

Phytochemicals and antibacterial activity screening of three edible mushrooms *Pleurotus ostreatus*, *Ganoderma lucidum* and *Lentinula edodes* accessible in Bangladesh

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Abstract

In this study methanolic extract of dried fruiting bodies of *Pleurotus ostreatus*, *Ganoderma lucidum* and *Lentinula edodes* were evaluated for their phytochemical analysis. The crucial objectives of this investigation were to check out the relative abundances of the selected eleven phytochemical constituents and preliminary antibacterial activity screening of selected three edible mushrooms. The screening was accomplished for alkaloids, polyphenols, flavonoids, tannins, saponins, phyto-sterols, vitamin-C, coumarins, terpenoids, cardiac glycosides and anthocyanin. The color strength or the precipitate formation was used as analytical answers to these tests. Eleven selected phytochemicals were found in fruiting body extracts of *Ganoderma lucidum* with polyphenols, flavonoids, tannins, coumarins, vit-C, and anthocyanin were in highest concentration category. Excepting polyphenols, *Lentinula edodes* showed the rest of 10 selected phytochemicals thru vit-C and tannin were in maximum concentration category. Saponins, coumarins, and anthocyanin were absent in fruiting bodies of *Pleurotus ostreatus* while steroids, terpenoids, cardiac glycosides were found in uppermost concentration category. The distribution difference of eleven selected phytochemicals in three edible mushrooms, *Pleurotus ostreatus* { $p > 0.05$ (0.965)}, *Ganoderma lucidum* { $p > 0.05$ (0.307)} and *Lentinula edodes* { $p > 0.05$ (0.484)} were statistically non-significant. Mushroom extracts were also exposed to preliminary antibacterial activity screening by disc diffusion method and found to be active against selected bacterial strains. Fruiting bodies of *Ganoderma lucidum* was exhibited highest antibacterial activity against both selected gram negative (*Escherichia coli* and *Pseudomonas fluorescens*) and gram positive bacteria (*Bacillus cereus*, *Staphylococcus aureus*) with zones of inhibition ranging from 11 mm to 16 mm. The *Pleurotus ostreatus*, and *Lentinula edodes* showed less antibacterial activities with less than 10 mm of inhibition zones. The relative phytochemicals distribution is responsible for its' biological activities. It is predicted that the phytochemical belongings is acknowledged in our study in the innate medicinal plants of *Pleurotus ostreatus*, *Ganoderma lucidum* and *Lentinula edodes* will be advantageous for clarification and footing of Pharmacognosy outlining of edible mushrooms.

Keywords

Mushrooms, Medicinal Plants, Statistical Inference, Phytochemicals, Fruiting Bodies

1. Introduction

There are over 200 edible mushroom species. The mushrooms *Pleurotus ostreatus* (oyster), *Ganoderma lucidum*

(reishi), and *Lentinula edodes* (shiitake) possess immense medicinal properties. The fruiting bodies of *Pleurotus spp.* possesses a number of therapeutic properties like anti-inflammatory, immunostimulatory, immunomodulatory

[1], anticancer activity [2], and ribonuclease activity [3]. Oyster mushroom has been explored to combat simple and multiple drug resistant isolates of *Escherichia coli*, *Staphylococcus epidermidis*, *S. aureus* [4] and species of *Candida*, *Streptococcus*, and *Enterococcus* [5]. It has been reported that *Ganoderma lucidum* has exhibited a greater antibacterial potential with their inhibition zones greater than that of some commercial antibiotics [6]. Shiitake mushrooms are the second most commonly cultivated edible mushrooms in the world. Extracts from this mushroom, and sometimes the whole dried mushroom, are used in herbal remedies. This mushroom is used as an anti-carcinogenic, anti-inflammatory, antioxidant, antifungal, antibacterial, antiviral as well as antithrombotic in cardiovascular disorders [7]. Studies in animals have found antitumor, cholesterol-lowering, and virus-inhibiting effects in compounds in shiitake mushrooms [8]. The medicinal properties that the mushroom species possess are due to the presence of certain phytochemicals. The phytochemical screening of edible mushrooms revealed the presence of alkaloids, flavonoids, cardiac glycosides, tannins, phenols, steroids, and terpenoids [9, 10, and 11]. Hence, the purposes of the existing work were to evaluate the phytochemicals and antibacterial properties of the edible mushrooms (*Pleurotus ostreatus*, *Ganoderma lucidum*, and *Lentinula edodes*) in order to establish the logical basis for some of their therapeutic properties in folkloric uses.

2. Materials and Methods

2.1. Sample Collection and Preparation of Extracts

Fruiting bodies of *Pleurotus ostreatus*, *Ganoderma lucidum* and *Lentinula edodes* were collected from National Mushroom Development and Extension Centre and identified by strengthening mushroom development officer, Savar, Dhaka-1304, Bangladesh.

The fruiting bodies of the selected mushrooms were collected and air dried for few days. The fruiting bodies were grinded off into powder and kept in polythene bags for future uses. The extracts of selected sample powder were organized by saturated 50 gm of dried powder in 100 ml 80 % methanol and traumatized well. The solution then filtered with the help of filter paper and filtered extracts of the selected plant sample were reserved and used for additional phytochemicals and antibacterial screenings.

2.2. Phytochemicals Screening

Phytochemicals screening of the three edible mushrooms extracts were done by using standard established methods [12, 13, 14, and 15].

2.3. Anti-Bacterial Activity Screening

Bacterial cultures of *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas fluorescens* were obtained from the culture collection centre, Department of Microbiology, Primeasia University, Bangladesh, were used

for antibacterial test organisms. The bacteria were maintained on nutrient broth (NB) at 37°C. The gram positive (*Bacillus cereus* and *Staphylococcus aureus*) and gram negative bacteria (*Escherichia coli*, *Pseudomonas fluorescens*) were pre-cultured in nutrient broth overnight in a rotary shaker at 37°C, centrifuged at 10,000 rpm for 5 min, pellet was suspended in double distilled water and the cell density was standardized spectrophotometrically (A_{610} nm). The methanolic extracts of fruiting bodies of *Pleurotus ostreatus*, *Ganoderma lucidum*, and *Lentinula edodes* were tested by the disc diffusion method [16]. A defined concentration of the extracts ($100 \mu\text{g ml}^{-1}$) was prepared by reconstituting with sterile distilled water. The test microorganisms were seeded into respective medium by spread plate method $10 \mu\text{l}$ (10^6 cells/ml) with the 24h cultures of bacteria growth in nutrient broth. After solidification the filter paper discs (5 mm in diameter) impregnated with the extracts were placed on test organism-seeded plates. The diameters of the inhibition zones were measured in millimeter.

3. Statistical Analysis

The screening of eleven selected phytochemicals among three nominated mushrooms *Pleurotus ostreatus*, *Ganoderma lucidum* and *Lentinula edodes* was statistically evaluated. Chi-square test was used to find out correlation of phytochemicals distribution in selected three mushrooms. Mean of zone inhibition with error bars generated with standard error of mean. This analysis was carried out using SPSS version 21.

4. Results

This study has uncovered the presence of phytochemicals painstaking as active medicinal chemical ingredients. The all selected mushrooms show the 11 selected phytochemicals with different concentrations. Graphical presentation of 11 selected phytochemicals such as alkaloids, flavonoids, polyphenols, saponins, steroids, coumarins, terpenoids, vit-C, tannins, anthocyanin and cardiac glycosides are exhibited in figure 1.

In *Ganoderma lucidum*, flavonoids, polyphenols, coumarins, vit-C, tannins, and anthocyanin while steroids, terpenoids and cardiac glycosides in *Pleurotus ostreatus* as well as vit-C and tannins in *Lentinula edodes* remained in utmost concentration category. The phytochemicals distributions in *Pleurotus ostreatus*, *Ganoderma lucidum* and *Lentinula edodes* were statistically non-significant ($p > 0.05$).

In the fruiting bodies of *Pleurotus ostreatus*, polyphenols and vit-C are present as medium concentration sort whereas alkaloids, flavonoids, and tannins are existed with lowest concentration category. On the other-hand, alkaloids, and terpenoids are present in *Ganoderma lucidum* and *Lentinula edodes* as medium concentration type of phytochemicals while saponins and cardiac glycosides in *Ganoderma lucidum*, and anthocyanins and cardiac glycosides in *Lentinula edodes* are recorded in lowest concentration sort of phytochemicals.

Figure 2 shows the comparative frequencies of

phytochemicals among three selected mushrooms. 6 (54.5 %) out of 11 (100 %) phytochemicals were uppermost (+++) concentration in *Ganoderma lucidum* whereas 3 (27.3 %) and 2(18.2 %) from 11 (100 %) selected phytochemicals were in *Pleurotus ostreatus*, and *Lentinula edodes*.

A trend line of 18.2 %, 27.3 %, and 45.5 % in *Pleurotus ostreatus*, *Ganoderma lucidum*, and *Lentinula edodes* was observed as sort of medium (++) concentration category despite the fact that in lowest (+) concentration group, the highest phytochemical frequency , 27.3 %, was observed in *Pleurotus ostreatus*.

Fruiting bodies extracts of *Pleurotus ostreatus* ,*Ganoderma*

lucidum, and *Lentinula edodes* exhibited speckled in the zone of inhibition from 7-10 mm, 11-16 mm and 8-11mm respectively (Figure 3). *Ganoderma lucidum* fruiting bodies extract showed highest activity against *Bacillus cereus* (16 mm) whereas *Pleurotus ostreatus* and *Lentinula edodes* did not show activity against *Bacillus cereus* (0 mm). The maximum inhibition zone of *Pleurotus ostreatus* and *Lentinula edodes* was 10 mm against *Escherichia coli* and 11 mm against *Pseudomonas fluorescens* respectively while *Ganoderma lucidum* showed 13 mm, 12 mm, and 11 mm zone of injibition against *Stapylococcus aureus*, *Pseudomonas fluorescens*, and *Escherichia coli*.

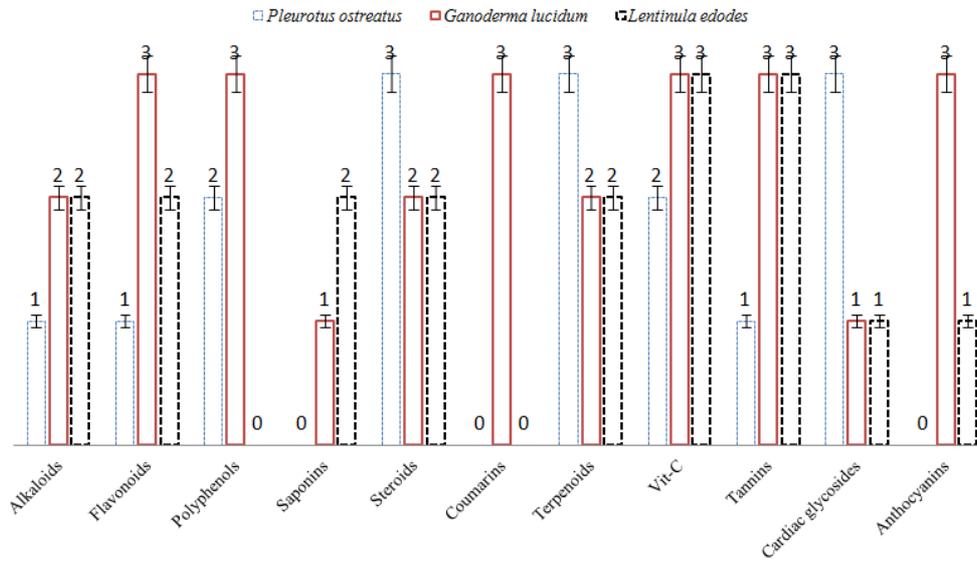


Figure 1. Distribution of phytochemicals in three selected mushrooms. [“3” = high amount after added of reagent immediately; “2” = moderate amount after 5 minutes of reagent added; “1”= low amount after 10 minutes of reagent added and “0”= absence and parenthesis; 0 = “-”, 1 = “+”, 2 = “++”, and 3 = “+++”].

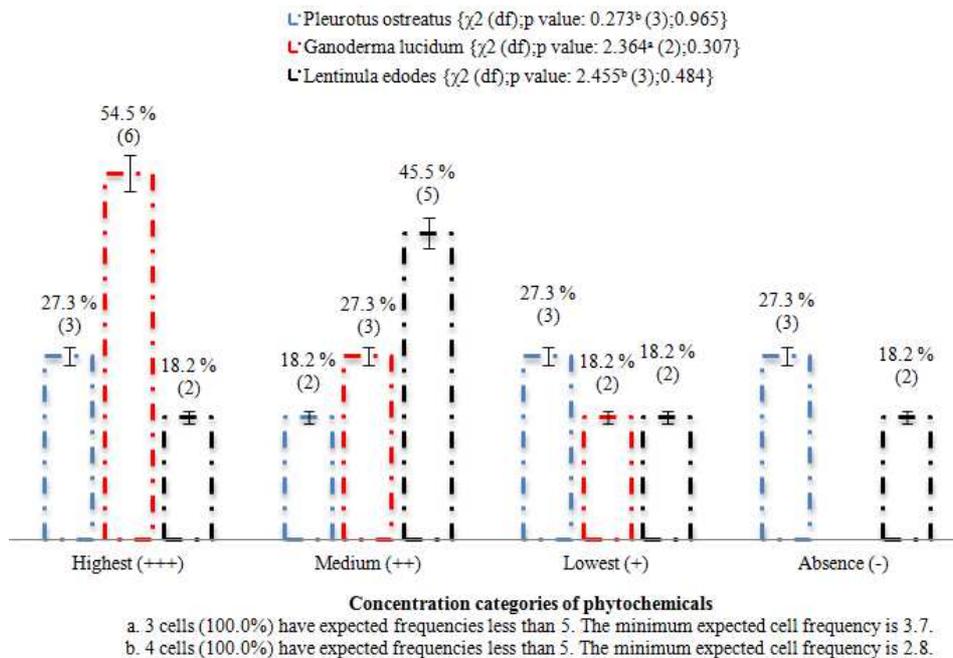


Figure 2. Graphical presentation of eleven selected phytochemicals with their reasonable abundances categories specifications. The column (X-axis) represents the concentration categories of selected plants with three color stratified and the row (Y-axis) with frequency of phytochemicals. Error bars are generated with 5 % value from selected data chart.

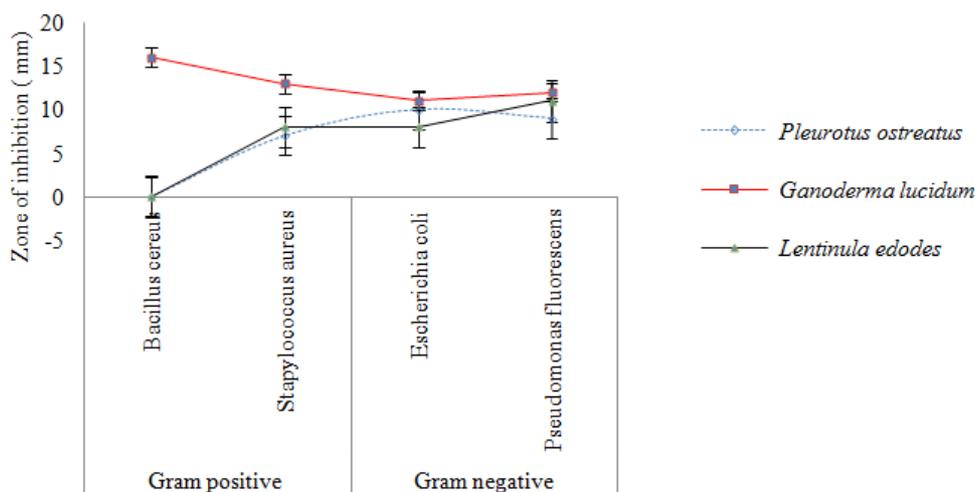


Figure 3. Antibacterial activity of edible mushrooms, *Pleurotus ostreatus*, *Ganoderma lucidum*, and *Lentinula edodes* against gram positive and gram negative bacteria. Mean of zone inhibition with error bars generated with standard error of mean.

5. Discussion

The relative distribution of eleven selected phytochemicals among the three selected mushrooms was correlated statistically in this study. In the highest concentration category, 6 of 11 phytochemicals were documented in the *Ganoderma lucidum*. Phytochemicals found in edible mushroom are known to play a vital role in upholding health. The presence of essential nutrients and minerals in the wild edible mushroom could be utilized to improve health [16, 17]. Alkaloids showed stimulant to CNS, anti-microbial activities, sympathomimetic, vasodilator, antihypertensive, antipyretics, and anti-malarial activity [18]. *Ganoderma lucidum* and *Lentinula edodes* with medium level of alkaloids bestow high medicinal values.

Several epidemiological studies have shown beneficial effects of polyphenol in cancer, cardiovascular, and neurological diseases. The health benefits associated with polyphenol containing preparation consumption have also been corroborated in animal studies of cancer chemoprevention, hypercholesterolemia, atherosclerosis, Parkinson's disease, Alzheimer's disease, and other aging-related disorders [19]. The existence of phenolic compounds in *Ganoderma lucidum* and *Pleurotus ostreatus* notice that these selected mushrooms may be used as a medicine. Tannin employs anti-inflammatory effects probably by inhibiting the release, synthesis and /or production of inflammatory cytokines and mediators, including prostaglandins, histamine, polypeptide kinins and so on [20]. Excepting *Pleurotus ostreatus* and the *Ganoderma lucidum*, and *Lentinula edodes* extracts shows highest level of tannin.

The methanolic extracts of *Pleurotus ostreatus*, *Ganoderma lucidum*, and *Lentinula edodes* were also reported that the flavonoids, cardiac glycosides, anthocyanins, steroids, coumarins, terpenoids, and vit-C were present at different concentration categories. It was reported that coumarins used in the treatment of asthma and lymphedema as well as this medication was a blood thinner used to keep blood flowing smoothly and prevent the formation of blood clots [21, 22].

Cardiac glycosides include a large family of naturally resulting compounds, the central structures of which contain a steroid nucleus with a five-membered lactone ring (cardenolides) or a six-membered lactone ring (bufadienolides) and sugar moieties [23]. Interestingly, the concentrations of cardiac glycosides used for cancer treatment are extremely close to those found in the plasma of cardiac patients treated with the same drugs, suggesting that the anticancer effects of these drugs are exerted at non-toxic concentrations [24]. The vitamin C content in *Pleurotus ostreatus*, *Ganoderma lucidum* and *Lentinula edodes* claimed that they have antioxidant activity. So, Vitamin C in humans must be ingested for survival. Vitamin C is an electron donor, and this property accounts for all its known functions. As an electron donor, vitamin C is a potent water-soluble antioxidant in humans. Antioxidant effects of vitamin C have been demonstrated in many experiments in vitro [25]. In this study, it is also reveals that the antibacterial activity of edible mushrooms show significant zone of inhibition against gram positive bacteria and gram negative bacteria. This study rationalizes the appealed uses of *Pleurotus ostreatus*, *Ganoderma lucidum* and *Lentinula edodes* in the traditional system of medicine to treat various infectious disease caused by microbes.

Based on the results obtained from the present study, it can be concluded that the methanolic extracts of mushroom can be fruitfully applied in the development of more effective and competent antimicrobial agents. The results of preliminary phytochemical analysis are in agreement with the reports of other workers. Thus we hope that the important phytochemical properties acknowledged by our study in the *Pleurotus ostreatus*, *Ganoderma lucidum* and *Lentinula edodes* will be helpful in the managing different diseases.

Supplementary effort is progress to identify the bioactive compounds and explicate their mechanism of action to scavenge the free radicals. This study is powerfully remindful that edible mushrooms can be used as antibacterial agent in the expansion of new drug for the therapy of urinary tract infections.

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