RESEARCH ARTICLE

Antioxidant and antimicrobial activity of *Lycoperdon pyriforme* fruiting body

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Lycoperdon pyriforme used in traditional medicine by locals in different parts of Ethiopia. The study aimed to evaluate the antioxidant, antimicrobial and phytochemical constituents of the methanol extract of *Lycoperdon pyriforme* which may infer the antioxidant and antimicrobial activity of this fungi. DPPH radical scavenging, total phenol content assay and total flavonoid content assay were done to determine the antioxidant activity of the fungi extract. Agar disc diffusion method was used to determine the antimicrobial properties against *S. aureus*, *B. subtilis*, *C. albicans*, *E. coli* in a dose-dependent manner. The fungi extract was found to show significant antimicrobial and antioxidant properties. The presence of phenol and flavonoid compounds in the fungi could be the reason behind the antioxidant and antimicrobial activity. The current study shows that *Lycoperdon pyriforme* could be a good source for preparation of antibacterial, antifungal and antioxidant extracts in future.

Key Words: *Lycoperdon pyriforme; Antioxidant; Antimicrobial; Free radicals; Fungi extract*

INTRODUCTION

Nature has been a source of healing agents for thousands of years and continues to be a major source of novel chemotypes and pharmacophores [1]. Ingredients with biological functions are derived from natural products such as plants, animals, and microorganisms. Less than 1% of bacteria and 5% of fungal species are currently known, and the potential for new microbial sources, especially those found in the worst-affected areas, appears to be limitless [1].

Natural products have been used since ancient times and in myths to cure many ailments. Many of these natural products have continued to be current drug patients [2]. Their use has been described throughout history in the form of traditional medicine, herbs, ingredients, and many of the oils of these active natural products that have not been identified [2]. It has become a major cellular resource used in drug discovery. It is undoubtedly one of the most popular natural products found in the tiny fungus penicillin from the fungus *Penicillium notatum* discovered by Fleming in 1929 [3].

Macrofungi mushrooms have a distinct fruit-bearing body, either hypogeous or epigeous, large enough to be seen with the naked eye and taken by hand [4]. Mushrooms have long been used as an important food source and as a traditional medicine throughout the world. Records of health-promoting properties such as antioxidant, antimicrobial, anticancer, cholesterol lowering and immuno stimulatory effects have been reported in other types of mushrooms [5]. Mushrooms are also given important amounts of healthy food, as they are rich in bioactive metabolites with high therapeutic properties such as lectins, polysaccharides, phenolics and poly-phenolics, terpenoids, ergosterol, and flexible organic compounds [6]. In addition, mushrooms can be considered a good source of many different nutrients for food and are directly used in human diets to improve human health due to the synergistic effects of their bioactive compounds [7].

Lycoperdon pyriforme, more commonly known as pear-shaped puffball or stump puffball, is a worldwide saprobic fungus. As it emerges in the fall, this puffball is common and littered with rotting logs of both leafy and coniferous wood. It is considered edible if it is not yet ripe and the flesh inside is white. It is considered a type of puffball fungus in the family Agaricaceae. This type of mushroom is found in many parts of the world. It is also pear-shaped and 1.5-4.5 cm wide and 2-4.5 cm high and grows mainly in areas covered with pine trees. Additionally, *L. pyriforme* has been given enzymatic activities at a certain pH and temperature, which can be used in large and industrial industries such as medicinal plants, various bioactive compounds such as polysaccharides, alkaloids, terpenoids, tannins and flavonoids [8]. In modern times; it is thought that researchers have conducted studies on the antibacterial and antioxidant properties of different types of mushrooms, and since no research has been done on this species so far, it was decided to shed light on the antimicrobial effect of *L. pyriforme*, by extracting methanol, is grown in areas of northern Ethiopia.

In this study, the phytochemical components, antioxidant activity and antimicrobial activity of *Lycoperdon pyriforme* have been evaluated considering its widespread use in traditional medicine by local residents in Ethiopia (Figure 1). The antioxidant activity, antimicrobial activity and phytochemical properties of this plant are measured in terms of their role in ethnomedicine nationwide. Antioxidant activity was determined using DPPH. Phytochemical volume analysis was performed to determine the total phenolic value and total flavonoid content. The antimicrobial activity of the fungi extract is determined by the efficient distribution of agar well against three species of bacterial and one fungus, two gram positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*,) one one gram negative bacteria (*Escherichia coli*) and phatogenic fungus, *Candida albicans*.

Figure 1) *Stump puffball (Lycoperdon pyriforme)*

MATERIALS AND METHODS

Chemicals and instruments

Chemicals used in this study includes methanol, distilled water (Aq), n-hexane, dimethyl sulfoxide (DMSO), Folin-Ciocalteu (FC) reagent, anhydrous sodium carbonate, gallic acid (GA), aluminium trichloride hexahydrate, sodium

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acetate, DPPH were of AR grade. All the above reagents and potato dextrose agar (PDA), glucose (Glc) and agar were purchased from Sinopharm Chemical Reagent Beijing Co., Ltd. Standard antibiotic drugs, gentamycin and terbinafine were obtained from Pharmagen, Hefei. Laminar flow cabinet was produced by Stramline Laboratory. Petri plates and glass columns were bought from Hefei Meifeng Chemical Instrument Co. Ltd. Autoclaver was produced by Shanghai ShenAn medical equipment factory.

Collection and identification of materials

Lycoperdon pyriforme was collected from south wollo, Sayint Adjibar woreda, Waro kebele, Ethiopia and identified by a botanist in the Department of botany, Wollo University, Dessie. The puffball samples were frozen at -20°C, prior to freeze-drying procedure. Freeze dried samples were ground to a fine powder, wrapped in plastic bags and stored in a dark place at room temperature, until further use.

Determination of extractive value

Naturally all solvents are divided into two types such as polar and non-polar based on dielectric constant. In the present study, both polar and non-polar solvents were used to obtain the output of the edible wild mushroom, *Lycoperdon pyriforme*. To obtain the output, 100 grams of dried powder of *Lycoperdon pyriforme* was extracted separately and dipped in a beaker containing both polar and non polar solvents such as distilled water, methanol and hexane. Leave all the beakers for 48 hours, the supernatant collected separately from each beakers. The same process is repeated three to five times until the soaked powder changes color, then, the extract obtained dried at room temperature and the extract extracted under reduced pressure [9]. The appropriate dried residues were dispersed in 5% of DMSO, prior to analysis.

The extractive value (EV) (%) was calculated by using the following formula:

EV (%)=(Wt of dried extract)/(Amount of dried sample) × 100

Total phenol content

The total phenol (TP) content of LycMtOH content was determined [10], converted to a 96 source plate reader (Multiskan Ascent, Thermo Electron Corporation). Folin-Ciocalteu reagent (125 µl, 0.1 M) was added to the blended extracts (25 µl). After 10 minutes, 100 µl of 7.5% w/v sodium carbonate was added and the reaction mixture was incubated for 2 hours. Absorbance read at 690 nm. TP is defined as mg gallic acid equivalents (GAE)/g of dry weight (d.w.).

Total flavonoid content

The total flavonoid (TF) content of LycMeOH was measured spectrophotometrically, at a 96-well plate reader, using a modified method with Chang , et al. [11]. The appropriate sample (30 µl) was mixed with methanol (90 µl), aluminumtrichloride (6 µl, 0.75 M), sodium acetate (6 µl, 1 M) and purified aqua (170 µl). Absorbance is measured at 414 nm, after incubating for 30 minutes. The results are shown to be equivalent to mg quercetin (QE)/g of dry weight (d.w.).

DPPH scavenging activity

The free radical scavenging capacity of mushroom extracts is measured on the basis of stable disposal activity 1, 1-diphenyl-2-picrylhyorazyl (DPPH) free radical according to the modified method described by Brand-Williams , et al. [12]. 1 ml of plant extracted with different concentrations such as (10, 25, 50, 75, 100 µg/ml) taken from a separate test tube. For each test tube, 1 ml of DPPH solution 0.1 ml is added to ethanol. At the same time, a randomized sample was prepared and BHT (1-100 µg/ml) was used as an indicator. A mixture of 1 ml ethanol and 1 ml of DPPH solution was used as a control. The reaction was triple and the absorption drop was measured at 517 nm after 30 minutes in the dark using a UV-Vis spectrophotometer. % of free radical block chain arithmetic is calculated using the following formula. Decreased absorption indicates an increase in free radical release.

$$\% \text{ Inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of the sample})}{(\text{Absorbance of the control})}$$

Antimicrobial tests

Pure cultures of *Staphylococcus aureus*, *Candida albicans*, *Escherichia coli* and *Bacillus subtilis* were obtained from Bless Agri Food Laboratory Services PLC.

Assessment of antimicrobial activity

The antimicrobial activity of Stump puffball (*Lycoperdon pyriforme*) extracted was tested using two types of disc distribution methods which were zone of inhibition [13] and minimum inhibition concentration (MICs), ZOI analyzes quality and MIC is quantitative analysis antimicrobial function [14].

In this study, three types of bacteria and one type of fungus were used to determine the antimicrobial activity of stump puffball (*Lycoperdon pyriforme*). The agar cultures of *S. aureus*, *B. subtilis*, *C. albicans*, *E. coli* prepared to evaluate the effects of Stump puffball antimicrobial. 50 ml of nutrient broth medium is poured into the Erlenmeyer flask, four flask prepared for tested samples. flasks including agar medium sterilized at autoclave at 121°C for 15 minutes.

For antibacterial testing, bacterial cultures have been raised to 35°C for 24 hours by injection into the Nutrient Broth [15]. Petri containers containing 20 ml of Nutrient Agar were prepared, previously vaccinated with 100 µl of standard suspension (1%, containing 106-107 cfu/ml) and the same volume of contaminated water was used as a controller. Three springs (5.0 mm wide) were cut from the agar in a hollow state. Prepared stump puffball solutions were filled in wells. Incubated plates were placed 24 hours at 35°C, after which the antimicrobial activity of the samples was observed. At the end of the incubation period, measurements are made basically from the edge of the site to the end of the spring.

The same method was used for all mold samples. C. *albicans* are grown in Potato dextrose broth (PDB) at 25°C for 48 hours. 100 µl of various fungal cultures (1%, containing 106-107 cfu/ml) were added to various flasks containing 25 sterile PDA at 45°C and poured into Petri containers. Agar is allowed to harden at 4°C for 1 hour. The springs (5.0 mm wide) were cut from the agar in a hollow state incubated plates 24 h at 25°C. PDA plates with added salt are used as controls. The plates are then incubated at 25°C for two days. ZOI restricted area is calculated.

Minimum Inhibitory Concentration (MIC)

A standard agar purification solution with double dilution was used. Extracts are incorporated into nutrient agar at concentrations ranging from 0.39 mg/ml to 25 mg/ml. A non-extract controller was also configured. 10 µL of tested organic matter, previously purified to 10 CFU/ml, was used for injection plates. These are incubated at 37°C 24 hours initially and another 24 hours before growth and recording. The minimum inhibitory concentrations (MICs) of each microorganism extract have been considered as a plate of agar with very low concentration without growth [16].

RESULTS AND DISCUSSION

The composition of phyto-compounds are different for different biomaterials. Water is a universal solvent, though; all phytoconstituents do not dissolve in water. Some compounds can be dissolved in polar solvent; some in solvent nonpolar and a few in solvent dipolar. So in the present study, the extraction was performed on different polar and nonpolar solvents of fruiting bodies of mushroom, *Lycoperdon pyriforme* to obtain a high melting environment to select the right solvents to extract the highest number of phytocompounds. Many previous studies on the release rate provide an indication of the nature of the chemical elements present in biomaterial, biomass or drug. The soluble extractive alcohol (2.8%) was higher compared to the soluble extractives in water (0.4%) of Hypsizygus ulmarius due to its relatively soluble components of alcohol [17].

The fruit bodies of *Lycoperdon pyriforme* were extracted from pure water, methanol and hexane. The extraction yields obtained from *Lycoperdon pyriforme* fruit bodies are shown in Table 1. Extraction of methanol extract resulted in a high yield of 39.7% and yields of pure water and hexane were 24.2% and 11.04% respectively. These results show that most of the substances in the body of mushroom fruit are extracted by solvents with high polarity [18]; supporting, current studies have shown high excretion of methanol solvents from fruit-bearing bodies of *Lycoperdon pyriforme*.

TABLE 1

Yield of extracts from *Lycoperdon pyriforme*

	Methanol	Distilled water	n-hexane
Yield (%)	39.7	24.2	11.04

Based on these research results, methanol was selected as an appropriate solvent to extract the phytocompounds of the current *Lycoperdon pyriforme*, continuously, the selection of methanol as a safe release solution, easy access and high affinity for antioxidants compared to other solvents [19]. Different methanol concentrations had different polarity. Low concentrations of methanol are ideal for extracting polar antioxidant compounds, whereas high concentrations of methanol prefer low polarity [20].

Total Phenolic Assay (TPC)

A variety of bioactive substances have an impact on cancer-fighting and antibacterial activities. As the second metabolite in the vegetable kingdom, polyphenol (phenolic hydroxyl, -OH) provides the electron, and suppresses oxidation with active oxygen. They also have various biological functions such as not only the brightness of anti-oxidation but also the anti-bacterial and anticancer effects [21,22].

The total phenolic content of *Lycoperdon pyriforme* was spectrophotometrically analyzed using the Folin-Ciocalteu reagent expressed in gallic acid equilibrium (normal curve value: y=0.923x+0.0071, R^2=0.9801). The total amount of phenolic content in methanolic extracts expressed as gallic acid equivalent (GAE) i.e., mg gallic acid/g weight loss showed a high phenolic content of the extract with disposal function. The resulting concentration of soluble phenolic content was expressed as mg of GAE/g of dried sample estimated to be 5.92 ± 0.02 mg GAE/g in methanolic extract. The soluble phenolic compounds in *Lycoperdon pyriforme* may be effective anti-tumor, anti-tumor, antibacterial, antioxidant and antiviral agent [23]. The total amount of phenol in the experimental species was significantly higher than that of the few edible wild mushrooms [24], which may have been due to specific genes (species) or certain epigenetic factors including its preservation, diversity, climate, solvent concentration, solvent to solid form, solvent polarity, mushroom moisture content [25].

Total Flavonoid Content (TFC)

As one of the compounds of polyphenols, flavonoids are different in the plant, and they suppress pathogenic bacteria, block ultraviolet radiation, and are suitable for antivirus and anti-inflammatory [26]. Flavonoids are known to have strong antioxidant activity to prevent or repel free radicals. Flavonoids content of *Lycoperdon pyriforme* extract was obtained from standard materials, quercetin effect as (Table 2). The total flavonoid content of *Lycoperdon pyriforme* was found to be 3.71 ± 0.03 mg QE/g of dry weight according to Quercitin (QE) which was considered the best control.

TABLE 2

Total phenolic and flavonoid contents of extracts from *Lycoperdon pyriforme*

	Total contents* (Methanol extract)
TP	5.92 ± 0.02[a]
TF	3.71 ± 0.03[b]

Note: Total phenol (TP) content is expressed as mg gallic acid equivalents/g extract of dry weight (mg GAE/g d.w.), while total flavonoid (TF) content is expressed as mg quercetin equivalents/g extract of dry weight (mgQE/g d.w).
a,b The results are expressed as a mean value ± SD. The means with different superscript within the same row are statistically different (p<0.05).

DPPH activity of *Lycoperdon pyriforme* extracts

1, 1-Diphenyl-2-picrylhydrazyl (DPPH) is a free radical that reacts with an active antioxidant to supply its hydrogen or electron and is reduced from dark violet to pale yellow in color. There is a difference between BHT and plant extraction with respect to antioxidant activity. The percentage of The inhibition was found to be significantly higher in normal BHT (100 µg/L) (measuring 98.3 ± 0.3%) followed by *Lycoperdon pyriforme* (95.1 ± 0.19%) and the activity of disposing of free DPPH free radicals of *Lycoperdon pyriforme* extract was also found. increasing and increasing concentration. The IC50 value of the *Lycoperdon pyriforme* plant extract was 22.8 µg/mL and the IC50 value of BHT was 12.2 µg/mL. Phenols and flavonoids your mass-determined presence may be involved in demonstrating DPPH detoxification activity as shown in Table 3.

TABLE 3

Antioxidant activity of *Lycoperdon pyriforme* extracts and BHT

	DPPH	BHT
%of Inhibition	95.1 ± 0.19	98.3 ± 0.3
IC50 Values(µg/ml)	22.8	12.2

As noted in Table 4, it can be seen that there is a positive correlation (r=0.92) between the total phenolic content and antioxidant activity (r=0.92) and the overall flavonoid and antioxidant activity (r=0.78) in the plant sample. Several studies have reported an association between phenolic content and antioxidant activity. Some authors have found an association between phenolic content and antioxidant activity, while others do not Velioglu , et al. [27]. Reported a strong relationship between the content of phenolic content and antioxidant activity in certain plant products that is consistent with current research. There is a need to expose the phenolic compounds present within the extracted plant in order to give different antioxidant functions, to ensure that the phenolic structure affects antioxidant activity and determines whether synergism actually occurs between certain phenolic compounds.

TABLE 4

The Pearson correlation coefficients (r^2) between TP/TF and DPPH assay

	DPPH	
TPa	0.92	0.92
TFb	0.78	0.78

Note: The correlations are significant at p<0.05.

Antimicrobial activity of *Lycoperdon pyriforme* extracts

Antibiotic resistance has become a global problem in recent years [28]. In the latter case, the world is facing significant challenges to modern health services because many antimicrobial agents have lost their effectiveness in treating infectious diseases primarily due to the development of antimicrobial resistance [29]. Examination of new antimicrobials containing bioactive compounds from plants, fungi, bacteria to treat available microorganisms that are resistant to daily is a new drug discovery [30].

According to Craig [31], to test the antimicrobial activity MIC values should be measured from 4th to 16th dilution. The antimicrobial effects of *Lycoperdon pyriforme* against bacteria and yeast were measured according to the following parameters [32]. MIC values below 100 µg/mL have a high, between 100 and 500 µg/mL medium, between 500 and 1000 µg/ml of weak antimicrobial activity and more than 1000 µg/mL have no antimicrobial effect. In addition, the tendency of experimental microorganism is related to the site of inhibition. Micro-organisms are said to be involved in plant extraction when local inhibition is equal to or greater than 7 mm (≥ 7) in diameter, or withstanding a protective area less than 7 mm (<7) of treatment [33], our results are summarized in Table 5.

TABLE 5

Antimicrobial activity of *Lycoperdon pyriforme* extract through determination of zone of inhibition and MICs (µg/mL)

Microorganisms	*S. aureus*	*E. coli*	*B. subtilis*	*C. albicans*
Zone of inhibition	21.5 ± 0.1	17 ± 0.5	25 ± 0.2	20.5 ± 0.1
Z. inhib. standard drug	25 ± 0.2	24 ± 0.1	27 ± 0.01	30 ± 0.2
MICs	45	82	32	51

The diameters of zone of inhibition were expressed in millimeter (mm) as mean ± standard deviation (SD).

The antimicrobial activity of *Lycoperdon pyriforme* was determined against pathogenic and non-pathogenic bacteria. Plant extraction has shown antimicrobial action against all bacterial and fungal species under investigation. The diameter of the *Lycoperdon pyriforme* (100 µg/ml) inhibitory area was 21.5 mm, 17.5 mm, 25 mm and 20.5 mm compared with *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Candida albicans* respectively. In addition, the MIC value that was most effective in experimental microorganisms detected with methanol extracted from *Lycoperdon pyriforme* was 45, 82, 32 and 51 µg/mL of *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Candida albicans* respectively. Treatment showed an effect in a manner based on a very low dose of 20 µg/ml of extracted plant. *Lycoperdon pyriforme* has been found to have better antibacterial activity against *Bacillus subtilis*. Plant extraction has shown the ability to inhibit the growth of both gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*), gram-negative bacteria (*Escherichia coli*) and fungal phatogen, *Candida albicans*. As shown in Table 5, crude quotes show the highest anti-bacterial activity against all viruses and fungi tested. The presence of a class of compounds such as phenols and flavonoids may contribute to the very high effect of antimicrobial activity. The presence of high levels of phenol and flavonoids has been reported to have high antimicrobial activity against gram-negative and gram-positive bacterial and phatogenic fungus [34]. The results obtained during the present study are somewhat consistent with the traditional use of *Lycoperdon pyriforme* especially in rural Ethiopia.

CONCLUSION

Several studies have shown the potent antioxidant and antimicrobial activity present in various plants. Here, the extract of *Lycoperdon pyriforme* revealed the presence of significant amount of flavonoid and phenol which contribute to its antioxidant and antimicrobial activity. It was effectively able to inhibit the growth of both pathogenic and nonpathogenic bacteria and fungus in a dose-dependent manner. The study also corroborates the use of these plants in traditional medicine for the treatment of various diseases. Further investigation needs to be done to confirm the various pharmacological activities of compound present in *Lycoperdon pyriforme*.

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