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تقييم محتوى المركبات النشطة بيولوجيًا والجوانب السمية والأنشطة المضادة للبكتيريا والفطريات للمستخلص الإيثانولى للفطر الريشي (جانوديرما لوسيدوم)

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Abstract:

Reishi mushroom (*Ganoderma lucidum*, family *Ganodermaceae*) is spread worldwide and is widely used for several nutritional and medical purposes. Therefore, knowledge of all aspects of this mushroom's composition, toxicity and biological activities is required. The present study aims to determine the bioactive compound content, toxicity studies, and antibacterial/antifungal activities of Ganoderma lucidum ethanol extract (GL-EE). Data on the nutrients composition of *Ganoderma lucidum* powder indicated that crude fiber and carbohydrates were the largest compounds (51.06 ± 2.42 and 36.17 ± 3.91%, respectively), followed by total protein (8.69 ± 0.69 %), ash (2.21 ± 0.14%) and crude fat (1.87 ± 0.20%.). Also, Furthermore, the nutraceuticals (bioactive compounds) content of *GL-EE* indicated that terpenoids were the largest compound (241.47± 20.72 mg linalol.100 g^{-1}) followed by polysaccharides (154.30± 19.06 mg starch equivalent.100 g^{-1}), phenolics (49.98 ± 5.61 mg gallic acid equivalent.100g^{-1}), triterpenoids (51.16 ± 6.49 mg ursolic acid.100 g^{-1}). Furthermore, GL-EE recorded antibacterial and antifungal. On the other side, the toxicity studies of GL-EE were evaluated in Albino rats using standard methods. Various doses (2000 and 5000 mg/kg) of the extract were orally administered to rats and kept under close observation for the next 10 days. Data from the acute toxicity study did not show any toxicity signs and symptoms at doses 2000 mg/kg and 5000 mg/kg of GL-EE. Such signs and symptoms, including no morbidity or mortality, were observed. As a result, the LD_{50} of the Gl-EE could be greater than 5000mg/kg body weight. The effective biotransformation and elimination of this natural product within a short period. Therefore, the present study recommended that Ganoderma lucidum extract (GL-EE) could be used safely in different nutritional and medical applications.

Keywords:

Chemical Composition, Bioactive Compounds, Acute Toxicity, Body Weight, Organs Weight.

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مستخلص البحث:

ينتشر فطر ريشي (جانوديرما لوسيدوم ، عائلة جانوديرماسيا) في جميع أنحاء العالم، ويستخدم على نطاق واسع لاغراض غذائية وطبية عديدة. لذلك ، يلزم معرفة بعض الجوانب المتعلقة بالتركيب الكيميائى والنواحى السمية والأنشطة البيولوجية المتعلقة بهذا الفطر. من اجل ذلك، تهدف الدراسة الحالية إلى تحديد محتوى المركبات النشطة بيولوجيًا ودراسات السمية والأنشطة المضادة للبكتيريا/الفطريات للمستخلص الإيثانولى لفطر جانوديرما لوسيدوم .(GL-EE) ، ولقد أشارنتائج التركيب الكيميائى لمسحوق ثمار الجانوديرما لوسيدوم إلى أن الألياف الخام والكربوهيدرات كانت أكبر المركبات (51.06 ± 2.42 و 36.17 ± 3.91٪ على التوالي) يليها البروتين الكلي (8.69 ± 0.69٪) والرماد (2.21 ± 0.14٪) والدهون الخام (1.87 ± 0.0.20٪). علاوة على ذلك ، أشار محتوى المركبات النشطة بيولوجيًا لـ GL-EE إلى أن التربينويدات كانت أكبر مركب (241.47 ± 20.72 مجم لينالول 100 جم $^{-1}$) تليها السكريات (154.30 ± 19.06 مجم مكافئ نشا. 100 جم $^{-1}$) ، الفينولات (49.98 ± 5.61 مجم مكافئ حمض الجاليك. 100 جم $^{-1}$) ، ترايتيربينويدات (51.16 ± 6.49 مجم حمض أورولیك ، 100 جم $^{-1}$) . علاوة على ذلك ، سجل GL-EE نشاطا مضادا للبكتيرا والفطريات. من ناحية أخرى ، تم تقييم دراسات السمية لـ GL-EE في الجرذان البيضاء باستخدام الطرق القياسية. تم إعطاء جرعات مختلفة (2000 ، 5000 مجم / كجمن وزن الجسم) من المستخلص عن طريق الفم للفئران وظل تحت المراقبة الدقيقة لمدة 10 أيام. لم تظهر بيانات دراسة السمية الحادة أي علامات وأعراض سمية عند كلتا الجرعتين (2000 مجم / كجم ، 5000 مجم / كجم من-Gl EE). والتى تمثلت فى عدم ظهور علامات اوأعراض مرضة أو وفيات. نتيجة لذلك ، يمكن أن يكون LD_{50} لـ Gl-EE أكبر من 5000 مجم / كجم من وزن الجسم. هذا يعني ان التحول البيولوجي الفعال وتخلص الجسم من هذا المنتج الطبيعي يتم في غضون فترة زمنية قصيرة. لذلك ، فإن الدراسة الحالية توصى بان مثل هذا المستخلص لفطر الجانوديرما لوسيدوم (GL-EE) يمكن استخدامه بشكل آمن في العديد من التطبيقات الغذائية والطبية المختلفة.

الكلمات المفتاحية:

التركيب الكيميائي، المركبات النشطة بيولوجيا، السمية الحادة، وزن الجسم، وزن الأعضاء.

Introduction

The food and drug crisis is represented in the presence of a defect in providing the needs of community members with food and medicine through local production or the presence of disturbances that make it difficult to obtain food and medicine from abroad. And whether this is due to political or economic conditions, the failure to provide the health and safety factors required to be available in food commodities and medicines used by community members, or the suffering of community members in providing the price for the food and medicine they need (Fahmy, 1993). The food crisis may take one of these previous forms or some of them, or they may combine all of them in one case. Therefore, the attention of scientists in various countries of the world turned to searching for new or innovative sources of food and medicine, and among these sources were algae (mushrooms), which are characterized by their abundance of productivity as a result of the short production cycle (speed of reproduction), ease of cultivation and the absence of the need for high technical expertise. Providing a safe, plant-based alternative to animal protein at low prices, and finally, contributing to the treatment of many diseases, thus improving the health level of its users (Chang and Phillip, 1989 and Mattila *et al.*, 2018).

It is worth noting that there are about 5000 species of mushrooms around the world, and the number of species cultivated on a commercial scale is only 10 species that follow 3 genera: agaric - Chinese oysters. Mushrooms are produced in more than 120 countries worldwide, and the United States comes to the fore and produces 200 thousand tons annually, followed by France, 180 thousand tons, then the Netherlands, China, and Taiwan (Chang 1995 and Wasser & Weis 1999). Among these fungi comes the reishi mushroom (*Ganoderma lucidum*, family *Ganodermaceae*), an oriental fungus with a long history of use in different Asian countries, particularly China and Japan, for promoting health and longevity (Wasser, 2005). It has been recognized as a medicinal mushroom and is widely used in folk medicine. It was attributed to therapeutic properties, such as anti-aging effects, strengthening cardiac function, enhancing vital energy, increasing memory, and relieving cough and asthma (Wachtel-Galor *et al.*, 2011*and* Money, 2016). Recently, additional powerful effects have been reported as anticancer, antitumor, immune-modulatory, antioxidant, antibacterial, antiviral, and antidiabetic (Elhassaneen *et al.*, 2016-a; Elsemelawy *et al.*, 2021 and Gharib *et al.*, 2022).

For all of the above reasons, *Ganoderma lucidum* is spread worldwide in Egypt and is widely used for several nutritional and medical purposes (El-Fallal *et al.*, 2015). Therefore, knowledge of all aspects of this mushroom's composition, toxicity and biological activities is required. The present study aimed to evaluate the bioactive compounds content, toxicological aspects, and antibacterial/antifungal activities of the ethanol extract of the *Ganoderma lucidum* (GL-EE).

Materials and Methods

Materials

Reishi mushroom (*Ganoderma lucidum*) samples: Dried reishi mushroom (*Ganoderma lucidum*) fruiting bodies samples were obtained from Agricultural Seeds, Spices and Medicinal Plants Company (Harraz), Bab El-Khalek, Cairo, Egypt. The fungus samples were subjected to verification by the staff in the Agricultural Plant Department, Faculty of Agriculture, Minoufiya University, Shebin El-Kom, Egypt.

Chemicals: All chemicals and buffers (Except as otherwise stated), reagents, and analytical grade solvents were purchased from El-Ghomhorya Company for Trading Drugs, Chemicals, and Medical Instruments, Cairo, Egypt.

Methods

Preparations of *Ganoderma lucidum* ethanol extract (GL-EE)

Dried fruiting bodies of ***Ganoderma lucidum*** were ground at high mixer speed (Moulinex Egypt, Al-Araby Co., Egypt) to a fine powder. The material that passed through an 80 mesh sieve was retained for use. GL-EE was prepared according to the method mentioned by Gharib *et al.* (2022). Twenty grams of *Ganoderma lucidum* dried powder were extracted with 180 ml ethanol (80%) on an orbital shaker (Unimax 1010, Heidolph Instruments GmbH & Co. KG, Germany) for 120 min at 70 ^{0}C. The mixture was subsequently filtered (Whatman No. 5) on a Buchner funnel. The residual solvents were removed under reduced pressure at 40^{0}C using a rotary evaporator (Laborata 4000; Heidolph Instruments GmbH & Co. KG, Germany). The obtained extract (GLEE) was stored at 4 ^{0}C until 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 (soxhlet miautomatic apparatus Velp company, Italy, petroleum ether solvent), ash, fiber, and dietary fiber contents were determined using the methods described in the AOAC, (1995). Carbohydrates calculated by differences: Carbohydrates (%) = 100 - (% moisture + % protein + % fat + % Ash + % fiber).

Bioactive compounds determination GL-EE

Bioactive compounds were determined in GL-EE according to the following methods. Total phenolics were determined using the Folin-Ciocalteu reagent according to Singleton and Rossi (1965) and Wolfe *et al.* (2003), and results are expressed as mg gallic acid equivalents (GAE)/g of dw. Total polysaccharides were estimated according to the method described by Vazirian *et al.* (2014), and the results

are expressed as mg of starch equivalents (S.E.)/g of dw. Total terpenoids were estimated by Ghorai *et al.* (2012) method, and results are expressed as mg linalool equivalents (L.E.)/g of dw. Triterpenoids were estimated according to the method described by Schneider *et al.* (2009), and results were expressed as mg ursolic acid equivalent (UAE)/g of dw.

Biological experiments (Toxicological studies)

Ethical approval

Biological experiments for this study were ethically approved by the Scientific Research Ethics Committee (SREC, Animal Care and Use), Faculty of Specific Education, Port Said University, Port Said, Egypt (Approval no. 01- SREC- 12-2021).

Animals

Animals used in this study, adult male white albino rats, Sprague Dawley strain (130±12.78 g per each) were obtained from the Research Institute of Ophthalmology, Medical Analysis Department, Giza, Egypt. All animal experiments were conducted following the rulings of the Institute of Laboratory Animal Resources, Commission on Life Sciences, and National Research Council (NRC, 1996).

Basal Diet (B.D.) BD

B.D. for rats feeding protocol was formulated (per kg) such as mentioned by AIN (1993) as follows: corn starch (465.692g), casein-85% protein (140g), dextrinized corn starch (155g), sucrose (100g), soybean oil (40g), fiber (50g), mineral mixture (35g), vitamin mixture (10g), L-cystine (1.8g), choline bitartrate (2.5g) and tert-butylhydroquinone (0.008g). Vitamins and minerals mixtures were formulated such as mentioned in the same reference.

Acute toxicology test of Gl-EE in rats

According to OECD guidelines, the acute toxicology test of Gl-EE was evaluated in rats (OECD, 2008). Rats were randomly selected and grouped into three groups (5 rats per each) and then kept in their cages for seven days before dosing to allow acclimatization to the laboratory conditions. Before administration, rats were fasted overnight, weighed, and the doses were calculated based on their body weight. The control group (group I) received 80% methanol. *Gl-EE* (80%) was then administered orally at the doses of 2000 mg/kg (group II) and 5000 mg/kg (group III) body weight of rats in the test groups. These doses were selected based on previous efficacy studies (Fenglin *et al.*, 2011). After administering the Gl-EE, the rats were kept under close observation for the next 10 days. Clinical observations were made, including mortality, behavioral, neurological, and any other abnormalities, and their

weight was measured weekly. Finally, their gross physical examinations were carried out on the last day, and body weights were measured. The rats were then anesthetized, sacrificed, and gross pathological observation was carried out on different vital organs.

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 ***GL-EE*** were elucidated by the agar cup methods described by Spooner and Sykes (1972).

Statistical Analysis

All measurements were done in triplicate and recorded as mean ± standard deviation (S.D.). Statistical analysis was performed using a Student *t*-test and MINITAB 12 computer program (Minitab Inc., State College, PA, USA). Differences between treatments at $P \leq 0.05$ were considered statistically significant.

Results and Discussion

Proximate composition of *Ganoderma lucidum* fruiting bodies

The proximate composition of Ganoderma lucidum fruiting bodies (% D.W.) was tabulated in Table 1. From such data, it could be noticed that crude fiber (51.06%) was the most abundant compound, followed by carbohydrates (36.17%), protein (8.69 %), ash (2.21%), and fat (1.87%). Such data are that determined by Gharib et al. (2022) and Oludemi *et al.* (2017) while not consistent with that noticed 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 data are fairly consistent with the ones reported by Stojkovic *et al.* (2014-a), who studied the proximate composition of wild and cultivated *Ganoderma lucidum* from Serbia and China. In another study, there was 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). The major sources of fiber are cellulose and other indigestible cell wall polymers (Mukhopadhyay and Guha, 2015). Regarding the total energy value, *Ganoderma lucidum recorded* 196.27 Kcal/100g. Such data are fairly inconsistent with that observed by Sumaira *et al.* (2016). They reported that the total energy value of five mushrooms ranges from 363-394 Kcal/100 g; the lowest value was in *Ganoderma lucidum.* This low total energy value of *Ganoderma lucidum* is attributable to the content of high fiber and low fat (Zahid *et al.,* 2010). Present data with the others indicated that the difference in the proximate chemical composition of *Ganoderma lucidum* could be due to several factors, including the

type of mushroom, the stage of development, the origin of the samples, the level of nitrogen available, and the habitat (Colak *et al.*, 2009; Stojkovic *et al.*, 2014-b; Sumaira *et al.*, 2016; Gharib *et al.*, 2022). All of these studies indicated that *Ganoderma lucidum* is characterized by a high fiber content that is indigestible and plays a significant nutritional role since it helps provide bulk to stool and aid in the movement through the digestive tract. Additionally, *Ganoderma lucidum* was low-fat calorie food and subsequently more suitable for humans in diet, atherosclerosis, and cardiovascular diseases.

Table 1. Proximate composition of *Ganoderma lucidum* fruiting bodies in (D.W., %)

Component	Content
Moisture	7.88 ± 0.65
Dry matter	92.12 ± 0.73
Total protein (g/100g)	8.69 ± 0.69
Crude fat (g/100g)	1.87 ± 0. 20
Ash (g/100g)	2.21 ± 0.14
Crude Fiber (g/100g)	51.06 ± 2.42
Carbohydrates (g/100g)	36.17 ± 3.91
Total energy value (Kcal/100g)	196.27±5.03

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

Bioactive compounds in GL-EE

Bioactive compounds (phenolics, polysaccharides, terpenoids, and triterpenoids) were determined in GL-EE, as shown in Table (1). Terpenoids were reported to be the most abundant ones, followed by polysaccharides, triterpenoids, and phenolics. Such data are reported by Gharib *et al.* (2022) in *Ganoderma lucidum* fruiting bodies. Also, Oludemi *et al.* (2017) noticed that polysaccharides, terpenoids, and triterpenoids in *Ganoderma lucidum* fruiting bodies were 15.4 mg starch, 27.2 ± 0.7 mg linalool, and 5.6 ± 0.5 mg ursolic acid per gram, respectively.

Several studies have reported that bioactive compounds such as phenolics, which was determined in the present study inside GL-EE, play an important vital role in preventing and treating many diseases, including diabetes, cardiovascular disease, atherosclerosis, cancer, obesity, osteoporosis, and aging (Aviram *et al.*, 2000; Gao *et al.*, 2005; Elhassaneen *et al.*, 2016-a, 2019, 2022; Aly et al., 2017; El-Nassag et al., 2019; El-Gamal *et al.*, 2020; Elsemelawy *et al.*, 2021). These vital roles are due to the possession of these active compounds in many biological activities, including antioxidant and scavenging activities, inhibition of lipid oxidation, and anticarcinogenic and anti-inflammatory effects. And in another study, Lien *et al.* (2008) confirmed the inhibitive action of the polyphenolic compound extract against LDL oxidation. Such mechanisms of action, protecting LDL against oxidation by phenolic compounds, could be included increased levels of reduced glutathione (GSH) and glutathione reductase (GSH-Rd) in the liver and lungs as well

as the increase in inhibition of NADPH-dependent lipid peroxidation (Majid *et al.*, 1991). In other compounds, the polysaccharides-mediated immune function potentiation is thought to be the principal mechanism of anti-tumorgenicity by *Ganoderma lucidum* (Liu, 1999 and Wasser, 2002*).* Also, several biological activities include anti-obese, anti-osteoporosis, anticarcinogenic and hypolipidemic responses **(**Elhassaneen *et al.*, 2020 and 2022). Furthermore, polysaccharides from *Ganoderma lucidum* restored the TNF-α production, which is inhibited by cyclophosphamide to normal levels in mice (Gao *et al.*, 2002). Additionally, *Ganoderma lucidum* extracts of polysaccharides or triterpenoids exhibited protective effects against liver disorders induced by toxic chemicals (Liu, 1999; Zhou *et al.*, 2002; Elhassaneen *et al.*, 2016-a). Triterpenoids can also be a starting material for synthesizing more potent bioactive derivatives, such as antitumor agents (Ma *et al.*, 2005). For all these reasons, GL-EE could act as a complete package of healthy food by being an excellent source of bioactive compounds. In line with this context, there are already a lot of nutraceutical products containing *Ganoderma lucidum* extracts commercially available. 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.

Table 2. Bioactive compounds in GL-EE

Component	Content
Phenolics (mg GAE. g^{-1} dw)	49.98 ± 5.61
Polysaccharides (mg SE. g^{-1})	154.30± 19.06
Terpenoids (mg LE. g^{-1} dw)	241.47± 20.72
Triterpenoids (mg UAE.g^{-1} dw)	51.16 ± 6.49

Each value represents the mean of three replicates ±SD. GAE, gallic acid equivalents; S.E., starch equivalent; L.E., linalool equivalent; UAE, ursolic acid equivalent.

The effect of Gl-EE on organ weights of control and treated rats during acute toxicity test

Data in Table (3) was shown the effect of GL-EE on the body weight of control and treated rats during the acute toxicity study. From such data, there was a gradual increase in the B.W. of both treated and control rats. The initial mean B.W. of control rats was 135.74±5.67g; at the end of the experiment, their final mean B.W. was 156.65±9.12g. The control rats' mean body weight gain (BWG) was 20.91 g (15.41%). At the same time, the mean BWG for rats treated with doses of 2000mg/kg and 5000mg/kg were 18.79 g (13.77%) and 16.62 (12.31%), respectively. In the

same context, Vahalia *et al.* (2011) reported that B.W. change is an important index for assessing toxicity. In the present study, there was a gradual normal increase in the mean B.W. of the Gl-EE treated groups, such control group. However, the BWG difference between the control and Gl-EE treatment groups was statistically insignificant.

Table (3). The effect of Gl-EE on body weight (B.W.) of control and treated rats during acute toxicity test

Body weight (g)	Groups (Doses)	Mean ±SD	% of change	Significance
Initial week 1	Group I (Control)	135.74±5.67	------	0.89
	Group II (2000 mg/kg)	136.41±3.98	+ 0.49	
	Group III (5000 mg/kg)	135.05±6.21	- 0.51	
Week 2	Group 1 (Control)	143.84±4.13	------	0.86
	Group II (2000 mg/kg)	141.90±4.95	- 1.35	
	Group III (5000 mg/kg)	140.98±5.21	- 1.99	
Week 3	Group 1 (Control)	156.65±9.12	-----	0.91
	Group II (2000 mg/kg)	155.20±7.90	- 0.93	
	Group III (5000 mg/kg)	151.67±5.87	- 1.90	

Values are expressed as mean ± S.D., N= 5/group.

On the other side, data in Table (4) indicated no significant abnormal changes in the mean absolute weights of the liver and kidney in all control and GL-EE treated groups. The mean absolute weights of the liver were 6.01±0.45 g (at 2000mg/kg) and 5.86±0.60 (at 5000mg/kg) compared with 5.62±0.53 g for the control group. The mean absolute weights of the kidneys were 1.52±0.25g (at 2000mg/kg) and 1.57±0.17 g (at 5000 mg/kg) compared with1.49±0.23g for the control group. Also, the treated rats' gross pathological examination of such vital organs, liver and kidney, showed no significant abnormal changes in color, size, shape, and texture compared with the control. The present data agree with Satyapal *et al.*, (2008) that many researchers use the liver and kidney of rats to assess the safety or toxicity of drugs or plant materials. Also, Fenglin *et al.* (2011) found that the ethanolic extract of Gl was nontoxic, up to 5000 mg/kg B.W. Therefore, the present study's data with the others indicated that the acute toxicity study did not show any toxicity signs and symptoms at both doses, 2000mg/kg and 5000mg/kg, of Gl-EE. Such signs and symptoms, including no morbidity or mortality, were observed. As a result, the LD_{50} of the Gl-EE could be greater than 5000mg/kg body weight.

Table (4). The effect of GL-EE on organ weights of control and treated rats during acute toxicity test

Organ weight (g)	Groups (Doses)	Mean ±SD	% of change	Significance
Liver	Group I (Control)	5.62±0.53	------	0.76
	Group II (2000 mg/kg)	6.01±0.45	6.94	
	Group III (5000 mg/kg)	5.86±0.60	4.27	

Kidney	Group 1 (Control)	1.49±0.23	------	0.81
	Group II (2000 mg/kg)	1.52±0.25	2.01	
	Group III (5000 mg/kg)	1.57±0.17	5.36	

Values are expressed as mean ± S.D., N= 5/group.

Antibacterial and antifungal activities of GL-EE

Data in Table (5) indicated the antibacterial and antifungal activities of GL-EE. Such fungal extract recorded high activity (inhibition zones) against the gram-positive bacteria *Staphylococcus aureus* (18.1mm). The same behavior was observed for the antifungal activity against *Candida albicans.* Also, no activity was detected against the Gram-negative bacteria *Escherichia coli*. Such amount of the antibacterial and antifungal activities measured (inhibition zones) for the GL-EE extracts are mainly due to the difference in polarities of extracting solvent. The ethanol solvent is high in improving the recovery of the bioactive compounds in *Ganoderma lucidum,* subsequently increasing their efficiency in inhibiting bacteria and fungi. Such data are relatively following that reported by Abd Elalal *et al.* (2021) and Gharib *et al.* (2022) by using brown alga (*Sargassum Subrepandum*) and *Ganoderma lucidum* extracts, respectively. Also, Kamenarska *et al.* (2002) found that the ethanol extract (more polar compounds) showed moderate activity against gram-positive bacteria and moderate antifungal activity. All of these data confirmed the potential use of *Ganoderma lucidum* as a good source of antibacterial agents against gram-positive bacteria and antifungal agents.

Table 5. Antibacterial and antifungal activities of *GL-EE*

Extract	Bacteria		Fungi
	Escherichia coli	*Staphylococcus aureus*	*Candida albicans*
GL-EE	5.4±0.6*	17.8±0.9	17.1±0.8

Each value represents the mean ±SD. *Diameter of the inhibition zone less than 10 mm means the absence of activity. GL-EE, *Ganoderma lucidum* ethanol extract.

Conclusion

This study's data has demonstrated that the nutrient composition of *Ganoderma lucidum* powder indicated that crude fiber and carbohydrates were the largest compounds, followed by total protein and crude fat. Also, the nutraceuticals (bioactive compounds) content of *GL-EE* include terpenoids, polysaccharides, phenolics, and triterpenoids. Furthermore, GL-EE recorded antibacterial and antifungal. On the other side, data from the acute toxicity study did not show any toxicity signs and symptoms at doses 2000 mg/kg and 5000mg/kg of Gl-EE. Therefore, the present study recommended that Ganoderma lucidum ethanol extract (GL-EE) could be used safely in different nutritional and medical applications.

Author contributions

All authors contributed equally to this article. The contribution includes collecting the reference studies (review), formulating the paper idea, designing all the experiments, preparing the materials, conducting all chemical, biological and statistical analyses, tabulating results, and conducting discussions. They are also participating in writing the manuscript, making all the reviews, agreeing on the publishing journal, and applying for it.

Conflict of interests

Authors declared no competing of interest whatsoever

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