

Nutrients and Nutraceuticals Content and *In Vitro* Biological Activities of Reishi Mushroom (*Ganoderma lucidum*) Fruiting Bodies

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ABSTRACT

The present study aims to determine the nutrients and nutraceuticals content and *in vitro* biological activities of reishi mushroom (*Ganoderma lucidum*) fruiting bodies. Data of the nutrients composition of *Ganoderma lucidum* powder indicated that crude fiber and carbohydrates were the most largest compounds (50.19 and 37.33%, respectively) followed by total protein (8.54 %), ash (2.03 %) and crude fat (1.91%). Also, *Ganoderma lucidum* powder is rich in different estimated elements (K, P, Ca, Mg, Na, Fe, Zn, Mn, Cu and Se) and vitamins (A, B1, B2, B3, B6, B9, C and E). Furthermore, nutraceuticals (bioactive compounds) content of *Ganoderma lucidum* powder indicated that terpenoids were the most largest compound (2541 mg linalol.100 g⁻¹) followed by polysaccharides (1635 mg starch equivalent. 100 g⁻¹), phenolics (572.67 mg gallic acid equivalent. 100g⁻¹), triterpenoids (495 mg ursolic acid.100 g⁻¹), Flavonoids (279.34 mg catechin.100 g⁻¹), and Lycopene (253.11 µg.100 g⁻¹). The different extracts of *Ganoderma lucidum* samples particularly ethanolic and methanolic also recorded several very high biological activities which include antioxidant activity, scavenging of free radicals, inhibition of low density lipoprotein oxidation (anti-atherosclerotic) and as antibacterial and antifungal. Such important biological effects could play important roles in strategies to combat/ prevent/treat many diseases, especially those for which oxidative stress is one of the mechanisms for its occurrence i.e. diabetes, cancer, atherosclerosis, cardiovascular diseases etc. Therefore, the present study recommended like of that *Ganoderma lucidum* fruiting bodies powder and/or extracts to be included in our daily diets, drinks, food supplementation and pharmacological formulae.

Keywords: *Ganoderma lucidum*, vitamins, minerals, bioactive compounds, anti-atherosclerotic, free radicals scavenging, antibacterial, antifungal.

INTRODUCTION

Various countries of the world, including Egypt, are currently suffering from a large food gap, which means the disproportion between the necessary food quantities and the number of the population (Madi, 2014). Such food gap leads the country to import food from abroad to compensate for this shortage in order to avoid the

emergence of under/malnutrition diseases among some classes of society. Among the proposed solutions, which received great popularity locally and globally, to bridge the food gap is the search for new sources of food. On the top of these sources are the plant organisms known as algae, due to their rapid growth and reproduction in various climatic conditions, their lack of need for special care treatments, and their richness in important nutrient groups such as protein, high-quality fats, vitamins and minerals (Stojkovic *et al.*, 2014a; Sumaira *et al.*, 2016). On the other hand, it is rich in various groups of bioactive compounds with important biological activities, which play a major role as natural sources of medicine instead of costly chemically manufactured treatments/medicines, which may cause health complications and dangerous side effects that result from their use. All of these factors and others make algae an affordable and accessible source of nutritional ingredients, as well as a promising source of pharmaceutical compounds.

The reishi mushroom (*Ganoderma lucidum*) is a whiteout, wood-decaying fungus that is classified within the family *Ganodermaceae* of Polyporales which shows hard fruiting bodies (Chang, 1995;Wasser and Weis, 1999). *Ganoderma* species are found all over the world, and different characteristics (Zhao and Zhang, 1994). They grow either as biotrophes on live trees, or necrotrophes on dead trees, logs and stumps. *Ganoderma* species can survive under hot and humid environments such as sub-tropical and tropical regions (Moncalvo and Ryvarden, 1997).



Reishi mushroom (*Ganoderma lucidum*) fruiting bodies

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In Egypt, specifically in 2015, two *Ganoderma* species causing butt rot were collected and isolated from *Ficus bengalensis* tree at Mansoura and *Citrus limon* trees at Damietta (El-Fallal *et al.*, 2015). For conserving the two *Ganoderma* species, they were cultivated in sawdust bags of citrus, beech and spruce trees for the first time in Egypt. They can be cultivated in local agro-industrial residues and are considered as a promising contribution to the records of successfully domesticated wild nutraceutical mushrooms. Artificial cultivation of *Ganoderma lucidum* has been achieved using substrates such as grain, sawdust, wood logs (Chang and Buswell, 1999; Wasser, 2005; Boh *et al.*, 2007), and cork residues (Riu *et al.*, 1997). Worldwide consumption is now estimated at several thousand tonnes, and the market is growing rapidly. The global reishi mushroom market was valued at \$3,096.9 million in 2019, and is projected to reach \$5,059.4 million by 2027, registering a compound annual growth rate (CAGR) of 8.1% from 2021 to 2027 (Bhavana and Roshan, 2022). Also, the powder reishi mushroom segment accounted for the highest share in the reishi mushroom market size.

Chemical and nutritional studies indicated that *Ganoderma lucidum* contains mainly protein, fat, carbohydrate and fiber (Stamets, 2000; Hung and Nhi, 2012). Artificially cultivated variety has similar contents of nutritional components compared with wild types, and the extraction significantly increases the amounts of crude protein and carbohydrates and deleted crude fiber. Also, Mizuno (1995) reported the composition of *Ganoderma lucidum* extract (% of dry weight), which consisted of folin-positive material (68.9%), glucose (11.1%), protein (7.3%), and minerals (10.2%). However, there are qualitative and quantitative differences in the chemical composition of *Ganoderma lucidum* products depending on the strain, origin, extracting process, and cultivation conditions (Hobbs, 1995; Stamets, 2000; McKenna *et al.*, 2002; Hung and Nhi, 2012). Beside the all mentioned nutrients, the fruiting body, mycelia, and spores of *Ganoderma lucidum* contain approximately 400 different bioactive/phytochemical compounds, which mainly include triterpenoids, polysaccharides, nucleotides, sterols, steroids, fatty acids, proteins/peptides, and trace elements (Mizuno, 1995; Paterson, 2006; Ihayere *et al.*, 2010; Wasser, 2005). For the unique content of bioactive compounds and their biological roles, *Ganoderma lucidum* has been reported to have a number of pharmacological effects including immunomodulating, antiatherosclerotic, anti-inflammatory, analgesic, chemopreventive, antitumor, radioprotective, sleep-promoting, antibacterial, antiviral (including anti-HIV), hypolipidemic, antifibrotic, hepatoprotective, diabetic, antioxidative and radical-scavenging, anti-aging, hypoglycemic, and anti-ulcer

properties (reviewed in Liu, 1999; McKenna *et al.*, 2002; Gao *et al.*, 2005; Jiang *et al.*, 2005; Ofodile *et al.*, 2005; Wasser, 2005; Elsemelawy *et al.*, 2021). So, reishi mushroom is a type of mushroom that has been used in Chinese medicine for approximately 200 years (Wachtel-Galor *et al.*, 2011).

Although all the previous studies and others dealt with many of the biological effects of *Ganoderma lucidum*, there is still a need to conduct more and more research to explore other roles that this important food source can play. Therefore, the present study aims to determine the chemical composition, nutritional evaluation and bioactive compounds in *Ganoderma lucidum*. Also, the different biological activities including antioxidant and scavenging activities, inhibition of lipid oxidation, and antimicrobial effects (*in vitro*) of such mushroom will be in the scope of this investigation.

MATERIALS AND METHODS

Ethical approval

Biological model, rats, used in experimental design of the study was ethically approved by the Scientific Research Ethics Committee (Animal Care and Use), Faculty of Home Economics, Menoufia University and Shebin El-Kom, Egypt (Approval no. 05- SREC- 01-2020).

Materials

Ganoderma lucidum samples

Dried reishi mushroom (*Ganoderma lucidum*) fruiting bodies samples were purchased from Agricultural Seeds, Spices and Medicinal Plants Company (Harras), El-Darb El-Ahmar, Cairo Governorate, Egypt. The samples were verified by the staff in Faculty of Agriculture, Minoufiya University, Shebin El-Kom, Egypt.

Chemicals

Bioactive compounds standard [gallic acid (GA), catechine (CA), lycopene, α -tocopherol, linalool, ursolic acid and butylated hydroxytoluene (BHT), DDPH (2,2-diphenyl-1-picrylhydrazyl), AAPH [2,2'-Azobis(2-methylpropionamide) dihydrochloride], CuSO₄ and dimethyl sulfoxide (DMSO) were purchased from Sigma Chemical Co., St. Louis, MO. All other chemicals (Except as otherwise stated), reagents and solvents were of analytical grade were purchased from El-Ghomhorya Company for Trading Drug, Chemicals and Medical Instruments, Cairo, Egypt.

Machines

Throughout this study absorbance for different assays were measured using UV-160A; Shimadzu Corporation, Kyoto, Japan. Also, atomic absorption spectrophotometer, type Perkin – Elmer, Model 2380,

Waltham, MA, USA was used for mineral determination. Furthermore, SP Thermo Separation Products Liquid Chromatography (San Jose, CA, USA) was used for vitamins determination.

Biological model

Adult male albino rats (130±10 g body weight per each) were obtained by special arrangement from Laboratory Animal Colony, Vaccine and Immunity Organization, Cairo, Egypt. Rats were housed, maintained, kept under normal healthy conditions in accordance with the National Research Council's Institute of Laboratory Animal Resources, Commission on Life Sciences rules (NRC, 1996). The basic diet for rats feeding was prepared according to the formula as mentioned by AIN (1993).

Methods

Preparation of *Ganoderma lucidum* fruiting bodies extracts

Twenty gram of *Ganoderma lucidum* dried powder were extracted with 80% aqueous solvents i.e. methanol and ethanol as well as water (180 ml) on an orbital shaker (Heathrow Scientific, Heathrow, UK) for 120 min at 70 °C for the organic solvents and 120 min at 100 °C for the water. The mixture was subsequently filtered (Whatman No. 5) on a Buchner funnel. The residual solvents were removed under reduced pressure at 55°C for water and 40°C for organic solvents using a rotary evaporator (Heathrow Scientific, Heathrow, UK). The obtained extracts were stored at 4 °C before use.

Chemical analysis of *Ganoderma lucidum* fruiting bodies powder

Ganoderma lucidum fruiting bodies samples were analyzed for proximate chemical composition including moisture, protein (T.N. × 6.25, micro - kjeldahl method using semiautomatic apparatus, Velp company, Italy), fat (soxhelt miautomatic apparatus Velp company, Italy, petroleum ether solvent), ash, fiber and dietary fiber contents were determined using the methods described in AOAC (2000). Carbohydrates calculated by differences: Carbohydrates (%) = 100 - (% moisture + % protein + % fat + % Ash + % fiber).

Determination of nutritional value of *Ganoderma lucidum* fruiting bodies

Total energy value

Total energy (Kcal/100 g) of *Ganoderma lucidum* fruiting bodies powder samples was calculated according to Manzi *et al.* (2004) using the following equation: Total energy value (Kcal/100 g) = 4 (Protein % + carbohydrates %) + 9 (Fat %)

Satisfaction of the daily needs of adult man (25-50 year old) in protein

Grams consumed (G.D.R. g) of *Ganoderma lucidum* fruiting bodies powder (dry weight basis) to cover the daily requirements of adult man (63 g) in protein was calculated using the RDA (1989) values. Percent satisfaction of the daily requirement of adult man in protein (P.S., %) when consuming the possibly commonly used portions in Egypt i.e. one bag (100 g weight), was also calculated.

Satisfaction of the daily requirements of adult man (25-50 year old) in energy

Grams consumed of *Ganoderma lucidum* fruiting bodies powder (dry weight basis) to cover the daily requirements of man in energy (G.D.R. g) were calculated using the RDA (Recommended dietary allowances) which are 2900 Kcal /day for man as given by RDA (1989). The percent satisfaction (P.S., %) of the daily needs of adult man (25 -50 year old, 79 Kg weight and 176 cm height) in energy upon consumption the commonly used portion at homes in Egypt, i.e. one bag (100 g weight), was also calculated.

Determination of minerals content

Minerals content of *Ganoderma lucidum* samples were determined according to the method mentioned by Singh *et al.* (1991) as follow: 0.5 g of defatted sample were transferred into a digested glass tube of Kjeldahl digestion unit and 6 ml of tri-acids mixture (containing nitric acid: perchloric acid : sulfuric acid in the ratio of 20 : 4 : 1 v/v respectively) were added to each tube. The tubes content were digested gradually as follow, 30 min at 70 °C; 30 min at 180 °C and 30 min at 220 °C. After filtration in ashless filter paper, aliquots were analyzed for minerals (K, Na, Zn, Ca, Mg, Mn, Fe, Se, Cu and P) using of atomic absorption spectrophotometer.

Vitamins determination

Fat soluble vitamins (A and E) were extracted from the *Ganoderma lucidum* according to the methods described by Epler *et al.* (1993) and Hung *et al.* (1980) while water soluble vitamins (B and C) according to Moeslinger *et al.* (1994), and analyzed by HPLC techniques. Under the chromatographic conditions used in those methods, mean values ±SD of vitamins A, C, B₁, B₂, B₃, B₆, B₉ and E recoveries were 90.21± 3.12, 88.23 ±1.54, 86.89± 3.76, 85.45 ±0.99, 86.10±3.01%, 84.33± 4.95, 89.01 ±1.87 and 88.63±2.23%, respectively.

Bioactive compounds determination

Total phenolics in brown algae *Ganoderma lucidum* extracts were determined using Folin-Ciocalteu reagent according to Singleton and Rossi (1965) and Wolfe *et al.* (2003). Results are expressed as gallic acid and equivalents (GAE). The total carotenoids in 80%

acetone extract were determined by using the method reported by Litchenthaler (1987) and was expressed as μg of carotenoid/g of dry extract. Lycopene was determined such as described by Anthon and Barrett (2007) and was expressed as $\mu\text{g/g}$ of dry extract. Total flavonoids contents were estimated using colorimetric assay described by Zhishen *et al.* (1999) and expressed as catechin equivalent, CAE (standard curve equation: $y = 0.0003x - 0.0117$, $r^2 = 0.9827$), mg of CA/g of dry extract. Total polysaccharides were extracted and measured according to the method of Vazirian *et al.* (2014). Starch was used as a standard and the results were expressed as mg of starch equivalents per g of dw. Total terpenoids were extracted and measured according to the method of Ghorai *et al.* (2012). Linalool was used as a standard and results were expressed in mg of linalool equivalents per g of dw. Total terpenoids were extracted and measured according to the method of Schneider *et al.* (2009). Ursolic acid was used as a standard and results were expressed in mg ursolic acid. 100 g^{-1} .

Biological activities determination

β -carotene bleaching (BCB)

Antioxidant activity (AA) of *Ganoderma lucidum* extracts and standards (α -tocopherol and BHT) was determined according to the BCB assay following a modification of the procedure described by Marco (1968).

DPPH radical scavenging assay

Free radical scavenging activity of *Ganoderma lucidum* extracts was determined by DPPH radical scavenging assay as described by Desmarchelier *et al.* (1997). The percent of inhibition (%) was plotted against concentration, and IC_{50} was calculated by using the graph.

Inhibition of low density lipoprotein (LDL) oxidation

Inhibition of LDL oxidation was determined in *Ganoderma lucidum* extracts according to the method of Princen *et al.* (1992). The final result was expressed by calculation of the net area under the curve.

Antibacterial and antifungal tests

Escherichia coli, *Staphylococcus aureus* and *Candida albicans* (from the collection of the Microbiology Department, Faculty of Agriculture, Damietta University, Damietta, Egypt) were used as test microorganisms. Antibacterial and antifungal activities for *Ganoderma lucidum* extracts were elucidated by the agar cup methods described by Spooner and Sykes (1972).

Statistical Analysis

All tests/measurements were carried out in triplicates and presented as mean \pm standard deviations

(SD). Statistical analysis was done using Student *t*-test and MINITAB 12 computer program (Minitab Inc., State College, PA).

RESULTS and DISCUSSION

Proximate composition of *Ganoderma lucidum* fruiting bodies (% DW)

The results for the proximate composition of *Ganoderma lucidum* are presented in Table 1. Crude fiber was the most abundant compounds, followed by carbohydrates, protein, ash, and fat. The results are not consistent with that reported by Sumaira *et al.* (2016) where the protein, fat, ash, and carbohydrates were 15.04, 0.53, 2.01 and 82.47%, respectively. Also, the results are relatively consistent with the ones reported by Oludemi *et al.* (2017) who noticed that protein, fat and ash were 6.72, 2.50, and 2.40%, respectively. Furthermore, the results are fairly consistent with the ones reported by Stojkovic *et al.* (2014) who studied the proximate composition of wild and cultivated *Ganoderma lucidum* from Serbia and China. Regarding the fiber content, the results of the present study indicate that *Ganoderma lucidum* is a good source of fiber ($50.19 \pm 3.91\%$). Similar studies indicated that there is a great variation in the fiber contents of five mushrooms ranging from 6.11-54.12% but the maximum fiber content (54.12%) was observed in *Ganoderma lucidum* (Sumaira *et al.*, 2016). Major sources of fiber are cellulose and other indigestible cell wall polymers (Mukhopadhyay and Guha, 2015). Regarding the calorie value, *Ganoderma lucidum* recorded 200.67 ± 3.80 Kcal/100g. Such data are fairly inconsistent with that observed by Sumaira *et al.* (2016) who showed that calorie value of five mushrooms ranges from 363-394 Kcal/100 g and the lowest value was in *Ganoderma lucidum*. This low calorie value of *Ganoderma lucidum* is attributable to the content of high fiber, low fat, no free fatty acids and no cholesterol (Zahid *et al.*, 2010). The difference in proximate composition of *Ganoderma lucidum* could be due to a number of factors, namely the type of mushroom, the stage of development, the origin of the samples, level of nitrogen available and the substrate/habitat (Colak *et al.*, 2009; Stojkovic *et al.*, 2014a; and Sumaira *et al.*, 2016). In general, these results indicate that the *Ganoderma lucidum* was good source of different nutrients for humans including carbohydrates, fiber, protein and ash. Also, it is a low fat calorie food subsequently more suitable for humans in diet and cardiovascular diseases. Additionally, *Ganoderma lucidum* is characterized by a high in fiber content. Although fiber is indigestible, it plays significant nutritional role since, it helps to provide bulk

to stool and aid in the movement through the digestive tract.

Table 1. Proximate composition of *Ganoderma lucidum* fruiting bodies

Component	Content
Moisture	8.14 ± 1.12
Dry matter	91.86 ± 1.65
Total protein (g/100g)	8.54 ± 0.87
Crude fat (g/100g)	1.91 ± 0.21
Ash (g/100g)	2.03 ± 0.28
Crude Fiber (g/100g)	50.19 ± 3.91
Carbohydrate (g/100g)	37.33 ± 4.06
Energy (Kcal/100g)	200.67±3.80

Moisture and dry matter were presented based on air-dried weight; others were presented based on dry weight (DW). Each value represents the mean of three replicates ±SD.

Nutritional evaluation of *Ganoderma lucidum* fruiting bodies powder

The nutritional evaluation of the *Ganoderma lucidum* fruiting bodies powder is shown in Table (2). From such date it could be noticed that the total energy was recorded 183.12 ±5.45Kcal/100g, G.D.R. (g) for protein (63 g) was 797.78 ±10.56g, G.D.R. (g) for energy (2900 Kcal) was 1583.63 ±20.67, P.S./ 100 g for protein (63g) was 12.53 ±1.11% and P.S./100 g for energy (2900 Kcal) was 6.31 ±0.85%. The nutritional evaluation reported was partially accordance with that observed by (Barros et al., 2007 and Oludemi et al., 2017). Such data prove that the *Ganoderma lucidum* represents a good source of protein. The consumption of 100 g powder covers 12.53% of the daily requirement of the adult person for energy (63 g). Also, *Ganoderma lucidum* represents low-calorie foods i.e. consumption of 100 g powder cover only 6.31% of the daily requirement of the adult person for energy (2900 Kcal). This is due to their fat content, the high calories component, is generally low. Such data confirm the possibility of successfully using *Ganoderma lucidum* in nutritional applications for obese and overweight patients.

Table 2. Nutritional value of *Ganoderma lucidum* fruiting bodies powder

Parameter	Value
Total Energy (Kcal/100g)	183.12 ±5.45
G.D.R. (g) for protein (63 g)	797.78 ±10.56
G.D.R. (g) for energy (2900 Kcal)	1583.63 ±20.67
P.S./100 g (%) for protein (63g)	12.53 ±1.11
P.S./100 g (%) For energy (2900 Kcal)	6.31 ±0.85

* Data expressed as the mean value of three replicates ±SD.

Mineral content in *Ganoderma lucidum* fruiting bodies

All scientific studies indicate that there is a general public interest in the availability of essential and non-essential elements in the foods consumed daily. Therefore, the present study concerned with estimating the mineral content in *Ganoderma lucidum* fruiting bodies (Table 3). Our results of minerals analysis showed that the *Ganoderma lucidum* is rich in different estimated elements. K recorded higher contents followed by P, Ca, Mg, Na, Fe, Zn, Mn, Cu and Se. Such data are in accordance with that reported by Sumaira et al. (2016) except related to Se. Also, Mallikarjuna et al. (2013) studied the elements levels in four mushroom species and reported K, Ca, Na, and P were in higher concentrations.

Minerals such as K, Ca, P and Mg are said to be major elements because they are in high concentrations of *Ganoderma lucidum*. However, Na is relatively less in *Ganoderma lucidum*; thus, mushroom such *Ganoderma lucidum*, is said to be good for patients with hypertension. Similar observations were made by Rajarathnam et al. (1998). Iron, zinc, copper, manganese, and selenium are dealt with under minor/trace elements. *Ganoderma lucidum* found in a reasonable concentration for such elements and were quite comparable with their recommended daily intake (RDI) Cu (2.2 mg/day), Fe (28–30 mg/day), Zn (15.5 mg/day) and Mn (5.5 mg/day) (ICMR, 1990). Although trace metals are found in *Ganoderma lucidum* fruiting body in moderate amounts in their inorganic form, they are found in larger quantities in their organic form mushrooms are known for bioconversion of such minerals from the growth substrate from inorganic form to organic form (Falandysz, 2008). As a source of trace elements, the situation of mushroom becomes quite practical when considered consumption of daily or several times a week by inserting it into different dishes and drinks. All of those trace elements are biologically very vital to the human body through prevention and/or fighting many diseases including anemia, immunodeficiency cancer and artherosclerosis (Forrest, 2000; Lipinski, 2005). Mn has an important role in the metabolism of lipids and lipoproteins and it participates in the pathogenesis of atherosclerosis and numerous other cardiovascular diseases (Daiana et al., 2013). The importance of Zn in the immune system is a consequence of its role in cell growth, division and maturation, cell membrane stabilization, as well as in DNA and RNA synthesis (Osredkar and Sustar, 2011; Nazanin et al., 2013). Cu has an important role in the process of erythropoiesis, maturation and signal-mediated activity of immune cells. Also, it participates in accelerating metabolism and strengthening tissue respiration, contributes to iron resorption in the

digestive tract and catalyzes hemoglobin biosynthesis, helping to incorporate heme iron (Danks, 1988). The main role of Fe, as an integral part of hemoglobin in red blood cells, the transfer of oxygen from the lungs to the tissues of all organs in the body. Also, It is necessary for DNA synthesis and plays an important role in the human immune system (Nazanin *et al.*, 2014).

Table 3. Mineral concentrations in *Ganoderma lucidum* fruiting bodies (mg/100g on dry weight basis)

Minerals	Content	Minerals	Content
K	654.16 ± 22.34	Mn	1.86 ± 0.32
Na	18.56 ± 3.42	Fe	15.87± 2.06
Zn	3.05 ± 0.64	Se	0.94± 0.08
Ca	125.76 ± 4.88	Cu	1.73± 0.11
Mg	76.45 ± 5.16	P	51.75± 31.25

* Data expressed as the mean value of three replicates ±SD.

Vitamins concentrations in *Ganoderma lucidum* fruiting bodies

The vitamins concentration of *Ganoderma lucidum* is given in Table 4. Vitamin B3 was the most abundant vitamins, followed by vitamins B2, B1, folate, C, A and E. Vitamins including A, B1, B2, B3, b6 and B9 found in high concentration in *Ganoderma lucidum* and were good comparable with their Dietary Reference Intake (DRI) by the rate of 43.38, 114, 169.09, 178.5, 40 and 89.82%, respectively. While other vitamins such C, E and B1 were found in fair concentration and were not comparable with their DRI. Such data are accordance with that observed by Lillian *et al.* (2008) who found that tocopherols, ascorbic acid and carotene were detected in higher amounts in different species of mushroom. Also, the present data are fairly inconsistency with Darija *et al.* (2018) who mentioned that *Ganoderma lucidum* recorded 3.49, 17.10, 61.9, 0.71 and 0.31 mg/100 g of vitamins B1, B2, B3, B6 and B9, respectively. In general, there are dearth of information regarding the vitamins content in mushroom particularly *Ganoderma lucidum*. That limited studies indicated that the difference in vitamins contents of *Ganoderma lucidum* could be due to a number of factors including the type, stage of development, the origin and the method determination (Lillian *et al.*, 2008 and Darija *et al.*, 2018).

In nutritional point of view, vitamins are essential for life because we need them for good health and for growth. *Ganoderma lucidum* is a good source of almost member of vitamin B including B1, B2, B3, b6 and B9. Vitamins B are a diverse group in terms of structure and function. Vitamin B9 (folate) which provide methyl groups necessary for DNA methylation, play an important role in the pathogenesis of neurological diseases (Robinson *et al.*, 2018). It is involved in the

metabolism of several amino acids, including, serine, methionine, glycine and histidine. The roles of folate and vitamin B12 in the conversion of homocysteine to methionine, along with the role of vitamin B6 (Pyridoxine) in the conversion of homocysteine to cystathionine, continue to receive considerable attention because low intakes of these three vitamins (B6, B9 and B12), especially B9 (folate), are inversely associated with plasma homocysteine concentrations, and elevated plasma homocysteine concentrations (>15 µ) are associated with several dangerous diseases including premature coronary artery disease, premature occlusive vascular disease and cerebral or peripheral vascular disease (Verhaar *et al.*, 2002; Kilmer and Kilmer, 2004; Meltem *et al.*, 2017). Another condition being investigated as possibly linked to poor folate status is dementia, including Alzheimer's dementia (Ravaglia *et al.*, 2005). Memory and abstract thinking appear to be influenced by folate. Cognitive dysfunction and dementia have been shown to correlate with plasma homocysteine concentrations, which in turn are influenced in part by folate status (Selhub, 2002 and Anita *et al.*, 2020). Folate deficiency or poor folate status is also suspected in the development (initiation) of some cancers, especially colon and colorectal cancers (Mason and Levesque, 1996; Yacong *et al.*, 2020).

Table 4. vitamins concentrations in *Ganoderma lucidum* fruiting bodies

Component	Content	DRI
Vitamin A (β-carotene, µg/100g)	271.14 ±19.10	625
Vitamin C (Ascorbic acid, mg/100g)	2.98 ±0.23	75
Vitamin E (Tocopherols, mg /100g)	0.36±0.02	12
Vitamin B1 (Thiamine, mg/100g)	1.14 ±0.38	1.0
Vitamin B2 (Riboflavin, mg/100g)	1.86 ±2.33	1.1
Vitamin B3 (Niacin, mg/100g)	21.42 ±7.08	12
Vitamin B6 (Pyridoxine, mg/100g)	0.44 ±0.14	1.1
Vitamin B9 (Folate, µg/100g)	287.45±14. 98	320

* Data expressed as the mean value of three replicates ±SD. DRI, Dietary Reference Intake

Bioactive compounds in *Ganoderma lucidum* fruiting bodies

Total phenolics, flavonoids, lycopene, polysaccharides, terpenoids and triterpenoids were determined in *Ganoderma lucidum* as shown in Table (5). Terpenoids was reported the most abundant ones, followed by polysaccharides, phenolics, triterpenoids, flavonoids and lycopene. Such data are partially in accordance with that observed by Oludemi *et al.* (2017)

who found that polysaccharides, terpenoids, and triterpenoids in *Ganoderma lucidum* were 15.4 mg starch/g, 27.2 ± 0.7 mg linalool/g and 5.6 ± 0.5 mg ursolic acid/g, respectively. Also, Skalicka-Woźniak *et al.* (2012) the polysaccharide content for strains of *Ganoderma lucidum* sourced in Poland was about 18.45 mg glucose equiv/g dw). Additionally, the triterpene content reported in the present work was consistent with the one described by Liu *et al.* (2017) who found that the triterpene amount of *Ganoderma lucidum* was about 5.90 mg ursolic acid equiv/g dw. Many of the previous studies, along with others, have proven that bioactive compounds (Phenolics, flavonoids and lycopene) which was spotted in this study inside *Ganoderma lucidum* play an important vital role in preventing and/or treating many diseases such as diabetes, atherosclerosis, cancer, obesity, bone and aging (Elhassaneen *et al.*, 2016 a,e; 2019; 2020a). All of the previous effects of these compounds are due mainly to their magical antioxidant activities. On the other side, the polysaccharide-mediated potentiation of immune function is thought to be the major mechanism of antitumor action by *Ganoderma lucidum* (Liu, 1999 and Wasser, 2002). Also, ganoderma polysaccharides restored the TNF- α production inhibited by cyclophosphamide to normal levels in mice (Gao *et al.*, 2002). *In vitro* and *in vivo* studies indicate that *Ganoderma lucidum* extracts (mainly polysaccharides or triterpenoids) exhibit protective activities against liver injuries induced by toxic chemicals (Liu, 1999 and Zhou *et al.*, 2002). Furthermore, triterpenoids such as ursolic acid can serve as a starting material for synthesis of more potent bioactive derivatives, such as experimental antitumor agents (Ma *et al.*, 2005). All these factors made the *Ganoderma lucidum* a complete package of healthy food through being an excellent source of nutritional and non-nutritional compounds. When orally ingested it is able to improve body functions and wellbeing. Commercially, there are already available a lot of nutraceutical products containing *Ganoderma lucidum* extracts, either alone or in combination with other mushroom species. For example, Rathore *et al.* (2017) formulated capsules from *Ganoderma lucidum* rich in triterpenes and β -glucans which have been reported to protect the body against oxidative stress and support the immune system.

Biological activities of *Ganoderma lucidum* fruiting bodies extracts

Antioxidant activity

The antioxidant activity of *Ganoderma lucidum* extracts was assayed by β -carotene bleaching (BCB). The decrease in absorbance of β -carotene in the presence of different *Ganoderma lucidum* extracts and references/standards antioxidants with the oxidation of β -carotene and linoleic acid is shown in Figure (1). The *Ganoderma lucidum* extracts showed considerable differences in antioxidant activity (AA). Ethanol extract (EtE) showed strong activity, even equal to α -tocopherol at 50 mg/L and higher than that of BHA mg/L followed by methanol extract (ME) even close to BHA and α -tocopherol at 50 mg/L while aquatic extract (AqE) showed lower antioxidant activity. Our previous studies with the others proved that the BCB method has been also used successively to evaluate the antioxidant activity in various plant parts including algae *in vitro* (Ismail *et al.*, 2004, Barros *et al.*, 2007; Abd Elalal *et al.*, 2021; Elhassaneen *et al.*, 2019, 2021_a and _b; El-Nassag *et al.*, 2019). All of these studies reported that polyphenols, flavonoids, carotenoids, lycopene and polysaccharides content, such as found in a highly content *Ganoderma lucidum* extracts, are highly correlated. So, the difference seen in the degrees of antioxidant activity of *Ganoderma lucidum* extracts probably due to the different content of each of those bioactive compounds. Many studies indicated that such compounds represent the major plant components with antioxidant activity. Also, polysaccharides found in *Ganoderma lucidum* extracts were also reported to protect the immune cells from oxidative damage (Ooi and Lui, 2000). Also, Shi *et al.* (2002) reported that aqueous extract of *Ganoderma lucidum* exhibited highly antioxidant activities which significantly protected cells from hydrogen peroxide (H₂O₂)-induced DNA damage *in vitro*. Furthermore, Sheena *et al.* (2003) mentioned that the antioxidant activities exhibited by *Ganoderma lucidum* methanol extract prevent kidney damage induced by cisplatin through restoration of the renal antioxidant defense system. Data of the present study revealed that ethanol and methanol extracts of *Ganoderma lucidum* had almost similar antioxidant activity when compared with standards, tocopherols and BHT. Therefore, those results suggest that all the extracts from *Ganoderma lucidum* showed antioxidant activity through radicals scavenging.

Table 5. Total content of bioactive compounds in *Ganoderma lucidum* fruiting bodies

Component	Content
Phenolics (mg gallic acid.100 g ⁻¹)	572.67 ± 16.34
Flavonoids (mg catechin.100 g ⁻¹)	279.34 ± 6.87
Lycopene (µg.100 g ⁻¹)	253.11± 7.11
Polysaccharides (mg starch.100 g ⁻¹)	1635± 24.50
Terpenoids (mg linalol.100 g ⁻¹)	2541± 16.72
Triterpenoids (mg ursolic acid.100 g ⁻¹)	495 ± 5.11

* Data expressed as the mean value of three replicates ±SD.

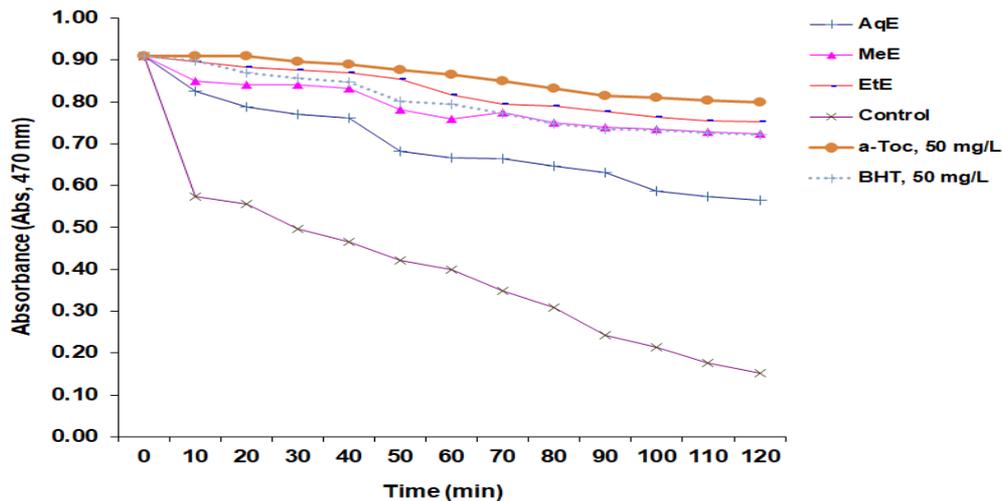


Figure 1. Antioxidant activity of *Ganoderma lucidum* extracts and references/standard antioxidants assayed by the β-carotene bleaching (BCB) method

AqE, aqueous extract, MeE, methanol extract, EtOH, ethanol extract, α-Toc, alpha-tocopherol, BHT, Butylhydroxy toluene

DPPH radical scavenging activity

The free radical scavenging activity of *Ganoderma lucidum* and butylated hydroxy toluene (BHT) as a standard are illustrated in Figure 2 and Table 6. Such data indicated that ethanol extract possessed the highest activity followed by methanol and aquatic ones. At a concentration of 100 µg/mL, the radicals scavenging activity of aquatic, ethanol and methanol extracts were 76.53, 90.72 and 85.94% respectively, whereas, BHT standard was 96.69%. For the IC₅₀, the aquatic, ethanol and methanol extracts were recorded 24.89, 10.39 and 15.11 µg/mL, respectively while BHT standard was 8.54 µg/mL. Therefore, the free radical scavenging activity of *Ganoderma lucidum* extracts and standard was in the following order: standard (BHT) > ethanol extract > methanol extract > aquatic. The theory of the DPPH radicals scavenging activity test is based on measurement of the absorption of diene conjugation (λ_{max}) in the presence of 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Antolovich *et al.*, 2002). Our several previous studies with the others indicated that DPPH assay has

been used successfully to evaluate the scavenging activity of various plant parts including algae (Aaby *et al.*, 2004; Laura *et al.*, 2010; Aly *et al.*, 2017; El-Gamal, 2020; Abd Elalal *et al.*, 2021; Elhassaneen *et al.*, 2021a). Also, those studies proved that the free radical scavenging activity are very important to prevent the adverse role of free radicals in different diseases including obesity, diabetes, cancer, neurological, pulmonary, nephropathy and cardiovascular diseases. The results of the present study suggest that all the *Ganoderma lucidum* extracts showed free radical scavenging activity which due to their rich in different categories of bioactive compounds knowing as antioxidants including phenolics, polysaccharides, carotenoids, flavonoids etc. Such data are in accordance with that observed by Huang *et al.* (2005) and Laura *et al.* (2010). Finally, data of the present study suggest that all *Ganoderma lucidum* extracts showed radical scavenging activity through electron transfer or hydrogen donating ability.

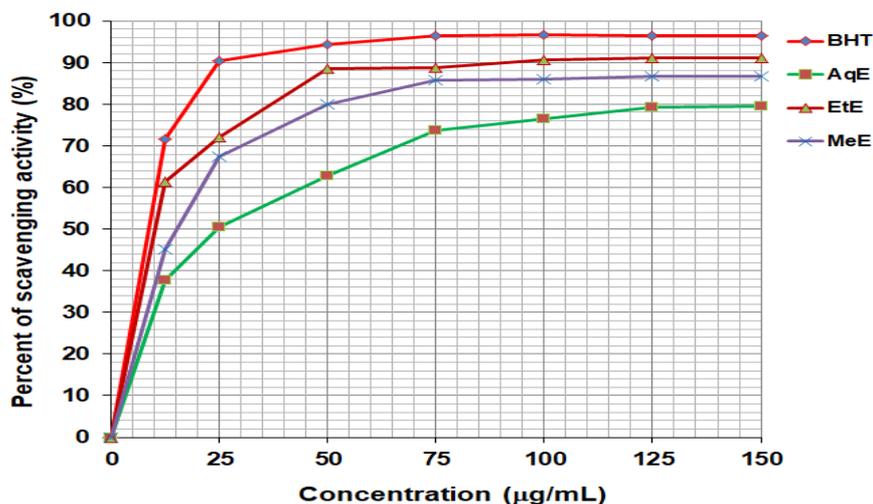


Figure 2. DPPH radical scavenging activity (%) of *Ganoderma lucidum* extracts and standard (BHT)*

* Each value represents the mean value of three replicates. BHT, Butylated hydroxytoluene, EtE, Ethanol extract, MeE, Methanol extract, WE, Water extract

Table 6. IC₅₀ (DPPH) of *Ganoderma lucidum* extracts and BHT (Standard)

Name of sample	BHT	WE	EtE	MeE
IC ₅₀ (µg/mL)	8.54 ± 0.23 ^c	24.89 ± 0.30 ^a	10.39 ± 0.18 ^c	15.11 ± 0.27 ^b

* Each value represents the mean value of three replicates ±SD. Values with different superscript letters in the same row are significantly different at p ≤ 0.05. BHT, Butylated hydroxytoluene, EtE, Ethanol extract, MeE, Methanol extract, WE, Water extract

Inhibition of low density lipoprotein (LDL) oxidation

Data illustrated in Figure (3) shown the dose-dependent inhibition of CuSO₄-induced LDL oxidation *in vitro* by *Ganoderma lucidum* extracts. From such data it could be noticed that the inhibitive action of *Ganoderma lucidum* extracts against CuSO₄-induced LDL oxidation, as evidenced by decreased conjugated dienes (≡) production in a dose-dependent behavior. The comparative study amongst *Ganoderma lucidum* extracts demonstrated that the aquatic, ethanol and methanol extracts acted more dramatically in protecting LDL against oxidation. The protecting LDL against oxidation activity of different *Ganoderma lucidum* extracts was in the following order: ethanol > methanol > water extracts. The present data with the others

proved that such effect could be attributed to the different bioactive compounds as antioxidants (phenolics, polysaccharides, carotenoids, terpenoids, vitamins etc.) contained in such *Ganoderma lucidum* extracts (Plaza *et al.*, 2010; Shinichi, 2011; Aly *et al.*, 2017; El-Gamal, 2020; Abd Elalal *et al.*, 2021; Elhassaneen *et al.*, 2021a).

In general, reactive oxygen species (ROS) cause cellular abnormality/damage membranes (cell wall, mitochondria, lysosomes etc.) by peroxidizing lipid moieties, particularly the polyunsaturated fatty acids in

a chain reaction known as lipid peroxidation (Lien *et al.*, 2008). Thus, the inhibition of lipid peroxidation is considered the most important index of antioxidant activity. Data of the present study show lipid peroxidation inhibition activity of ethyl and methyl extracts of *Ganoderma lucidum* were higher than aquatic extract. These results indicated that *Ganoderma lucidum* extracts can prevent cellular damage caused by free radicals through slump the chain reactions responsible for lipid peroxidation. In this manner, several studies found that phenolic compounds such as detected in *Ganoderma lucidum* extracts exhibited a protecting LDL against oxidation through increasing the levels of reduced glutathione (GSH) and glutathione reductase (GSH-Rd) in liver and lungs as well as increase in inhibition of NADPH-dependent lipid peroxidation (Majid *et al.*, 1991; Elbasouny *et al.*, 2019; El-Gamal, 2020 and Elhassaneen *et al.*, 2020b). Also, various studies reported that the oxidative modification of lipoproteins hypothesis postulates that LDL oxidation plays a key role in early atherosclerosis (Aviram *et al.*, 2000; Chisolm and Steinberg, 2000). The oxidized LDL is atherogenic due to its cytotoxic effects toward arterial cells and stimulates the monocytes to be adhesive to the endothelium which leads to the development of atheromatous plaques (Hong and Cam, 2015).

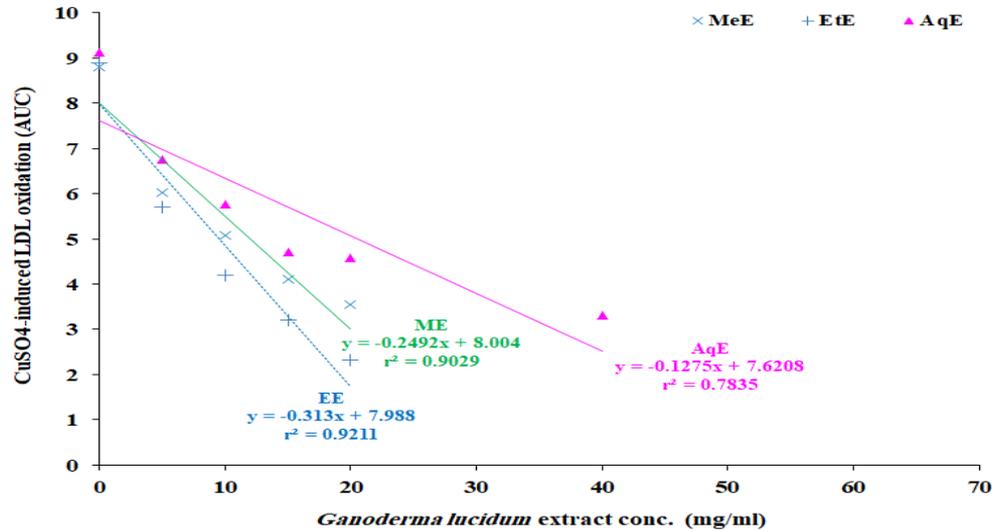


Figure 3. Dose-dependent inhibition of CuSO₄-induced LDL oxidation *in vitro* by *Ganoderma lucidum* extracts. EtE (Ethanol extract), MeE (Methanol extract), AqE (Aquatic extract).

Our present data with the others proved that the *Ganoderma lucidum* extracts could be used successfully as a promising agent in the prevention of atherosclerosis out of inhibiting LDL oxidation process.

Relationship between bioactive compounds content and antioxidant activities of *Ganoderma lucidum* extracts

The correlation coefficients (r^2) between bioactive compounds content and antioxidant activities [β -carotene bleaching rate, free radical scavenging efficiency and inhibition of low density lipoprotein (LDL) oxidation] of *Ganoderma lucidum* were shown in Table 7. Total phenolics, flavonoids, polysaccharides and terpenoids content of the extracts showed significant ($p \leq 0.05$) and strong positive correlation with the all antioxidant activities assays. Also, lycopene showed significant ($p \leq 0.05$) and moderate positive

correlation for the same relationship. The present data are consistent with that reported by Oludemi *et al.* (2017) for DPPH radical scavenging, even though the authors performed a different extraction procedure (methanol: water, 80:20, v/v). They also found that phenolic and polysaccharide extracts of *Ganoderma lucidum* exhibited antioxidant activity and it was slightly lower in the latter. Thus, our data suggesting that phenolic compounds beside flavonoids, polysaccharides and terpenoids play a more prominent role in antioxidant activities (free radical scavenging and lipid peroxidation inhibition). Other compounds such as lycopene was also participated those bioactive activities but to a lesser degree.

Table 7. The correlation coefficients (r^2) between bioactive compounds content and antioxidant activities of *Ganoderma lucidum* extracts

Bioactive compound	Antioxidant activity (β -carotene bleaching rate)	DPPH radical scavenging activity	Inhibition of low density lipoprotein (LDL) oxidation
Phenolics	0.9215 \pm 0.0416 ^a	0.9312 \pm 0.0465 ^a	0.9410 \pm 0.0179 ^a
Flavonoids	0.8565 \pm 0.0254 ^{bc}	0.8131 \pm 0.0291 ^c	0.8462 \pm 0.0188 ^b
Lycopene	0.7434 \pm 0.0226 ^d	0.7165 \pm 0.0344 ^d	0.7498 \pm 0.0352 ^c
Polysaccharides	0.8387 \pm 0.0543 ^c	0.8444 \pm 0.0281 ^{bc}	0.8353 \pm 0.0189 ^b
Terpenoids	0.8717 \pm 0.0317 ^b	0.8794 \pm 0.0179 ^b	0.8342 \pm 0.0265 ^b

Each value represents the means of three replicates \pm SD. Means with different superscript letters in the same columns are significantly different at $p \leq 0.05$.

Antibacterial and antifungal activities of *Ganoderma lucidum* extracts

Data in Table (8) indicated the antibacterial and antifungal activities of *Ganoderma lucidum* extracts. The highest activity (inhibition zones) against the gram-positive bacteria *Staphylococcus aureus* (18.1mm) was recorded for ethanol extract followed by methanol extract (16.5 mm) and aquatic extract (13.8 mm). The same behavior was observed for the antifungal activity against *Candida albicans*. Also, no activity was detected for the all tested extracts against Gram-negative bacteria *Escherichia coli*. Such variations recorded in the amount of the antibacterial and antifungal activities measured (inhibition zones) for the *Ganoderma lucidum* extracts are mainly due to the difference in polarities of extracting solvents. The ethanol and methanol solvent are higher in improving the recovery of the bioactive compounds in *Ganoderma lucidum* than water, subsequently increases their efficiency in inhibiting bacteria and fungi. Such data are relatively accordance with that reported by Abd Elalal *et al.* (2021) by using brown alga (*Sargassum Subrepandum*) extracts collected from the Mediterranean Sea, Egypt. Also, Kamenarska *et al.* (2002) found that the toluene (non-polar compounds, including volatile compounds) and methanol:chloroform (1:1) extracts (compounds with average polarity) showed a moderate activity only against the gram-positive bacteria. The ethanol extract (more polar compounds) showed not only a moderate activity against gram-positive bacteria but also a moderate antifungal activity. Furthermore, Omar *et al.* (2012) reported that the growth inhibitions of bacteria by algae extracts could be affected by seasonality (environmental conditions). The growth inhibitions were higher in samples collected during autumn than that investigated in summer. Finally, data of the present study confirmed the potential use of *Ganoderma lucidum* as a good source of antibacterial agent against the gram-positive bacteria and antifungal agent.

Table 8. Antibacterial and antifungal activities of *Ganoderma lucidum* extracts

Extract	Bacteria		Fungi
	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Candida albicans</i>
AqE	4.1±0.3*	13.8±0.8 ^b	10.7±0.9 ^b
MeE	5.6±0.4*	16.5±1.1 ^a	15.3±0.6 ^a
EtE	6.1±0.5*	18.1±1.7 ^a	16.4±1.2 ^a

* Data expressed as the mean value of three replicates ±SD. Means with different superscript letters in the same row are significantly different at $p \leq 0.05$. ^aDiameter of the inhibition zone less than 10 mm means absence of activity. EtE (Ethanol extract), MeE (Methanol extract), AqE (Aquatic extract).

CONCLUSION

Data of the study supported our hypothesis that reishi mushroom (*Ganoderma lucidum*) fruiting bodies contains several categories of bioactive compounds including phenolics, flavonoids, lycopene, polysaccharides, terpenoids etc., with other compounds that are responsible for its different biological activities. The biological activity studies enhanced here including antioxidant activities ((β -carotene bleaching rate, DPPH radical scavenging activity and inhibition of low density lipoprotein (LDL) oxidation), and antibacterial and antifungal activities. Such important biological activities could be played important roles in strategies to prevent/combat/treat many diseases, especially those for which oxidative stress is one of the mechanisms for its occurrence. Therefore, the present study recommended like of that reishi mushroom (*Ganoderma lucidum*) fruiting bodies powder and/or extracts to be included in our daily diets, drinks, food supplementation and pharmacological formulae.

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Conflict of Interests

Authors declared no competing of interest whatsoever

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الملخص العربي

محتوى العناصر الغذائية والمغذيات الصيدلانية والأنشطة البيولوجية المعملية خارج الجسم للأجسام الثمرية للفطر الريشي (جانوديرما لوسيدوم)

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مجم كاتشين ١٠٠ جم^{-١})، الليكوبين (٢٥٣،١١ ميكروجرام ١٠٠ جم^{-١}). كما سجلت المستخلصات المختلفة لعينات الجانوديرما لوسيدوم وخاصة الإيثانول والميثانولي العديد من الأنشطة البيولوجية العالية جدًا والتي تشمل النشاط لمضاد للأكسدة وكسح الجذور الحرة وتثبيط أكسدة البروتين الدهني منخفض الكثافة (مضاد لتصلب الشرايين) وكمضاد للبكتيريا والفطريات. لذا يمكن أن تلعب هذه التأثيرات البيولوجية أدوارًا هامة في استراتيجيات مكافحة /منع/ علاج العديد من الأمراض، خاصة تلك التي يكون الإجهاد التأكسدي أحد آليات حدوثها، مثل مرض السكري، والسرطان، وتصلب الشرايين، وأمراض القلب والأوعية الدموية وما إلى ذلك. كما أوصت الدراسة الحالية أن الفطر الريشي (جانوديرما لوسيدوم) مسحوق الأجسام المثمرة و/أو المستخلصات يجب تضمينها في وجباتنا الغذائية اليومية والمشروبات والمكملات الغذائية والصيغ الدوائية.

الكلمات المفتاحية: جانوديرما لوسيدوم، فيتامينات، معادن، مركبات نشطة حيويًا، مضاد لتصلب الشرايين، كسح الجذور الحرة، مضاد للبكتيريا، مضاد للفطريات.

تهدف الدراسة الحالية إلى تحديد محتوى العناصر الغذائية والمغذيات الصيدلانية والأنشطة البيولوجية المعملية خارج الجسم للأجسام الثمرية للفطر الريشي (جانوديرما لوسيدوم). أشارت نتائج التركيب الغذائي لمسحوق الجانوديرما لوسيدوم إلى أن الألياف الخام والكربوهيدرات كانتا أكبر المركبات (٥٠،١٩ و ٣٧،٣٣٪ على التوالي) يليها البروتين الكلي (٨،٥٤٪) والرماد (٢،٠٣٪) والدهون الخام (١،٩١٪). أيضًا، مسحوق جانوديرما لوسيدوم غني بالعناصر المقدرة المختلفة (البوتاسيوم، الفوسفور، الكالسيوم، الماغنسيوم، الصوديوم، الحديد، الزنك، المنجنيز، النحاس، السيلينيوم) والفيتامينات (أ، ب١، ب٢، ب٣، ب٦، ب٩، ج، هـ) علاوة على ذلك، أشار محتوى المغذيات الصيدلانية (المركبات النشطة حيويًا) من مسحوق جانوديرما لوسيدوم إلى أن التربينويدات كانت أكبر مركب (٢٥٤١ مجم لينالول . ١٠٠ جم^{-١}) تليها السكريات العديدة (١٦٣٥ مجم مكافئ النشا ١٠٠ جم^{-١})، الفينولات الكلية (٥٧٢،٦٧ مجم مكافئ حمض الجالليك ١٠٠ جم^{-١})، التراي تيربينويدات (٤٩٥ مجم حمض أوروليك ١٠٠ جم^{-١})، الفلافونويدات (٢٧٩،٣٤