoid content and higher values of TFC are present in those stages of mushrooms in which the spores are not fully developed.

14.3.3 Carotenoids

The two main Carotenoids present in mushrooms are Lycopene and β-Carotene. These Carotenoids are determined mainly by a spectrophotometer. Since Carotenoids are fat-soluble pigments, acetone and hexane are mostly used as extraction medium. The extract obtained is filtered through whatman filter paper no.4 and absorbance is recorded at 453, 505, and 663 nm. Lycopene and β-Carotene are estimated using the formulae given below:

$$\text{Lycopene} = -0.0458 \times A_{663} + 0.372 \times A_{505} - 0.0806 \times A_{453}$$

$$\beta - \text{Carotene} = 0.216 \times A_{663} - 0.304 \times A_{505} + 0.452 \times A_{453}$$

The values of Lycopene and β-Carotene are taken in mg/100 ml of extract obtained from the sample. These values are then expressed in μg/gram of extract. Barros et al. (2008) used different methods for determination of antioxidant potential of five varieties of mushroom such as *Agaricus bisporus, Agaricus arvensis, Agaricus romagnesii, Agaricus sivaticus, and Agaricus silvicola*. All the types of mushroom were procured from Portugal. After determination of Carotenoids, it was observed that Lycopene content in these types ranged from 0.38 to 4.70 μg/gram and β-Carotene content varied from 1.32 to 8.52 μg/gram. The order of Lycopene and β-Carotene content in the types of mushrooms taken for evaluation was *Agaricus romagnesii* > *Agaricus bisporus* > *Agaricus silvicola* > *Agaricus sivaticus* > *Agaricus arvensis*.

The individual bioactive components in mushroom can also be identified and then quantified by high-performance liquid chromatography (HPLC). The HPLC system consists of a pump that pumps the sample through a column. The column consists of a stationary and a mobile phase. The system is provided with a detection device used to record the output. The result is obtained in the form of a chromatogram showing different peaks for different components. The system gives the phenolic profile of the sample based on the retention time of the individual components through the column. At a particular wavelength, the retention time of a particular component and absorption is compared with a standard used, which leads to identification and quantification of components present in a sample (Kim et al. 2008).

14.3.4 DPPH Radical Scavenging Activity

Diphenyl picryl hydrazyl (DPPH) radical is a free radical used to determine the scavenging activity of antioxidants present in a particular sample. According to a method used by Liu et al. (2012) for determination of antioxidant activity of five different varieties of mushroom procured from china, about 6×10^{-5}Molar solution of DPPH is prepared in methanol. About 0.1 ml of extract obtained from mushroom is mixed with 3.9 ml of DPPH solution. The mixture after shaking vigorously is allowed to stand for about an hour in dark conditions. Absorbance of the mixture is taken after an hour in a spectrophotometer at 517 nm using DPPH solution without the extract as blank. Lower values of absorbance indicate higher radical scavenging activity. The formula for determination of scavenging activity is:

$$\text{Radical scavenging activity }(\%) = [(A_{\text{DPPH}} - A_{\text{SAMPLE}})/A_{\text{DPPH}}] \times 100$$

A_{DPPH} represents absorbance of DPPH solution without the extract and A_{SAMPLE} represents absorbance of the extract mixed with DPPH solution.

The EC_{50} value represents the extract concentration showing 50% radical scavenging activity. It is obtained from a graph plotted between different extract concentrations and radical scavenging activity. Butylated hydroxyl anisole and ascorbic acid are the most used controls for determination of EC_{50} values.

14.3.5 Ferric Reducing Antioxidant Power (FRAP)

For FRAP assay of extract from the mushroom sample, it is necessary to prepare a "FRAP reagent" by combining a required quantity of solutions of TPTZ [2,4,6-Tri (2-pyridyl)-s-Triazine], acetate buffer, and $FeCl_3 \times H_2O$ of known concentrations. The FRAP reagent (1.8 ml) is added to a test tube and heated at 30°C for 10 minutes. After the period of 10 minutes, 100 μl each of the sample extract and distilled water are mixed with FRAP reagent present in the test tube and the mixture is heated for half an hour at nearly ambient temperature (30°C). Absorbance of the FRAP reagent after heating and absorbance of the reagent containing the extract and distilled water after heating is taken at 593 nm (Benzie and Strain 1996). The FRAP value is calculated using the formula:

$$\begin{aligned}\text{FRAP value} = {} & \text{Absorbance of (FRAP reagent} + \text{Extract)} \\ & - \text{Absorbance of (FRAP reagent)}\end{aligned}$$

FRAP value obtained in the formula above is determined as Fe^{2+} E (micromoles)/gram of mushroom extract.

A higher FRAP value represents higher antioxidant activity of the sample. According to a study conducted by Muhammad et al. (2019) on evaluation of antioxidant capacity (both primary and secondary) of a variety of mushrooms obtained from three different parts of Penang island in Malaysia, button mushroom showed the highest reducing activity(84.60%) among all the varieties taken for evaluation and the order of the reducing activity in percentage was:

$$\begin{aligned}&\text{Button mushroom}(84.60\%) > \text{Kukur mushroom } (24.42\%) > \\ &\text{Maitake mushroom } (39.46\%) > \text{Oyster mushroom } (24.42\%) > \\ &\text{White mushroom } (17.06\%) > \text{Abalone mushroom } (16.35) > \\ &\text{Bunapi mushroom } (15.95) > \text{Bunashimeji mushroom } (14.99) > \\ &\text{Enoki mushroom } (12.86\%).\end{aligned}$$

14.3.6 Β-Carotene Bleaching Effect

This assay is carried out to check the ability of the extract to prevent β-Carotene from bleaching, which is predominant under conditions favorable for oxidation of plant pigments. Bleaching of β-Carotene is prevented by the presence of antioxidants in the extract. For this, an assay given by Barros et al. (2007c) is used with slight modifications, in which chloroform is used to prepare the solution of β-Carotene. An aliquot of a few milliliters is taken from the solution and is subjected to removal of solvent (chloroform). After removal of chloroform, it is added with linoleic acid, tween 80, and distilled water. Tween 80 acts as an emulsifier. The mixture is shaken well and transferred to test tubes with the sample extract at different concentrations and is heated for a required period of 2 hours at a proper temperature. Absorbance is measured at a wavelength of 470 nm after every 20 minutes till there is change in color of the mixture. A blank used for this assay contains all the components except β-carotene. Inhibition of β-carotene is evaluated in percentage using the formula: (Amount of β-carotene present after 2 h/Initial β-carotene content) × 100.

Cheung et al. (2003) evaluated the antioxidant activity of edible mushrooms using the β-carotene bleaching method. Two solvents, methanol and distilled water, were used to prepare the extracts. The mushroom varieties taken for evaluation were *Lentinusedodes* and *Volvariella volvacea.* The results showed that a sample concentration of 2 mg/ml does not possess antioxidant activity in any mushroom variety. On increasing the extract concentration from 2 mg/ml to 20 mg/ml, the antioxidant activity increased in both the solvents. *Volvariella volvacea* showed higher antioxidant activity than *Lentinusedodes*. At a concentration of 4 mg/ml, *Volvariella volvacea* showed antioxidant activity of 26.3% in methanol and 31.8% in distilled water while at 20 mg/ml the activity increased to 49.6% in methanol and 64.2% in distilled water. On the other hand, *Lentinusedodes* showed antioxidant activity of 13.1% in methanol and 52.7% in distilled water at 4 mg/ml extract concentration but at 20 mg/ml concentration the activity increased to 45.8% in methanol and 75.9% in distilled water.

14.3.7 Inhibitory Effect against Lipid Peroxidation Induced by ABAP

The chemical compound 2, 2′-azobis-(2-amidinopropane)-dihydrochloride (ABAP) can be used to evaluate the ability of the mushroom extract to prevent oxidation of unsaturated fatty acids. The unsaturated fatty acid used for this study is linoleic acid. A solution of linoleic acid is mixed with mushroom extract. The mixture is added with ABAP and stirred well. Absorbance of the mixture is taken in a spectrophotometer at 234 nm. The solvent used for preparation of the extract is used as a blank for this study (Pryor et al. 1993). The inhibitory effect is evaluated in percentage using the formula:

$$\%\text{inhibitory effect} = \{(A_{\text{Control}} - A_{\text{Extract}})/A_{\text{Control}}\} \times 100$$

A_{Control} represents absorbance of control contains linoleic acid and ABAP without sample extract while A_{Extract} represents absorbance of the mixture of linoleic acid and ABAP with the sample extract.

Palacios et al. (2011) evaluated the inhibitory effect of some wild and cultivated varieties of mushroom procured from Spain. The mushroom varieties taken for evaluation were *Cantharellus cibarius, Boletus edulis, Calocybe gambosa, Craterelluscornu copioides, Lactarius deliciosus, Agaricus bisporus,* and *Hygrosphorusmarzuolus*and *Pleurotus ostreatus*. The results obtained from the study showed that ABAP increased the oxidation of linoleic acid in control up to a constant value but in the presence of mushroom extract, oxidation of linoleic acid was decreased. *Cantharelluscibarius* showed higher inhibition (74%) against oxidation of unsaturated fatty acids while *Agaricus bisporus* showed lower inhibition (10%).

14.3.8 Inhibitory Effect against Erythrocyte Hemolysis Caused by Peroxyl Free Radicals

The ability of mushroom extract to prevent erythrocytes from hemolysis caused by chain forming free radicals is determined according to the method given by Ng et al. (2000). According to this method, erythrocytes are separated from the blood samples of animals. The erythrocytes after proper washing and centrifugation are formed into a suspension using phosphate buffer saline of required percentage. The suspension is then added with 2, 2′-azobis (2-amidinopropane) dihydrochloride (AAPH) solution prepared in buffer. The mixture is then added with mushroom extracts at different concentrations. After giving proper reaction time and temperature, the mixture is then centrifuged. Absorbance of supernatant is observed through a spectrophotometer at 540 nm. AAPH is a compound that acts as a prooxidant as it results in formation of free radicals that form chain reactions leading to oxidation of proteins, fats, and other compounds (Miki et al. 1987). Studies have shown that mushroom extracts prepared in water show higher activity against prevention of hemolysis than extracts prepared in methanol. Water extracts showed inhibitory effects at any concentration; on the other hand, methanol extracts showed inhibitory effects at concentrations above 2 mg/ml.

14.3.9 Superoxide Anion Radical Scavenging Activity

Superoxide radical scavenging activity of mushroom extract is determined according to a method given by Zhishen et al. (1999). For generation of superoxide anions, a mixture of nitro blue tetrazolium (NBT), riboflavin, and methionine is illuminated for the required period of time. Superoxide anion is generated from riboflavin, which leads to formation of blue colored formazan from NBT (reduced). The mixture is added with mushroom extract. Addition of antioxidant-rich mushroom extract leads to scavenging of superoxide anion radicals and hence prevents formation of formazan from NBT. Absorbance of both blank (mixture of NBT, riboflavin, and methionine without illumination) and the sample is measured in a spectrophotometer at 560 nm. Scavenging activity is expressed in percentage (%) using the formula:

$$\text{Scavenging activity } (\%) = \{(A_{\text{blank}} - A_{\text{extract}})/A_{\text{blank}}\} \times 100$$

Mahfuz et al. (2007) studied superoxide radical scavenging activity of some mushroom varieties obtained from Turkey. Results showed that scavenging activity of *Verpa conica* (99%) was highest and *Agaricus bisporus* (71%) possesses the lowest scavenging activity.

The studies discussed above suggest that one must increase consumption of mushrooms in diet as it is a good source of antioxidants as well as prevents the process of aging and other diseases in humans. Mushroom has metal scavenging activity as metals act as cofactors for enzymes involved in increasing the rate of oxidation. However, studies on development of antioxidant-rich nutraceuticals for humans from mushroom are still underway and need proper attention in future.

14.4 Health Benefits

The presence of biologically active compounds having different medicinal properties provides an opportunity to develop edible mushrooms into functional foods with enhanced nutritional value and numerous health benefits. Edible mushrooms when ingested as a part of daily diet have been found to be helpful in the prevention and treatment of various diseases. Some of these health benefits are explained below.

14.4.1 Cancer

There has been sufficient research, which proves the role of mushrooms in the control and treatment of cancer, which is a major cause of worldwide mortalities. This was attributed to the presence of bioactive polysaccharides present in the mushrooms (Guillamón et al. 2010; Meng et al. 2016). Recently, it has been established from various studies that the intake of edible mushrooms significantly reduced the prevalence of breast cancer, but further studies are needed to corroborate the findings (Li et al. 2014a, 2014b). The mechanism of cancer prevention is proposed to be due to the immunomodulatory role played by the bioactive polysaccharides present in mushrooms. They trigger the immune system response, which facilitates the antitumor activity, thereby enhancing the defense mechanism of the host (Meng et al. 2016).

14.4.2 Metabolic Syndrome

Metabolic syndrome refers to that clinical condition in human beings, which comprises of disorders such as obesity, hyperglycemia, high cholesterol, and hypertension. The intake of edible mushrooms has been proven to effectively prevent the metabolic syndrome. The mushroom extracts comprising of polysaccharides and other bioactive compounds help in lowering of cholesterol levels and reducing hypertension. These bioactives include β-glucans, lectines, eritadenine, tri-terpenes, sterols, etc. (Kundakovic and Kolundzic 2013).

14.4.3 Obesity and Hyperlipidemia

There are various studies carried out in relation to the prevention of different cardiovascular disorders and minimizing obesity and hyperlipidemia and the risk associated with such diseases by introduction of mushrooms in the diet. Most of such health benefits are found to be helpful in reduction of sugar and cholesterol levels in the blood. The presence of other compounds including antioxidants plays an important role in lowering the levels of triglycerides (Zhang et al. 2016).

Several studies have propounded the role of mushroom intake in the prevention of hyperlipidemia and obesity. Different studies have proved *L. edodes* to effectively reduce the lipid levels and help in the prevention of weight gain, which could lead to obesity. In this study, the antihyperlipidemia activity of *L. edodes* was established by feeding rats with high-fat diet along with *L. edodes*. The fat deposition was significantly reduced along with lowering of the triacylglycerol levels in the plasma (Handayani et al. 2011). In another study, the concentration of phosphatidylcholine, which is an essential phospholipid for secretions from liver, was found to be decreased due to the presence of bioactive compound of *L. edodes*, namely, Eritadenine. It helped in the prevention of the condition of dyslipidemia (Sugiyama et al. 1995).

14.4.4 Hypercholesterolemia

The extracts from mushrooms are considered to be a good source of bioactive derivatives such as eritadenine, ergosterol, β-glucans, etc., which help in the inhibition of enzymes responsible for biosynthesis of cholesterol inside the body, thereby preventing various cardiovascular diseases. The intake of mushrooms as such prevents the onset of atherosclerosis as they consist of lower fat content along with high amount of fibers (Guillamón et al. 2010; Gil-Ramírez et al. 2016).

The benefits of mushrooms, especially *A. bisporus*, which is a very widely consumed mushroom, in prevention of hypercholesterolemia have been shown by many studies. Jeong et al. (2010) reported a significant decrease in the cholesterol levels and concentration of lipoproteins in the plasma of rats upon administration of the mushroom *A. bisporus* orally. The mechanism behind this health benefit was expressed by de Miranda et al. (2016) through the study on health benefits of *A. brasiliensis*. They found that the lowering of cholesterol levels in serum was linked with biochemical changes, which stimulated the biliary excretion rate of cholesterol, thereby reducing its levels in the body.

14.4.5 Diabetes

A lot of studies have been conducted, which showed the role of mushroom bioactives in lowering the glycemic activity in the body. Among the different varieties of genus *Pleurotus*, the extracts from *P. eryngii* were found to possess significant hypoglycemic activity. It reduced the glucose levels in the serum of mice, thereby providing an opportunity to be used as an alternative food to diabetic people (Li et al. 2014a, 2014b). Other mushroom species found to possess hypoglycemic activity and reducing the levels of glucose in the serum include *P. pulmonarius* and *P. sajor-caju* (Badole et al. 2008; Ng et al. 2015). One more mushroom genus which has been found to possess a beneficial role in case of diabetes is *Agaricus*. A lot of studies have been conducted which demonstrated the antiglycemic activity of many species of *Agaricus* (Jeong et al. 2010; Mascaro et al. 2014; Niwa et al. 2011). It was reported by Jeong et al. (2010) that the oral administration of *A. bisporus* helped in significant reduction in the lowering of glucose levels in the plasma of rats induced with type-II diabetes. The species of *Agaricus sylvaticus* (Mascaro et al. 2014) and *Agaricus blazei* (Niwa et al. 2011) are found to be beneficial in reducing the levels of glucose along with glycerol and triglycerides in the blood of rats induced with type-II diabetes, thereby providing relief during type-I diabetes. These effects were proposed to be due to the protection of glandular cells in the liver, which are responsible for the secretion of insulin.

14.4.6 Hypertension

High blood pressure occurs due to the effect of Angiotensin-converting enzyme (ACE), which is a main component of the renin–angiotensin system causing constriction of blood vessels. The treatment of cardiovascular diseases includes the use of ACE inhibitors, which are the main constituents of drugs for the prevention of diseases related to it. Recently, various studies have been carried out to replace the synthetic antihypertension drugs with natural compounds. In this regard, various mushroom species are explored to be used in place of synthetic antihypertension drugs. These include *G. frondosa, H. erinaceus, Hypsizygus marmoreus, A. bisporus*, etc., which can be used as alternatives for treating hypertension (Yahaya et al. 2014; Friedman 2015; Lau et al. 2014). Several bioactives in the extracts of these mushrooms inhibit the ACE's active site involving the renin angiotensin–aldosterone system, which helps in lowering hypertension (Yahaya et al. 2014). The various bioactives possessing such benefits found in the mushrooms include some peptides from *A. bisporus* (Lau et al. 2014), proteins from *P. pulmonarius* mycelium (Ibadallah et al. 2015), etc.

14.4.7 Neurodegenerative Diseases

Different species of mushrooms such as *Ganoderma lucidum, Sarcodon scabrosus, H. erinaceus* etc. have been studied in relation to their beneficial effects on the nervous system of humans (Sabaratnam et al. 2013)[9]. These benefits are in the form of the regulation of growth and development of nervous tissue along with the prevention and treatment of various neurodegenerative disorders. *H. erinaceus* is the most studied mushroom in this context. The different bioactives present in *H. erinaceus* stimulate the synthesis of the best neurotropic factor, i.e., nerve growth factor (NGF), which is an essential regulatory substance in the nervous system (Zhang et al. 2015). The oral administration of *H. erinaceus* has been also found to play an important role in the prevention and treatment of various neurodegenerative disorders related with the loss of progressive function of neurons, which include

Alzheimer's disease, dementia, depression, and other cognitive impairment-related disorders (Jiang et al. 2014).

14.4.8 Other Health Benefits

There are lot of other health benefits provided by the various bioactive compounds present in mushrooms, which include antioxidant activity, anti-inflammatory and anti-allergic properties, and antimicrobial and antiviral benefits (Zhang et al. 2016). The intake of mushrooms has been found to reduce the oxidative stress due to the presence of a wide range of antioxidants present in mushrooms, which include the different phenolics, tocopherols, ascorbic acid, carotenoids, and other polysaccharides. The different mechanisms responsible for providing antioxidant properties include free radical scavenging, inhibition of lipid peroxidation, facilitating increased antioxidant enzyme activity, etc. (Zhang et al. 2015; Kozarski et al. 2015a, 2015b).

Mushrooms contain some antiallergic compounds, which help in the stimulation of the immune system, thereby providing benefits in the prevention and treatment of various allergic diseases. Some of the examples which have demonstrated significant antiallergic effects include *P. eryngii, Pholiotanameko, H. marmoreus, and Flammulina velutipes* (Sano et al. 2002). The antiallergic effect was shown by Jesenak et al. (2014), who reported a significant reduction in the occurrence of recurrent respiratory tract infections in children.

Different studies have demonstrated the potential effects of mushroom extracts against bacterial and viral infections (Hassan et al. 2016; Zhang et al. 2016). Although there has been no report of any direct correlation with the active adsorption of virus or virucidal effect, many studies have demonstrated the inhibitory role of mushrooms in hampering the virus replication in initial stages (Faccin et al. 2007).

14.5 Conclusion

Consumption of mushrooms as a part of diet across the world is due to their potential nutritional properties. There is a wide range of compounds which are obtained from mushrooms with their distinct properties providing different health benefits. This makes mushrooms an interesting field of study and very useful source of bioactives for disease prevention in human beings. The use and potential health benefits of mushrooms for nutritional as well as therapeutic purposes have been explored by carrying out various studies. The different proposed effects on health include antitumor and immunomodulating effects. The edible mushrooms when ingested as a part of daily diet have been found to be helpful in the prevention and treatment of various diseases. The intake of mushrooms has been found to reduce oxidative stress due to the presence of a wide range of antioxidants present in mushrooms, which include the different phenolics, tocopherols, ascorbic acid, carotenoids, and other polysaccharides.

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