

BETWEEN THE BIOACTIVE EXTRACTS OF EDIBLE MUSHROOMS AND PHARMACOLOGICALLY IMPORTANT NANOPARTICLES: NEED FOR THE INVESTIGATION OF A SYNERGISTIC COMBINATION - A MINI REVIEW

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ABSTRACT

The pharmacological potential of bioactive compounds extracted from mushrooms has been studied to a reasonable level. In the same vein, the bioactivity of nanoparticles has also been investigated and reported to be of potential pharmacological benefit. No doubt, there is a reasonable amount of claims regarding the vast activity of the mushroom extracts and nanoparticles on the tested cell lines and microorganisms. In this paper, a review of the recent application of bioactive compounds extracted from two edible mushrooms (*Coprinus comatus* and *Lactarius deliciosus*), as well as some of the recently reported studies on some nanoparticles of pharmacological potentials, was carried out. In order to check for synergy in the bioactivity of the mushroom extracts when co-administered with nanoparticles, an investigation on the synergistic application of the materials through the encapsulation of the bioactive extracts from the mushroom onto the nanoparticle was proposed. The supposed synergy in the activity of the extract-nanoparticle complex could hold the key to improved activity of nutraceuticals against resistant microorganisms and tumor cells.

Keywords: *Coprinus comatus*, *Lactarius deliciosus*, Nanoparticles, Synergy, Bioactive extracts.

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INTRODUCTION

In the days and even presently, mushrooms are consumed all over the world by people for their nutritional and culinary potentials. Due to the high protein, low fat, and cholesterol contents of mushrooms, they are considered by many people to be a vital component of daily meals as a source of nutritional ingredients such as minerals, polysaccharides, flavonoids, and polyphenols [1]. Some polyphenolic compounds derived from mushrooms have been found to possess powerful antioxidant activities, with free radical scavenging and metal chelating activities [2]. Recent researches provide consistent approval for the development of ethnomedicine [3]. More evidence has been provided using bioactive materials extracted from mushrooms [4], as well as polysaccharides isolated from *Ganoderma lucidum* and *Trametes versicolor* [5], which showed great expression of tumor markers [4], increased natural killer cells activities, and higher survival rate [5]. Following the successful trials, polysaccharides isolated from *T. versicolor* had further been approved in Japan and marketed as an antitumor drug known as Krestin (PSK). Further utilization of the pharmacological potentials of natural materials could be the key to solving the emerging challenges of new diseases and drug-resistant variants.

The current emergence of the superbug, increased cancer epidemics and multidrug-resistant bacteria strains is pushing for an urgent need in the provision of antibacterial compounds that could be novel enough to combat the threat of these organisms. As a result of mushrooms' natural living environment (life cycles which depend on degrading dead organic matters) and the need to defend against pathogenic organisms in their environment, it is believed that potent antimicrobial and cytotoxic compounds could be extracted from mushrooms [6]. This prospect is supported by a previously reported case, whereby antimicrobial farnesyl hydroquinones (ganomycin A and B) isolated from *Ganoderma pfeifferi* were found to inhibit the growth of methicillin-resistant *Staphylococcus aureus* [7].

With this supporting evidence, it is certain that work has previously been done on the cytotoxicity of bioactive substances extracted from *Coprinus comatus* and *Lactarius deliciosus*. However, it could be interesting to extend the present knowledge on the pharmacological properties of these bioactive compounds from *C. comatus* and *L. deliciosus* by studying the cyto-effects when coupled on a nanoparticle with pharmacological potentials. Some nanomaterials have been synthesized in a nanoscale (<100 µm) and a handful of these materials tested against notable human cell line for cytotoxic and antibacterial activities. Among the nanomaterials synthesized and tested were sulfated zirconia nanoparticles, cerium, single-walled carbon nanotubes (SWCNTs) to mention but a few [8]. Although nanoparticles are most suitable for delivering of hydrophobic drugs, several reports have shown success in encapsulating hydrophilic drugs through surface attachment or polymer drug conjugation methods [9]. Many approaches have also been taken to co-encapsulate multiple therapeutic agents into a single polymeric nanoparticle. Drugs can be attached to the nanoparticles using either of the following ways:

- Direct encapsulation of drugs into the hydrophobic polymeric core of the nanoparticle
- Incorporation of an additional medium to the nanoparticle, usually on the particle surface to create a separate partition for drug loading
- Covalent conjugation of drugs to the polymer backbone during the synthesis of the nanoparticle.

Hence, in this article, a mini review of the current studies on two edible mushrooms (*C. comatus* and *L. deliciosus*) as well as a review of selected nanoparticles of pharmacological interest was conducted. The recent applications of the bioactive compounds extracted from the selected mushrooms, as well as the application of nanoparticles for pharmacological benefits, were carried out. At the end of the review, a proposal for the synergistic utilization of the bioactive compounds extracted from the mushrooms, together with the nanoparticles for an improved antibacterial and antitumor activity against selected microorganisms and tumor cells, was made.

MUSHROOMS

Mushrooms, also known as toadstools, are group(s) of the fleshy, spore-producing, and fruiting body of fungi, commonly springing from the ground or on the food source where they grow as saprophytes. Generally, mushrooms are commonly identified through the application of the basic understanding of their macroscopic features. Most mushrooms are classified as Basidiomycetes, with characteristic possession of much-developed gills, which are arranged in a room-like pattern. Mushrooms possess spores (basidiospores) which are produced on the gills and when matured fall off in a fine rain manner from the underside of the caps, facilitating its dispersals [10]. Typically, mushrooms are the fruit bodies of the order *Agaricales*, but with the evolution of molecular classification, not all the members of this order can produce the typical fruit bodies but possess gills, placing them in the class *Agaricomycetes*. For instance, the milk cap mushrooms (*Lactarius* and *Lactifluus*) and russulas (*Russula*), as well as *Lentinellus*, belong to the *Russulales* [2].

Mushrooms can be grouped as edible or poisonous, suggesting that mushroom may be eaten, toxic, or unpalatable with the human body. The edible mushrooms are cultivated in controlled (or wild) environment for human consumption owing to their low caloric content and abundance of bioactive compounds which are beneficial to human health. The poisonous (toxic) mushrooms are known to contain substances which are toxic to the human body when eaten. Most of the toxins are believed to possess neurotoxic, nephrotoxic, hepatotoxic, as well as psychotic effects [11]. Medicinally, mushrooms possess bioactive compounds which have been studied and documented as potential sources of nutraceuticals owing to their potency against reactive oxygen species (ROS) and tumor cell inhibition. Mushrooms also possess antimicrobial activities which have been effective against the Gram-positive and the Gram-negative bacteria [2,12]. The ability of mushroom farmers to cultivate some of the medicinal mushrooms in commercial quantity has boosted the recent advances toward the utilization of the bioactive compounds from mushrooms for pharmacological and therapeutic purposes. Within the scope of this research, a critical review will be focused on two identified medicinal mushrooms (*L. deliciosus* and *C. comatus*). The review will focus on the geographical location (habitat), the nutritional benefits, the pharmacological importance of the bioactive compounds, and the economic importance of the discussed mushrooms.

Clinical evidence of mushroom extracts as pharmacological agents

Various mushroom polysaccharides have gone into clinical trials in cancer patients, most of which, however, have just been studied in non-randomized trials with a small number of patients. These trials have been carried out exclusively in the Far East although some have already begun in the United States. Safety during clinical assessments has been good, with no maximum tolerated dose for these compounds currently reached, and making the potential of a prolonged use of these compounds without side effects a possibility. Griffon-D, a mushroom-based product, has recently been excluded from Phase I study by the FDA because of the lack of preclinical toxicity. Even though some of these mushroom compounds have not been tried in the Western standard randomized trials, there are strong proof-of-principle data for some medicinal mushrooms to support further studies [13]. Trials with compounds isolated from *Agaricus bisporus* [14,15] and some species of *Pleurotus* [16] have shown both *in vitro* and *in vivo* anti-inflammatory activities, whereas extracts from *L. deliciosus* have also been put to clinical trials.

Methanol extracts of *L. deliciosus* have been tested to assess the potential anti-inflammatory activity of the mushroom using macrophages RAW 264.7 by Moro *et al.*, who reported that the extracts were able to affect the viability of the cells, except at a lower concentration of 0.5 mg/mL [14]. This report was supported by a bar chart presenting a comparative study of six mushrooms (*A. bisporus*, *Boletus edulis*, *Cantharellus cibarius*, *Craterellus cornucopioides*, *L. deliciosus*, and *Pleurotus ostreatus*) with the conventional anti-inflammatory agent, lipopolysaccharide (LPS). The data showed that *L. deliciosus* portrayed the highest anti-inflammatory activity against the macrophages and stood to argue that the high total phenolic content of the *L. deliciosus* could

be a reason for its strong anti-inflammatory activity [14]. The ability of the bioactive extracts of *L. deliciosus* has also been tested for its ability to stimulate the production of nitric oxide (NO) (a substance produced by macrophages when stimulated). The macrophages were incubated with the extracts for 1 hr and later stimulated with LPS (1 µg/mL) for 24 hrs, and the level of NO release was measured by the accumulation of the metabolite nitrite in the culture supernatant. The macrophages that were not stimulated were found to produce no NO, while those stimulated with the LPS presented a high level of NO production [14]. The results showed that extracts from some of the mushrooms studied inhibited LPS-induced NO production and the expression of pro-inflammatory cytokines, such as interleukin-1b (IL-1b) and IL-6. The overproduction of these mediators has been implicated in various inflammatory diseases and cancer [17]. The inhibition of the activation of these mediators seems to be an important target for the treatment of inflammatory diseases. The most efficient species among the studied species were *C. cibarius*, *L. deliciosus*, and *A. bisporus* while others such as *B. edulis* and *P. ostreatus* had no effect [18].

Although many natural substances may take part in anti-inflammatory activities, phenolic compounds have been recognized as natural molecules with most potent anti-inflammatory effects. Positive correlations have been established between total phenolic content and anti-inflammatory effects of different mushroom extracts [19,20]. Phenolic compounds consist of a range of substances which display a great diversity of structures and their biological activity may be highly influenced by the chemical nature of the components. Even though phenolic acids are the most abundant compounds in most mushroom species, their content may not correlate with the inhibitory potential of the extracts and stands to reason that the contribution of these compounds to the anti-inflammatory activity is not relevant.

To justify this, studies carried out to establish the effect of derivatives on the production of inflammatory agents concluded that derivatives, such as caffeic acid, ferulic acid, chlorogenic acid, and p-hydroxybenzoic acid, from an ethanolic plant extract, did not affect the production of inflammatory agents [21]. Furthermore, pyrogallol, which is present in extracts of *A. bisporus*, *C. cibarius*, *C. cornucopioides*, and *L. deliciosus* [14], may be responsible for the anti-inflammatory activity observed in the extracts of these mushrooms since these extracts were the most active in terms of NO inhibition. Synergisms between pyrogallol and other active compounds present in the extracts from *L. deliciosus*, and other studied mushrooms such as flavonoids or cinnamic acid derivatives could partly explain the differences in activity of the studied extracts [14].

L. DELICIOSUS

L. deliciosus, best known as the saffron milk cap or red pine mushroom, belongs to the genus *Lactarius* and order *Russulales*. It is among the best-known members of the large milk cap mushrooms found mainly in Europe and but accidentally has been introduced to other countries. It can be found growing in pine plantations and related environments where the environment is favorable [22]. *L. deliciosus* possesses a carrot orange cap, which is convex to vase-shaped. When young, it is in-rolled, measuring about 4-14 cm (1.5-5.5 inch) in diameter, usually with darker orange lines arranged in concentric circles. The cap is viscous and sticky when wet although it is often dry. The gills are crowded and decurrent with a squat hollow orange stipe often measuring 3-8 cm (1-3 inch) in length and about 1-2 cm (0.5-1 inch) in thickness. When handled, *L. deliciosus* stains with a deep green color; however, when fresh, the mushroom produces orange-red "milk" which does not change color [22]. Mycologically, *L. deliciosus* possesses gills on the hymenium and has a depressed cap, hymenium is decurrent, stipe is bare, spore print is tan, and ecology is mycorrhizal, while edibility is purely on choice.

Nutritional benefits of *L. deliciosus*

The major compounds found in mushrooms are carbohydrates and proteins. Total protein content varying between 21% and 50% has been reported, which competed with protein from meat, milk, and egg.

Mushrooms are also consumed due to their low-calorie content (1.40-10.58%) [23]. Analysis of the chemical composition of *L. deliciosus* showed that it contained 8.75%, 2.64%, 75.25%, and 4.61% of moisture, fat, protein, and ash, respectively, representing approximately 89.96% of the total dry material [24]. Although similarities in the composition of mushrooms exist, the chemical composition of mushrooms is usually affected by a number of factors, which includes mushroom strains, growth media composition, environment, harvesting time, handling conditions, and substrates preparation. There is diversity in the antimicrobial and antitumor activity of mushrooms at different stages of cultivation of the same species due to changes in the content and amount of active compounds according to the growth media and environment of the mushroom [25].

Wild edible mushrooms such as *L. deliciosus* are popular food stuff with reported high levels of proteins, zinc, vitamins, iron, sodium, and minerals, which could be a major component of the mushroom or could be absorbed from the surrounding environment. This could be the reason for a higher composition of minerals and chemicals in wild mushrooms [26]. Because mushrooms aid in the breakdown of organic matter and play a role in the natural decomposition of materials, a very effective mechanism for the accumulation of heavy metals from the environment persists in mushrooms, especially the wild mushrooms [27]. These metals are part of the nutritional benefits from mushrooms since most of the metals act as cofactors for most of the complex enzyme systems.

Pharmacological potentials of *L. deliciosus*

Pharmacologically, the application of mushroom extracts in medicine has been researched and various reports have been documented regarding the health benefits of extracts from the mushroom. In pharmacological trials, bioactive compounds from *L. deliciosus* have been tested against a wide range of bacteria and tumor cells owing to their radical scavenging properties and phenolic content [12,28]. Jayakumar *et al.* reported that wild mushrooms can be used for therapeutic purposes because they produce varieties of metabolites, such as organic acids, terpenoids, alkaloids, and phenolic and steroid compounds [29]. Health beneficial properties, such as antioxidant, antimicrobial, anticancer, cholesterol-lowering activities, and immune stimulatory effects, have all been reported for species of mushrooms including *L. deliciosus* [12,28].

The radical scavenging properties and the total phenolic content of *L. deliciosus* have been studied by various researchers and compared with other wild and cultivated mushrooms. The results of the previous studies on *L. deliciosus* compared with other mushrooms are presented in Table 1.

Table 1: Radical scavenging and phenolic content of selected mushrooms

Mushroom	DPPH radical scavenging	Phenolic content	References
<i>Lactarius deliciosus</i>	493.82±2.35	42.68±1.065	[27]
	(IC ₅₀ µg/mL)	(µg PE/mg of extract)	
	34.48±4.34	17.25±0.65	[30]
	(IC ₅₀ µg/mL)	(µg PE/mg of extract)	
<i>Macrolepiota procera</i>	0.210±0.006	12.75±0.38	[31]
	(500 µg/mL)	(mg PE/g dry weight)	
	Nd	17.25±0.65	[1]
<i>Cantharellus cibarius</i>		(mg/g)	
	311.40±1.28	67.98±1.013	[27]
	(IC ₅₀ µg/mL)	(µg PE/mg of extract)	
<i>Cantharellus cibarius</i>	69.74±0.65	8.90±0.10	[31]
	(500 µg/mL)	(mg PE/g dry weight)	
<i>Cantharellus cibarius</i>	56.34±0.05	0.69±0.20	[31]
	(500 µg/mL)	(mg PE/g dry weight)	

Nd: Not determined, DPPH: 1, 1-diphenyl-2-picryl-hydrazil

The rate of antioxidant activity depends on the mushroom species and also on the solvent used for extraction. Various reports suggested that the radical scavenging activities of most extracts depend on the total phenolic content as extracts with higher total phenolic were seen to possess high radical scavenging properties [12,32,33].

L. deliciosus is an important source of natural antioxidants such as phenolic compounds (tocopherols, flavonoids, and phenolic acids), nitrogen compounds (alkaloids, amino acids chlorophyll derivatives, and amines), or carotenoids as well as ascorbic acid. These are molecules produced as secondary metabolites with great therapeutics effects. The consumption natural foods such as mushroom provide supplements and antioxidants which help to protect the body against various diseases, such as cancer, heart, and cerebrovascular diseases. They may also help the human body in reducing oxidative damage or protect oxidative deterioration, usually develop as a result of stress. Both the fruiting body and the mycelium of *L. deliciosus* contain compounds which exhibit both antioxidant and antimicrobial activities. Specific compounds are extracted to produce antibiotic or pharmaceutical drugs for combating various forms of diseases [25,31].

Due to the high polyphenol content of *L. deliciosus*, it has been reported by Elmastas *et al.* to be superior in anthocyanin content (1.38±0.09 mg cyaniding 3-glucoside/g dry weight) among 12 screened mushroom species and this conferred a great pharmacological activity on *L. deliciosus* [31]. The radical scavenging property of *L. deliciosus* has also been reported to be higher than the activity of most conventional antioxidants such as butylated hydroxytoluene and trolox [12]. Early research has shown that isolates from mushrooms have anticancer, cardiovascular, antiviral, antiparasitic, antibacterial, anti-inflammatory, blood lipid-lowering, immune response stimulating, and even antidiabetic effects [34-36]. Currently, extracts from several mushrooms including *L. deliciosus* have widespread application in Japan, Korea, and China, being used as potential adjuvants to radiation and chemotherapy [10,13].

Antibacterial potential of *L. deliciosus* extracts

The bioactive compounds (phenolic and flavonoids) of *L. deliciosus* have been extracted using different extracting medium and tested for activities against selected microorganisms for potential use as natural antimicrobial agents. Baros *et al.* studied the antimicrobial properties of phenolic extracts of Portuguese wild edible mushroom species (*L. deliciosus*, *Sarcodon imbricatus*, and *Tricholoma portentosum*) against microbial pathogens. In the report, it was demonstrated that *L. deliciosus* had the greater effect on all the tested microorganisms owing to its higher total phenolic content. The observed total phenolic, flavonoids and ascorbic acid contents of *L. deliciosus* extracts were 17.25±0.65, 8.14±0.81, and 0.24±0.02 mg/g, respectively [12]. When the extracts were tested against *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Cryptococcus neoformans*, *L. deliciosus* extracts expressed a strong antibacterial inhibitory activity against all the species tested at a minimum inhibitory concentration (MIC) of 10 mg/mL, except for *E. coli*, where even a higher of 300 mg/mL showed no inhibitory activity. *E. coli* expressed strong resistance to all the tested extracts from the three mushrooms tested, implying that the resistance to the extracts of *L. deliciosus* was not in isolation, but a general phenomenon likely due to gene variation [12].

Dilsad *et al.* studied the antimicrobial, antioxidant activities and chemical composition of *L. deliciosus* collected from Kastamonu Province of Turkey and reported that the highest inhibitory activity was found against *P. aeruginosa* with an inhibition zone of 30±00 mm using acetone and ethanol as extraction solvents. The study equally reported weak inhibitory action against *S. aureus* and *C. albicans* with a zone of inhibition measuring <9 mm in diameter using dimethylsulfoxide and distilled water as extraction solvents [23]. The antibacterial activity of most bioactive compounds depends on the solvent used during extraction. Various reports of varying activities have been reported

regarding the antibacterial activities of *L. deliciosus* extracts, which seem to be higher when extracted with methanol, as compared to other extraction solvents. Dulger *et al.* reported that methanol extracts of *L. deliciosus* have been found to be effective against *Mycobacterium* sp. and greater antimicrobial activity against Gram-positive and Gram-negative bacteria, with little or no effect against yeasts [37].

Among the *Lactarius* spp., various studies have shown a different pattern of antimicrobial sensitivity among the members of the group, especially against *E. coli*. Looking at the individual reports among the *Lactarius* spp., Ozen *et al.* reported that among *Lactarius piperatus*, *Lactarius camphorates*, *Lactarius volemus*, and *L. deliciosus*, *L. camphorates* showed more antibacterial activity against *E. coli* and other studied organisms [25], whereas Dulger *et al.* reported that *L. deliciosus* was more effective against *E. coli* than other members of the *Lactarius* family [37]. Barros *et al.* studied the effect of fruiting body maturity stage on the chemical composition and antimicrobial activity of *Lactarius* spp. mushrooms and reported that *L. deliciosus* had the highest antibacterial effect against *E. coli* among the six species studied [1].

Antitumor potential of *L. deliciosus* extracts

Based on available literature, a few reports have been documented on the antitumor activities of whole extracts from *L. deliciosus*. However, there have been reports on the antioxidant and scavenging potentials of extracts from *L. deliciosus*. Having known the role of the total phenols and flavonoids in radical scavenging, more works on the antitumor potential of the extract from *L. deliciosus* becomes inevitable. Marijana *et al.* studied growth inhibitory effects of methanol extracts of *L. deliciosus* on human epithelial carcinoma (HeLa), human lung carcinoma (A549), and human colon carcinoma (LS174) cell lines and reported that the mushroom showed anticancer potential with an IC_{50} against tested cells ranging from 19.01 mg/mL to 80.27 mg/mL. The IC_{50} for *L. deliciosus* against HeLa, A549, and LS174 cells lines was 19.01, 33.05, and 74.01 mg/mL, respectively [27]. This is the only reported investigation into the anticancer potential of *L. deliciosus* crude extract against cell lines, thereby paving the way for the investigation of the antitumor activities against a broader range of cell lines.

Some works have also been reported on the activity of some polysaccharides extracted from this mushroom, having antitumor activities. Ding *et al.* reported a novel polysaccharide from *L. deliciosus* during the study of the structure, feature, and antitumor activity of a novel polysaccharide isolated from *L. deliciosus* gray. The polysaccharide codenamed LDG-A was used on mice S180 tumor to evaluate the antitumor effects of the polysaccharide [38]. From the results, LDG-A was found to be able to inhibit the growth of the tumors in a dose-dependent fashion. The rate of inhibition in the mice treated with about 80 mg/kg LDG-A was found to be 68.422%, which actually was the highest dose tested, thereby showing the greatest rate of inhibition. The antitumor activity of the isolated polysaccharide was believed to be a result of the activation of the cell-mediated immune response in the host [39]. A summary of the antitumor activity of the *L. deliciosus* polysaccharide isolated by Ding *et al.* is shown in Table 2.

From the results, Ding *et al.* observed that there was minimal change in the average liver weight of the test groups, which indicated that the LDG-A from *L. deliciosus* did not cause serious liver damage and could be a potential antitumor agent with a good chemotherapeutic index. The study concluded that the activation of the immune responses in the host might likely be one of the mechanisms of antitumor activity of LDG-A, which has been the case for many antitumor polysaccharides already in use.

Antioxidant potential of *L. deliciosus* extracts

The antioxidant potential of *L. deliciosus* has been investigated and reported by a number of authors. The most common method of investigating the antioxidant potential of the mushroom has been the scavenging of 1,1-diphenyl-2-picryl-hydrazil (DPPH). Marijana

et al. reported that methanol extracts of *L. deliciosus* showed lower scavenging property when compared to the standard ascorbic acid which served as the control. The extracts of *L. deliciosus* had a DPPH radical scavenging (IC_{50}) of 493.82 ± 2.35 μ g/mL, which was much lower than the 6.42 ± 0.18 μ g/mL of the ascorbic acid which served as the control [27]. Similarly, Ferreira *et al.* studied the free radical scavenging capacity and reducing power of wild edible mushrooms from Northeast Portugal and reported that the extracts of *L. deliciosus* had higher scavenging property on DPPH when compared to the scavenging power of *T. portentosum*. The *L. deliciosus* extract presented a scavenging ability of 79.1-84.3% at 50 mg/mL which was better than the 30.4-65.0% at 50 mg/mL of *T. portentosum*. Butylated hydroxyanisole (BHA) and α -tocopherols were used as controls, presenting higher scavenging effects of 3.6 mg/mL for BHA and 8.6 mg/mL for α -tocopherols being 96% and 95%, respectively. They also reported that the extracts obtained from the cap of the mushroom scavenged DPPH radicals at a higher percentage than extracts from the other parts (body and rhizomes) of the mushroom [30].

Studying the antioxidant, antimicrobial activities and chemical composition of edible mushrooms grown wild in the Black Sea Region of Turkey, Ozen *et al.* reported that all the tested mushrooms, including the extracts from *L. deliciosus*, showed moderate scavenging effects on DPPH. The level of scavenging effects was lower in some mushrooms studied compared to α -tocopherols including *L. deliciosus* (51%) than the scavenging effects of α -tocopherols (60%) which served as control. The scavenging effects of extracts from *Ramaria flava* (80%), *Macrolepiota procera* (69%), *L. volemus* (61%), *Lactarius camphoratus* (61%), and *L. piperatus* (61%) were higher than the scavenging effects of α -tocopherols (60%) [25].

C. COMATUS

C. comatus or lawyer's wig is a macrofungus often seen growing along gravel roads and lawns. Sometimes, they are seen growing on dirty areas with the young fruiting bodies appearing first as white cylinders growing up from the ground, followed by the bell-shaped caps which open out later. When the caps opened, the gills under the cap are white, then changes to pink, and finally turns black before secreting a black liquid that is filled with the spores. This is the reason it is sometimes regarded as the "ink cap." Due to the short lifespan of this mushroom, it is unusual because as soon as it turns black, it auto dissolves in a matter of hours. Long-term storage of the mushroom can be achieved through drying, freezing, or microwaving [40]. Taxonomically, *C. comatus* is a species belonging to the genus *Coprinus*.

This genus *Coprinus* was originally considered as a large genus with over 100 species. However, on molecular analysis of DNA sequences, it showed that the former species belonged to two families, known as the *Agaricaceae* and the *Psathyrellaceae*. The species *C. comatus* is the best known of the member of the true *Coprinus* [41]. Descriptively, *C. comatus* is easily recognized by its cylindrical mostly whitish shaggy cap that initially covers most of the stem. The cap of the mushroom is usually paler and uses to change from white to pink rapidly before finally turning black. It absorbs water from the environment when exposed, conferring to it the deliquescent status. It has stipe which usually measures about 10-37 cm in height and about 1-2.5 cm in diameter. At the microscopic level, it does not possess pleurocystidia, and the spore print is usually blackish brown, with the spores measuring about 10-13 μ m. It has a whitish flesh and also tastes mild [41].

Nutritional benefits of *C. comatus*

C. comatus when young contains about 25% proteins and is also rich in triglycerides, mainly made up of linoleic acid. Traditionally, it is used in Chinese medicine for the treatment of piles and to improve digestion [42]. The consumption of *C. comatus* has been reported to cause recovery from alcoholic effects when tried in experimental animals [43]. The total energy derivable from 100 g of the mushroom is about 349 Kcal, meaning it is usually low in caloric content. The protein content is about 37%, water content made up about 13.5%, ash content

Table 2: Anti-tumor activities of *L. deliciosus* (LDG-A) polysaccharide on S180 tumor

Group	Spleen index (mg/g)	Liver index (mg/g)	Thymus index (mg/g)	Average tumor weight (g)	Inhibitory rate of tumor (%)
Negative control	5.784±2.108	60.307±9.395	1.429±0.706	3.740±0.423	-
20 mg/kg	7.619±2.414	56.468±4.509	4.741±0.301	2.081±0.740	44.358
40 mg/kg	7.807±3.427	66.351±8.802	7.790±3.208	1.783±0.430	52.326
80 mg/kg	5.202±0.989	59.977±4.786	0.903±0.401	1.181±0.241	64.422
Positive control	7.0439±3.105	58.263±5.399	2.862±1.727	1.581±0.706	57.727

Adapted from [38]

accounted for about 5.5%, while fats and oil and carbohydrates contents accounted for 5% and 39%, respectively [43]. A profile of the fatty acid content of the extracts from *C. comatus* using GC-FID showed that it is majorly composed of linoleic acid (C18:2n6c) (66.6%), palmitic acid (C16:0) (11.8%), palmitoleic acid (C16:1) (5.4%), stearic acid (C18:0) (3.3%), oleic acid (C18:1n9c) (6.1%), and linolenic acid (C18:3n3) (1.1%). These are lipids that have been proven to be of beneficial use to the normal functioning of the brain and the nervous systems [44]. The vitamin content of *C. comatus* was also investigated and reported to contain the following vitamins at the following concentrations: Thiamin (vitamin B1) (7.5 mg/100 g), riboflavin (vitamin B2) (0.45 mg/100 g), retinol (vitamin A) (<50 mg/100 g), ascorbic acid (vitamin C) (6.15 mg/100 g), α -tocopherols (vitamin E) (<1 mg/100 g), and niacin+niacinamide (vitamin B₃) (<1 mg/100 g) [44]. These indicated that the mushroom is a good source of the important vitamins required for daily body function.

Antibacterial potentials of *C. comatus* extracts

The antibacterial activity of methanolic extracts both cultivated and wild *C. comatus* has been previously investigated by Dejan *et al.* and documented as follows: The samples from both cultivated and wild *C. comatus* showed strong antibacterial activity against all the bacteria that were tested. Wild and cultivated extracts of *C. comatus* exhibited the highest antibacterial activity against *S. aureus* (MIC of 0.0625 and 1.5 mg/mL; minimum bactericidal concentration [MBC] of 0.125 and 3.0 mg/mL) and *B. cereus* (MIC of 0.0625 and 0.75 mg/mL; MBC of 0.125 and 3.0 mg/mL). The lowest observed antibacterial activity was achieved recorded against *E. coli* with MIC of 3.0 mg/mL and MBC of 6.0 mg/mL. The methanolic extract of the cultivated *C. comatus* extract was reported with higher antibacterial potential in general, as compared to the extracts from the wild sample. This could be attributed to the controlled environment, in which the cultivated mushroom was grown. It was also reported that against *E. coli*, both extracts exhibited the same level of activities [45]. A summary of the activities of the wild and cultivated extracts of *C. comatus* as was documented by Mull *et al.* is presented in Table 3.

Similarly, Ehssan *et al.* studied antibacterial activity of wild mushrooms from the Khartoum state of Sudan and reported that the ethanol extract of *C. comatus* was very active against almost all of the tested organisms, except for *Aspergillus niger* where it showed some resistance. It was also reported that the petroleum extracts from the mushroom were not active against all the tested organisms [46]. These activities were concluded to be due to the presence of bioactive compounds such as alkaloids, flavonoids, phenolic, saponins, sterols, carbohydrates, tannins, coumarins, and cyanogenic glycosides, as well as the solubility of these compounds in the extraction solvent.

Kalaw and Albinto studied the functional activities of Philippine wild strain of *C. comatus* and *Pleurotus cystidiosus* grown on rice straw and noted that ethanol and acetone extracts of the mushrooms were able to inhibit the growth of *S. aureus* though *C. comatus* ethanol extract showed a wider zone of inhibition, recording a mean inhibition diameter of 14.09 mm, as compared to acetone extract, with a mean inhibition diameter of 13.43 mm. However, on the other hand, it was reported that *P. cystidiosus* acetone extract showed a larger inhibition zone with a mean diameter of 15.25 mm than that of ethanol extract which has a mean diameter of 13.16 mm. No activity was recorded against *E. coli*

when the ethanol and acetone extracts of both mushroom species were tested against the bacteria. This showed that both mushrooms can exhibit potential antibacterial activities, but the extent of activity depends on the solvent used during the extraction process [47]. Furthermore, Srivastava and Sharma showed that the antimicrobial activity of mushroom depends on the solvent used for extraction as well as the strain of the fungus. Similarly, Iwalokun *et al.* demonstrated that petroleum ether and acetone extracts of *P. ostreatus* inhibited the growth of both Gram-positive and Gram-negative bacteria, as well as fungi, tested [35,48].

Antitumor potential of *C. comatus* extracts

Studies on the antitumor potential of extracts from *C. comatus* against selected cell lines have been investigated and reported previously, with recommendations for further studies on other range of human cancer cell lines. Few of the works already reported showed that the mushroom has antitumor potential against tested cell lines due to the abundance of phenolic and flavonoids in the mushroom. Zaidman *et al.* studied the effects of *C. comatus* and *G. lucidum* interference with androgen receptor function in LNCaP prostate cancer cells and reported that the extracts of both mushrooms inhibited the proliferation of the prostate cancer LNCaP cells with IC₅₀ values of 28.3 g/mL for *C. comatus* and 44.81 g/mL for *G. lucidum*. However, interestingly, they also reported that a significantly higher concentration of the *C. comatus* extract was needed to cause 50 % growth inhibition of the other tested cell lines, meaning that the *C. comatus* extract had an appreciable level of selectivity toward LNCaP cell lines, as compared to the extracts of *G. lucidum* which showed lesser selectivity [42]. The study also showed that the extracts of *C. comatus* were able to selectively affect the cell cycle progression of prostate cancer PCa cell lines, comprised LNCaP, PC-3, and DU 145.

Small and Roach studied the effects of *C. comatus* and *G. lucidum* extracts on the level of secreted prostate-specific antigen (PSA) from LNCaP cell line in a dose-dependent manner study and found that the extracts successfully decreased the level of PSA secreted while treatment with dihydrotestosterone (DHT) caused an increase in the level of secreted PSA by 2.47-fold [49]. Zaidman *et al.* in a similar manner also studied the effect of DHT treatment of LNCaP cells (PSA release) and compared it with the treatment of *C. comatus* extract. The results showed that the treatment with DHT caused an appreciable increase in the level of PSA whereas treatment with flutamide caused a moderate reduction in the PSA production level. However, with the *C. comatus* extract treatment, a significant decrease in the level of PSA levels in a dose-dependent manner [42].

In another study, Rouhana-Toubi *et al.* studied the effect of dry powder of *C. comatus* fruiting bodies extracted using ethyl acetate on the viability of three cell lines from human ovarian cancer (ES-2, SKOV-3, and SW-626). In the conclusion of the study, they reported that the mushroom extract was very active against all the tested cell lines, in a dose-dependent manner. However, to know which fraction of the crude extract was more active against the cells, the crude extract was subjected to purification using chromatography on a silica gel column. At the end of the purification and further testing of the resulting six different fractions on the cells, they observed more significantly effective action with the last elute than the crude extract in the reduction of cell viability. To cap it all, the study summarized that the

Table 3: Antibacterial activity of the methanolic extracts and standard drugs (mean±SD)

Bacteria	<i>Coprinus comatus</i> (cultivated) (mg/mL)	<i>Coprinus comatus</i> (wild) (mg/mL)	Ampicillin (mg/mL)	Streptomycin (mg/mL)
<i>Staphylococcus aureus</i>				
MIC	0.0625±0.00	1.5±0.10	0.25±0.05	0.04±0.00
MBC	0.125±0.00	3.0±0.30	0.37±0.02	0.09±0.00
<i>Bacillus cereus</i>				
MIC	0.0625±0.00	0.75±0.00	0.25±0.00	0.09±0.00
MBC	0.125±0.01	3.0±0.10	0.37±0.02	0.17±0.01
<i>Myotis flavus</i>				
MIC	1.5±0.10	1.5±0.30	0.25±0.03	0.17±0.02
MBC	3.0±0.30	6.25±0.30	0.37±0.00	0.34±0.02
<i>Listeria monocytogenes</i>				
MIC	0.75±0.10	3.0±0.10	0.37±0.01	0.17±0.01
MBC	3.0±0.00	6.25±0.30	0.49±0.03	0.34±0.00
<i>Pseudomonas aeruginosa</i>				
MIC	0.35±0.03	1.5±0.30	0.74±0.02	0.17±0.04
MBC	3.0±0.10	3.0±0.30	1.24±0.00	0.34±0.03
<i>Salmonella typhimurium</i>				
MIC	3.0±0.30	1.5±0.00	0.37±0.01	0.17±0.00
MBC	6.25±0.10	3.0±0.00	0.49±0.03	0.34±0.01
<i>Escherichia coli</i>				
MIC	3.0±0.10	3.0±0.10	0.25±0.05	0.17±0.03
MBC	6.25±0.01	6.25±0.30	0.49±0.05	0.34±0.02
<i>Enterobacter cloacae</i>				
MIC	3.0±0.10	1.5±0.10	0.37±0.05	0.26±0.01
MBC	6.25±0.10	6.25±0.00	0.74±0.07	0.52±0.02

Adapted from [45]. SD: Standard deviation, MIC: Minimum inhibitory concentration, MBC: Minimum bactericidal concentration

ethyl acetate extract of *C. comatus* reduced the viability of the three cell lines of human ovarian cancer [50]. These reports from previous studies suggest that the tendency of producing antitumor drugs from natural sources, especially from mushrooms, is realizable.

Further studies on the ways to promote the delivery of the drugs to the target cells and the possibility of a synergy in the action of the compounds with other compounds or materials of pharmacological interests deserve investigation for enhanced activities. The potential of nanoparticles serving as therapeutic and delivery agents has been investigated, and various reports suggested that nanoparticles can be applied directly as a therapeutic agent against human cancer proliferation as well as a dependable delivery agent of bioactive compounds [8,51]. On this note, a mini review of some of the nanoparticles of pharmacological importance is been carried out, with a view of finding out the particular properties and characteristics that confer the status of a pharmacological important particle. Furthermore, a review of the possible ways to encapsulate the bioactive compounds extracted from the studied mushrooms on the nanoparticle of interest for better performance of the bioactive compound nanoparticle complex against selected human cell lines and microorganisms.

THE ERA OF NANOTECHNOLOGY IN PHARMACEUTICAL SCIENCES

Nanotechnology was introduced into pharmaceutical sciences in the 19th century when Paul Ehrlich during his study described it a magic bullet, which was employed to specifically deliver the drug of interest to the target cell without much effect to the host system. This necessitated further studies into the transition of particle size from microscale to nanoscale [52]. Drug targeting attracted interest in the recent years due to the fact that drugs possess both positive and negative effects on the host cells. The requirement/properties of ideal drug delivery system include being able to be extended into the blood circulation and must be targeted against a selected disease targets. Furthermore, it must be able to accurately deliver the drug of interest and has also been able to be excreted from the body through normal metabolic pathways. Hence, many materials have presently been studied for application as drug delivery systems with different manufacturing methods and modifications being established, while others are investigated for potential activities against target cells [52].

During the early days of nanoparticles development, they were initially used as carriers for the delivery of vaccines and anticancer drugs for improved uptake. Furthermore, studies were carried out on ways to reduce the intake of the nanoparticles by the cells of the reticuloendothelial system [53]. Nanoparticles for pharmaceutical use have to do with evolving technologies as a way of addressing emerging diseases and improvement of the drug delivery systems. Drug delivery systems ought to have an impact on the rate of absorption, metabolism, distribution, as well as excretion of the drug metabolites in a positive way. Similarly, drug delivery systems ought to permit the drugs to actively bind to their target cells and enhance receptor's signaling and response [53].

Nanoparticles as drug delivery agents

Peptides and proteins delivery

The increase in the number of biotechnological molecules such as monoclonal antibodies, vaccines, and hormone, as well as their pharmaceutical potentials, makes it interesting for studies into the protein delivery system [54]. In the structure of proteins, stability is mainly a result of the balance between stabilizing and destabilizing forces exerting effects on the secondary, tertiary, and quaternary structures of the protein. The formation and maintenance of the stability of these structures are based on weak interactions such as electrostatic interactions, van der Waals force, and hydrogen bonding which hold these structures together. Alteration of any of these weak forces shifts the delicate balance of these protein structures and destabilizes the proteins [54]. The physical and chemical stability of these proteins can be altered by external factors such as pH, temperature, pH, ionic strength, high pressure, metal ions, non-aqueous solvents, adsorption, detergents, and agitation.

Solid lipid particulate systems such as solid lipid nanoparticles (LNs), lipospheres, and lipid microparticles have been used as an alternative delivery system for therapeutic peptides, antigens, and proteins, and research in this regard confirms that under optimized conditions, these particulate systems could be produced to incorporate hydrophilic or hydrophobic proteins and could serve as a particulate carrier system. Proteins and antigens meant for pharmaceutical purposes may be encapsulated or adsorbed onto the particulate system and administered by various routes such as through parenteral, oral,

nasal, or pulmonary routes. The encapsulation of the proteins in the particulate system adds to the stability of the protein, avoids proteolytic degradation, and also ensures a sustained release of the incorporated material [51]. In application, lipid particulate systems have been used as a delivery system of drugs where higher amounts of the drug were found in the brain after intravenous injection, suggesting the potential use of the particulate system in delivering drugs to the brain without crossing the blood-brain barrier [55].

Antitumor drugs delivery

Some of the characteristics which prompted the use of nanoparticles for the delivery of antitumor drugs include the following: (a) delivery of reasonable amount of drug within the surrounding of the target tumor through the enhanced retention and permeability or ligands active targeting on the surface of the nanoparticles and (b) reduction of the exposure of healthy cells and tissues to the drugs through the limitation of drug distribution to only target tissues and organs. Researchers have demonstrated that the hydrophobicity, biodegradation profile, drugs molecular weight, localization in the nanosphere, mode of incorporation and absorption have an influence on how drugs attached to nanoparticles are distributed inside the body. Although the major mechanism is yet to be fully understood, the biodistribution of nanoparticles is fast and could be completed within 60-180 minutes, prompting that the activity of the mononuclear phagocytic system (MPS) and the endocytosis/phagocytosis process might be involved. Such propensity of nanoparticles biodistribution gives the opportunity to efficiently deliver drugs to the target cells without adverse effect to the surrounding healthy cells and tissues. The effectiveness of the biodistribution of drugs through the nanopathway could be of importance in the chemotherapeutic treatment of organs laden with MPS-localized tumors such as hepatocarcinoma and hepatic metastasis hepatic metastasis originating from digestive tract, bronchopulmonary tumors, gynecological cancers, primitive tumors and metastasis, myeloma, small cell tumors, and leukemia [56,57].

Dermatology drug delivery

In the field of nanotechnology, manufacturing of elaborate nanosized particles for several applications has been allowed in recent times. Some of the new applications of this technology include the controlled release of drugs to skin and its appendages, transcutaneous vaccination, hair follicle (HF) targeting, and transdermal gene therapy. The delivery systems of this new generation carrier take the advantages of an enhanced skin penetration potentials, surface functionalization, and depot effect with sustained drug release, thereby allowing targeting of specific cells. The treatment of various types of skin diseases could be revolutionized through the use of nanomaterials as the delivery systems [57].

Of particular interest in the field of dermal drug delivery through the nanopathway is delivery with LN. This has been demonstrated to be effective for the treatment of the diseases of the HF, for an increased local bioavailability of active pharmaceutical ingredient (API) at their drug target. Nelson *et al.* identified that isotretinoin can cause the arrest of a cell cycle as well as apoptosis in sebocytes [58], whereas Messenger and Rundegren reported that minoxidil activated the synthesis of the vascular endothelial growth factor and prostaglandin in the dermal papilla [59], cyclosporine A was also reported by Takahashi and Kamimura to support the growth of hair epithelial cell growth [60]. In dermal therapy, the major objective is to reduce the systemic side effects of drugs through local administration of the API. The idea of using nanotechnology for the delivery of these drugs is already making gains and further development guarantees effective drug delivery to target cells without adverse effects on the surrounding healthy cells.

In cosmetics

Studies have shown promising areas of nanoparticles been used in several cosmetic products such as deodorant, toothpaste, soaps,

shampoo, anti-wrinkles, hair conditioners, moisturizing creams, foundation, lipsticks, face powder, eye shadows, nail polishes, and perfumes. Nanoparticles (nanostructured lipid carriers) have been shown as a potential next-generation cosmetic delivery agent capable of providing improved skin hydration, stability of the agent, bioavailability, and controlled occlusion [61].

Other applications of nanotechnology in cosmetics are as follows:

- a. Sunscreens
Gibson *et al.* studied advances in diffuse optical imaging with emphasis on ultraviolet (UV) filters and concluded that filters such as titanium dioxide (TiO₂) and zinc oxide can be used in the nanoscale instead of higher forms to make sunscreens more transparent instead of being white [62].
- b. Breast cream
In the formulation of its breast cream using herbal products, St Herb Nano Breast Cream claims to have used a combination of nanomaterial and *Pueraria mirifica*, a Thai herbal plant during the production of the cream [63].
- c. Hair care
In hair care, the RBC Life Sciences Nanoceticals have used nanoclusters in the formulation of its citrus mint shampoo and conditioners, which confer hair with a healthy shining look [64,65].
- d. Make-up
The use of nanodispersion technology has aided Serge Lutens Blusher to produce an extremely fine and light powder which possesses extraordinary properties with excellent elasticity, light diffusion, and extreme softness [65].
- e. Moisturizers/anti-wrinkles
The application of nanotechnology in cosmetics has helped in the manufacture of various moisturizing creams and anti-wrinkles. Lancôme encapsulated triceramide in its Hydra Zen Cream to renew the healthy look of the skin healthy, while L'Oreal Revitalift Double Lifting anti-wrinkle cream contains nanosomes of pro-retinol A [65].

This is evidence of the application of nanotechnology not only in the treatment of diseases but also in the production of life-aiding products.

NANOPARTICLES WITH PHARMACOLOGICAL POTENTIALS

Various studies have been carried out on the pharmacopotential of various materials when used as nanoparticles. Most of the nanomaterials (sized >100 μm) when reduced to the nanoparticles (<100 μm) exhibited pharmacological properties which when tested against various cell lines and were able to inhibit or reduce the growth of major human cancer cell lines. The materials investigated showed antitumor, antioxidant, as well as antibacterial potentials. A mini review of some nanoparticles and their recent application for pharmacological benefits was carried out here with the intent of identifying the pharmacodynamics surrounding their application. This review should not be seen as an exhaustive review of all the nanomaterials of pharmacological potential because there are so many reviews and works covering various aspects of this topic.

Cerium oxide nanoparticles

Cerium oxide is an oxide of the rare earth metal cerium, which is a pale yellow to white powder with the chemical formula CeO₂. Cerium oxide is an important product commercially and exists as an intermediate during the purification of the element from the ores. The cerium (IV) oxide is formed through the calcination of cerium oxalate or cerium hydroxide at a high temperature. The mechanism of action and biological properties of cerium oxide is well understood owing to the fact that its ions switch between two valence state (Ce³⁺ and Ce⁴⁺). This two-valence state exists on the surface of the cerium oxide when it is synthesized as a nanoparticle. This deficiency in charge resulting from Ce³⁺ is rightly compensated through the formation of oxygen vacancies, which confer to cerium nanoparticles (CNPs), an intrinsic antioxidant property [66-68].

Mechanism of action

The mechanism of action of CNPs shares similarities with most naturally-occurring metalloenzymes, which utilizes the transition metals cofactors such as Fe, Cu, Zn, or Mn to scavenge ROS in cells. CNPs have been shown to mimic the enzyme superoxide dismutase (SOD), when the Ce^{3+} form of it reacts with superoxide, thereby changing to Ce^{4+} while simultaneously reducing the superoxide into hydrogen peroxide [27,69]. Similarly, the oxidation of Ce^{3+} to Ce^{4+} ions permits the scavenging of other reactive species such as hydroxyl radicals [70], NO [71], and peroxynitrite (ONOO) [68]. Consequently, Ce^{4+} can be reduced to Ce^{3+} during the oxidation of H_2O_2 to O_2 , just in the same manner as catalase enzyme [72]. Therefore, the synergistic pair of Ce^{3+} and Ce^{4+} can alternately and reversibly switch forth and back even while scavenging peroxides and superoxide [66,68,73].

When considering the catalase potentials and the SOD activities of CNPs together, it has been suggested that CNPs undergo a complete redox cycle during the scavenging of two superoxides and a hydrogen peroxide molecule [66], which will be a perfect way to limit/eliminate, in a sequential set of energy-free reactions, both superoxide and hydrogen peroxide [67], resulting in the freeing of the cells from the most abundant ROS. This ability of CNPs to self-regenerate antioxidants is a potential pharmacological tool, which will portray CNPs as a unique and attractive biological scavenger.

Recent application in pharmacological studies

During the study of the ability of cerium oxide nanoparticles to stimulate the proliferation of primary mouse embryonic fibroblasts *in vitro*, Popov *et al.* demonstrated that citrate-stabilized cerium oxide nanoparticles promoted the proliferative activity of primary mouse embryonic fibroblasts *in vitro*. They postulated that cerium oxide nanoparticles stimulated the proliferation of a cell in a wide range of concentrations, ranging from 10^{-3} M to 10^{-9} M, through the reduction of intracellular levels of ROS at the lag phase of the cell growth. Finally, they concluded that nanocrystalline ceria could be considered as a basis for the effective and affordable source of supplements during cell culturing [74].

Furthermore, several studies have been conducted with regard to neuropathies, often associated with oxidative conditions. In this regard, CNP pharmacologic potentials were tested on several neuronal models, both with and without the introduction of external oxidative stress sources. Treatment with CNPs was found not to have affected the viability of neurons, rather there was an increment in the life span of the neuron of cells isolated from the spinal cord of the rat [75], as well as protecting the neurons from mechanically induced trauma [76]. Accordingly, the brain lesions caused by oxidative stress in the cases of stroke-induced hypoxia and cerebral ischemia were reduced by CNPs [77]. CNPs have also been tried for the treatment of Alzheimer's disease, which is an oxidation related neurodegenerative disease, occasioned by the formation of beta-amyloid plaques [77]. Study on CNPs loaded with antibodies against beta-amyloids was tested in different cellular models, and the results show that the CNPs actually allowed the preservation of the normal neural cells morphology, while significantly inhibiting neuron agglomeration and apoptosis [78,79].

Platinum nanoparticles (PNPs)

The catalytic properties of the inert noble gasses such as palladium, gold, and platinum, when reduced to the nanoscale, are gaining much research interest for potential application as pharmacological agents [80-82]. PNPs are considered promising materials for the treatment of conditions related to oxidative stress due to their potent antioxidant potential and experiments conducted by Hamasaki *et al.*, and Kajita *et al.* have shown that PNPs can actually interact or react with various ROS such as superoxides and hydrogen peroxide in oxidative-related diseases [83,84]. PNPs also possess SOD activity in a similar way as the CNPs which made them an interesting biological agent [85]. Irrespective of these interesting potentials and abilities of PNPs, Caputo *et al.* stated that the basic mechanism of action of the nanoplatinum and

its catalase-like activity still remains to be either investigated or even postulated [86].

Recent application

Hamasaki *et al.* [83] investigated the use of PNPs in the scavenging of UV-induced ROS in HeLa cells and found that they were able to preserve the viability of the cells [83], while Onizawa *et al.* noted that the PNPs were able to shield experimental mice exposed to extracts from cigarette smoke extract, thereby reducing the development of neutrophilic inflammation in the lungs of the mice [87]. Furthermore, owing to their anti-inflammatory properties, the PNPs were found by Kim *et al.* to modulate the activation of the redox-sensitive NF-kappa B, as well as displaying interesting antiangiogenic properties which are associated with its SOD and catalase-like activities and significantly extending the lifespan of *Caenorhabditis elegans* [88,89].

PNPs verses CNPs

Not long ago, Clark *et al.* compared the antioxidant properties of PNPs and cerium oxide nanoparticles, employing similar quantities of CNPs and PNPs with an average diameter of 16 and 1.9 nm, respectively. The study found that at an equal doses of 48 mg/mL, the CNPs were able to show higher *in vitro* SOD activity when compared to the PNPs which showed a lower SOD activity and also that the CNPs were more effective than platinum NPs in the protection of the human breast fibrosarcoma cells from cell death induced by the activities of hydrogen peroxide [90].

Zirconia nanoparticles

Research in the area of the behavior of acids has found that just the strength of an acid does not matter during selection but the type of acidity possessed (Brønsted or Lewis) which actually matters for an improved acidity and selectivity. Brown and Hargreaves in 1999 noted that the introduction of super acidity on acids improves its activity and increases its selectivity [91]. The successful inclusion of the sulfate group on the zirconia skeleton has improved its application which has projected sulfated zirconia as a potential particle of great importance in many reactions, which tends to be improved when the size of the sulfated zirconia is reduced to nanosize [92,93]. The antioxidant, antibacterial, and antitumor potentials of sulfated zirconia have been investigated and reported recently [8], which opened the way for more studies on the other applications of the sulfated zirconia in nanosize for pharmacological benefits.

Recent application

The cytotoxicity potential of sulfated zirconia nanoparticles was investigated recently by Mftah *et al.* on human breast cancer (MCF-7), human liver cancer (HepG2), human colon cancer (HT29), and normal human breast (MCF-10a) cells. The investigation using sulfated zirconia nanoparticles showed that the particles have significant cytotoxic effects against the HT29 cells at almost every tested concentration, while investigations with the MCF-7 and HepG-2 cells showed that at a concentration of 15.6 $\mu\text{g/mL}$, the particles had no significant adverse effect on the growth of the cells, while inhibiting the growth of all cell lines [8]. The study also reported that the IC_{50} values of sulfated zirconia nanoparticles against tested cell lines showed that the nanoparticles were more active against the HT-29 cells with an IC_{50} value of 26.4 $\mu\text{g/mL}$, while the HepG-2 and MCF-7 cells showed IC_{50} values of 61.8 and 81.8 $\mu\text{g/mL}$, respectively. The normal cells MCF-10a cells which were also tested as controls showed the least sensitivity to the sulfated zirconia nanoparticles, having an IC_{50} value of 151.63 $\mu\text{g/mL}$, compared to the breast cancer MCF-7 cells which were more sensitive, with a reported IC_{50} value of 89.9 $\mu\text{g/mL}$ [8]. This is evidence of the antitumor potential of sulfated zirconia nanoparticle, demonstrating high sensitivity against tumor cells while at the same time showing lesser side effects to the normal cells.

The antimicrobial potential of sulfated zirconia was also tested against a range of microorganisms which are responsible for various human diseases. It was reported by Mftah *et al.* that the sulfated zirconia

nanoparticles presented powerful antimicrobial activity against Gram-negative and Gram-positive bacteria tested. *P. aeruginosa* and methicillin-resistant *S. aureus* (MRSA) were reported as the most sensitive organisms, followed by *Salmonella choleraesuis* and *B. subtilis*. Against the fungal elements tested (*C. albicans*), antifungal activity was not detected, showing that the organisms were resistant to the activities of the particles [8]. Following the reported activity of sulfated zirconia nanoparticles against the selected microorganisms and tumor cells, it stands to reason that sulfated zirconia has a potential application in the field of pharmacological studies for further investigation, understanding, and utilization of its pharmacological attributed for human benefits.

THE INTERACTION BETWEEN NANOMATERIALS AND THE HUMAN IMMUNE SYSTEM (NANOTOXICOLOGY)

To defend the body against a host of invaders, the immune system employs various mechanisms such as complex detection, communication, and execution systems to protect and defend the organism from diseases. The two most recognized immune systems are the innate and the adaptive immune systems, both working in a specialized and functionalized pattern to ward off infection [34]. Evidence of a cross-link between the specialized cells of the two immune systems exists, such as the neutrophils which are innate cells but also involved in the workings of the adaptive immune system [35]. Farrera and Fadeel had proposed, during the study of the relationship between the immune system and engineered nanomaterials, that most, if not all, of the adverse effects of engineered nanomaterials are exerted through direct effects on the cells of the innate system. The cells implicated here were listed as the dendritic cells (DCs), the macrophages, the phagocytes, and the antigen-presenting cells [94]. A brief review of the interaction of nanomaterials with the two recognized human immune systems is carried out here for a better understanding of the interaction because it takes two to tango [94].

Nanomaterials interaction with the innate immune system

Phagocytosis is the major process through which the innate immune system recognizes process and responds to invading allergens. The understanding of the major processes involved in the interaction of phagocytosis with nanomaterials as well as the different population of cells which made up the cell populations and how they are activated is essential. Macrophages, when activated, can be divided into classically activated macrophages M1 or alternatively activated macrophages M2 [95]. M1 macrophages are more pro-inflammatory and leaned toward the killing of pathogens, while M2 macrophages are responsible for anti-inflammatory, tissue repair, and wound healing. The presence of Th1 cytokines has the ability to polarize macrophages toward the M1 phenotype, and the Th2 immune responses can induce macrophage polarization toward the M2 phenotype. Recently, studies by Jones *et al.* demonstrated that experimental mouse that is prone to Th1 immune response cleared introduced nanoparticles at a slower rate than rats prone to Th2 [96]. In the human sense of application, the study showed that the nature or status of the human immune system can determine how the body can clear nanoparticles when introduced into the body [94].

Nanoparticles have been reported to cause undesired effect, mainly caused by the impairment of phagocytic activities. The nanoparticle, SWCNTs, for instance, has been investigated by Witasp *et al.* and found to cause impairment in the ability of macrophages to engulf dead cell targets [97]. Similarly, ultrafine carbon particles have been shown to impede the ingestion microorganisms by the macrophages of human alveolar [98]. The exposure of macrophages to nanoparticles has been shown to cause an impaired ability of the activated macrophages to transition from M1- to M2-like state which was associated with a reduced phagocytic activity of the macrophages toward some bacteria. It was however concluded that the biological effects of nanoparticles may be indirectly manifested solely after challenging the normal cell function, such as clearance of bacteria [99].

Nanomaterials interaction with the adaptive immune system

A basic knowledge of the composition of the adaptive system and how it works will help in the understanding of how it interacts with foreign bodies for either recognition or elimination. The major function of the adaptive immune system is to proffer antibody-mediated immunity against foreign bodies. It is constituted primarily with the Bursa of Fabricius (B) cells and the thymus-derived (T) cells. These cells are further specialized with the B-cells being responsible for antibody-mediated (humoral) immunity while the T-cells are more involved in the direct engagement (cell-mediated) with foreign bodies. The T-cells are further functionalized according to the nature of their role in body defense. The T-helper cells also called the CD4⁺ are involved in boosting antibodies production from the B-cells, while the T-helper cells are further classified into Th1, Th2, and Th17 depending on their cytokine profiles and role. Cytotoxic T-cells or CD8⁺ is involved in the direct killing of viral-infected and malignant cells, but the maintenance of the immune regulatory function is done by the regulatory T-cells (the "self" and "non-self" recognition process). The DCs form the link between the innate and the adaptive immune system, which are effective phagocytic cells that can as well possess the capacity to process and present an antigen for recognition [100].

The targeting of the DCs may have advantages and disadvantages as well. Rightful targeting of the DCs might be beneficial in the area of vaccination, whereas an undesired targeting of DCs might lead to toxicity, which might even include nanomaterial toxicity. Studies have shown that nanoparticles may alter the role of DCs and consequently affect B- and/or T-cells. For instance, multi-walled carbon nanotubes (MWCNTs) have been reported by Laverny *et al.* to alter the ability of the human monocytes to differentiate into DCs [101]. In addition, Tkach *et al.* demonstrated that the pulmonary exposure of mice to SWCNTs induced reduced proliferation of T-cells of the spleen although the direct effects the nanotubes had on the DCs [102].

The introduction of nanomaterials can either stimulate or suppress the function of the immune system as demonstrated by Mitchell *et al.*, who found out through their investigation with animal models that the inhalation of MWCNTs caused a systemic suppression of the mice immune system, causing a decreased T-cell proliferation rate in the spleen, believed to be caused by a signal from the spleen, most likely the transforming growth factor-beta secreted by alveolar macrophages. As already mentioned, nanoparticles have been shown to influence T-cell proliferation, typically through an effect on antigen-presenting cells, leading to an enhanced T-cell stimulatory capacity [103-107].

To show the effect or tolerance of nanoparticles in human models, Schanen *et al.* demonstrated that when human was exposed to TiO₂ it caused an elevated level of inflammatory cytokines, with an increased maturation of the DCs. In addition, the material (TiO₂) when in nanosize effectively primed the activation and proliferation of the native CD4⁺ T-cells, which were more effective than the same material when applied in the micrometer size [108]. All foreign materials introduced into the body elicit a kind of immune reaction and the outcome of such reaction determines how beneficial or harmful the material could be. Nanoparticles with more benefits are currently considered for pharmacological benefits considering the potential gains which outweigh the hazards.

THE PROPOSED SYNERGISTIC COMBINATION

A beneficial synergistic antitumor and antibacterial activities against bacterial and tumor cells could be the possible outcome of a combination/encapsulation of bioactive extracts from mushrooms onto the nanoparticles. The encapsulation process could be done either during the synthesis of the nanoparticle through incorporation into the nanoparticle skeleton or after the synthesis through the addition of a carrier on the surface of the nanoparticle. This is the basis for this review to serve as an eye opener on the possibility of achieving a greater pharmacological activity through the encapsulation of bioactive compounds on nanoparticles for an enhanced activity.

CONCLUSION

Nanomaterials possess both intrinsic and acquired identities, while the immune system is made up of the innate and adaptive systems, and it could be simply presumed that the interactions between nanomaterials and the immune system could be reciprocal in the sense that when the body is exposed to the nanomaterial, it may facilitate inflammation which involves the innate immune cells, which may also in some cases, digest the nanomaterials, leading to the reduction of its toxicity [109]. Encapsulation of the bioactive extracts from mushrooms onto the nanoparticle structure could proffer a synergistic pharmacological activity against targeted bacterial and tumor cells.

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