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COMPARATIVE STUDIES OF PHYTONUTRIENTS AND ANTIMICROBIAL ACTIVITIES OF ETHANOL EXTRACTS OF TWO MEDICINAL MUSHROOMS (*Pleurotus tuberregium* and *Ganoderma lucidum*)

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Abstract: The phytochemical, mineral and nutrient compositions of the two medicinal mushrooms: *Ganoderma lucidum* and *Pleurotus tuberregium* as well as its antimicrobial activity were studied. Phytochemical analysis of *Ganoderma lucidum* and *Pleurotus tuberregium* mushrooms yielded (µg/m) saponin (20.462; 2.603), flavonoid (25.000; 5.000), phenolic (38.500; 20.400), tannin (23.091; 6.8107), and alkaloid (0.360%; 0.220%)espectively. Their proximate analysis gave high moisture content of the both mushrooms (*Ganoderma lucidum* = 83.200±0.400% and *Pleurotus tuberregium* = 87.288±0.84%). Other proximate analysis (%) recorded for *Ganoderma lucidum* and *Pleurotus tuberregium* mushrooms include: ash content (8.352±0.4; 14.225±0.8), crude protein (20.588±0.8; 21.20±0.7), crude fibre (4.103±0.5; 3.499±0.3), carborhydrate (63.906; 58.621). while the yielded fat content were (3.051±0.2 % 2.455±0.8%) and vitamin c were (1550.998; 623.300 µg/ml) respectively. However mineral composition analysis yielded (g/kg) the following: potassium (92.300; 83.000), nitrogen (3.294; 3.392), magnesium (2.320; 2.460), iron (1.400; 1.900), sodium (0.800; 0.500), manganese (0.610; 0.162), and zinc (0.490; 0.690) respectively. The antimicrobial analysis of these mushroom proved the susceptibility effect of the ethanol extract of the two mushrooms against the selected bacteria and fungi. For *Ganoderma lucidum* the minimum inhibitory zone for ethanol extracts against *Staphylococccus aureus* was at 3.125% concentration however better zone of inhibition was recorded at 6.25% concentration while *Pleurotus tuberregium* ethanol extract against *Staphylococccus aureus* shows that increase in concentration increases the zone of inhibition. However, *Bacillus* sp. was recorded to be resistant against the lower concentration of both extracts (3.125% - 12.500%)

Keywords: Antimicrobial activities, Mushroom, Phytonutrient analysis.

Introduction

The term mushroom is broadly defined as follows: "a macro fungus with a distinctive fruiting body which can be either epigeous (above ground) or hypogeous (underground) and large enough to be seen with the naked eye and to be picked by hand" (Chang and Miles, 2004). Mushroom mode of nutrition is saprophytic because they lack chlorophyll, a few are parasitic; therefore, they are distinctly different from plant and animal (Gupta, *et al*., 2017). The vegetative part of the fungus, called the mycelium, comprises a system of branching threads and cordlike strands that branch out through the soil, compost, wood log or other lignocellulosic material on which the fungus is growing. After a period of growth, and under favorable conditions, the established (matured) mycelium produces the fruiting structure, which we call the mushroom. It can be classified into four which are: edible, medicinal, poisonous and other mushrooms (Chang and Miles, 2004). According to Lindequist *et al*. (2014) "medicinal mushrooms can be defined as macroscopic fungi, mostly higher Basidiomycetes, which are used in the form of extracts or powder for prevention, alleviation, or healing of diseases and/or for nutritional reasons". Since the prehistoric times, mushrooms have been important in the human diet and in traditional medicine especially in Asia, as well as America, Africa, and Europe. Mushroom are suitable for vegetarians; they contain dietary fiber, vitamins, minerals, and relatively highly protein content; low fat and sugar as such they are increasingly used for culinary purposes as healthy food (Pohleven, *et al.*, 2016). In 2010, *Pleurotus* was reported as one of the classes of edible mushroom that shows potential medicinal and functional properties while *Ganoderma* as species that are coarse, harder in texture and are bitter in taste as such are not edible but only known for their medicinal properties (Ganeshpurkar *et al*., 2010). *Ganoderma lucidium* is being referred to as the "mushroom of immortality"; recognized as an important medicinal mushroom. Popularly known as Lingzhi in China, Reishi in Japan, and Yeongji in South Korea; its pharmacological and nutraceutical applications can be traced back nearly two thousand years ago (Chen and Lan, 2018). The Edo people of Nigeria call it "Itu-oshi" (mushroom of medicinal fertility (Osemwegie *et al*., 2010). In South Eastern Nigeria, *Pleurotus tuberregium* is one of the popular mushroom consumed where it is popularly known as "ero osu". The possession of both an edible fruiting body and edible sclerotium has been its peculiarity; both of which make important contribution to the diet of the indigenous peoples of the region (Onuegbu, *et al*., 2020). The naturally occurring protective chemicals that are found in foods of plant origin and in plant based diets are called phytonutrients also referred to as phytochemicals (Poe, 2017). Mushroom can be grown or cultivated using organic wastes: farm waste such as rice straw; and industrial waste such as sawdust. The technology and the artificial production of desired mushroom is known as mushroom science; via the sclerotia of the edible and medicinal mushroom *Pleurotus tuberregium*; it is possible to grow mushrooms. The sclerotium helps the mushroom to thrive over hash environment such that sclerotium will grow out fruiting bodies of mushroom when favorable condition returns (Okhuoya and Akpaja, 2012).

Materials and Methods

Sources of Samples (Mushrooms) Used

The two mushrooms used for this work *Ganoderma lucidum* and *Pleurotus tuberregium have* different sources. While *Ganoderma lucidum* was supplied by a well-known mushroom growing or production firm/farm by name Mycofarms and Allied Synergy Limited, Isiohor Benin city; Edo state Nigeria. *Pleurotus tuberregium* was cultivated at the back of Science Laboratory Technology Faculty of Life Sciences; University of Benin.

Ganoderma lucidum:

For *Ganoderma lucidum* they were gotten fresh and divided into two, one part was air dried in readiness to be extracted for antimicrobial analysis and the other part was taken straight into the laboratory fresh for chemistry analysis.

Pleurotus tuberregium:
On 7th May 2021, three tubers of sclerotia of *Pleurotus tuberregium* were bought from Efeye in New Benin Market, Benin City; Edo State Nigeria. The sclerotia were identified in African Centre for Mushroom Innovation and Technology University of Benin. They were soaked in water for six days. On the sixth day, 13th May 2021; they were planted wholly into a heap of soil and shelter was provided to them using bamboo and straw grasses. Then they were watered morning and evening. The pinhead of the planted sclerotia was first noticed shooting out on 18th May 2021. Notwithstanding that it has started fruiting, watering them continued. Between 18th and 25th of May 2021 the fruiting body of sclerotia popular known as mushrooms had fully developed/matured. They were harvested that day 25th of May 2021. The yield was divided into two, one part was taken into the laboratory fresh for chemistry analysis; while the other part was dried and ground for extraction of the bioactive ingredient for antimicrobial analysis.

Extraction of the mushroom extracts

The air dried fruiting bodies of the two mushrooms were ground with electric blender. 250g of *Pleurotus tuberregium* was soaked in 300 ml of ethanol and 100g of *Gernoderma lucidium* was soaked in 120 ml of ethanol for 48hrs with continuous turning of the soaked materials at 1hr interval during the working hours (during the day). They were later extracted and concentrated with steam using water bath set at 80 ^{0}C until the extract is properly dried and collected, packaged in an airtight container and stored in refrigerator until required for further research studies.

Proximate Analysis: The moisture content, ash content, crude fiber, crude fat, crude protein and carbohydrate contents of both mushrooms *Ganoderma lucidum* and *Pleurotus tuberregium* was analyzed following the methods outlined by the Association of Official Agricultural Chemists (AOAC 1997).

Phytochemical Analysis: Quantitative analysis for alkaloids, phenolics, flavonoid, saponin and tannin was carried out on both mushrooms *Ganoderma lucidum* and *Pleurotus tuberregium* following the methods described by Ejikeme *et al*. 2014).

Mineral composition: Both *Ganoderma lucidum* and *Pleurotus tuberregium* mushrooms were analyzed for the following minerals composition: potassium, nitrogen, magnesium, manganese, iron and sodium.

Experimental Microorganisms: Staphylococcus aereus, Candida albicans, Mucor mucedo and *Penicilium chrysogenum* were obtained from the Pharmaceutical Microbiobiology Laboratory, Faculty of Pharmacy, University of Benin; *Bacillus subtilis, Pseudomonas aeruginosa, Micrococcus luteus, Enterococcus faecalis*, and *Klebsiella pneumonia* were obtained from Microbiology Laboratory, Faculty of Life Sciences, University of Benin; *Providencia rettgeri, Arthrobacter globiformis, Acinetobacter calcoaceticus*, were obtained from Science Laboratory Technology Laboratory, Faculty of Life Sciences, University of Benin; *Aspergillus tamari, Aspergillus niger, Trichoderma harzianum* and *Escherichia coli* were collected from the Plant Biology and Biotechnology Laboratory, Faculty of Life Sciences, University of Benin, Benin City. Each of these microorganisms was already identified by the various laboratory technologists/scientists before collection. The microorganisms were stored in the refrigerator.

Preparation of Crude Extract

Ethanolic extract of 3.000g was weighed into a sterile beaker containing 3 ml of ethanol which was used as stock concentration. Further concentrations were made from the stock concentration by double serial dilutions. This was carried out by transferring of 1ml of the stock concentration into a test tube containing 3 ml of ethanol to 25mg/ml, another 1ml was transferred from the 25mg/ml into a test tube containing 1ml of distilled water to obtain 12.5mg/ml and this process was continued to obtain concentrations of 25mg/ml, 12.5mg/ml, 6.25mg/ml, and 3.125mg/ml.

Antimicrobial Plating: Antimicrobial plating was carried out as described by Elek and Hilson (1954). The Inhibition Zone Diameter (IZD) was measured after incubation using a transparent meter rule.

Result and Discussion

Chemical Composition: The proximate analysis showed that the fruiting body of both mushrooms *Ganoderma lucidum* and *Pleurotus tuberregium* have high moisture content of 83.200 ± 0.800% and 87.288 ± 0.840% respectively (Table 1).

Table 1: Proximate composition of the two medicinal mushrooms *Ganoderma lucidum* and *Pleurotus tuberregium*.

Parameters	*Ganoderma lucidum*	*Pleurotus tuberregium*
Moisture Content (%)	83.200±0.800	87.288±0.840
Ash Content (%)	8.352±0.400	14.225±0.800
Crude Fiber (%)	4.103±0.500	3.499±0.300
Crude Fat (%)	3.051±0.800	2.455±0.800
Crude Protein (%)	20.588±0.800	21.200±0.700
Carbohydrate (%)	63.906	58.621
Vitamin C (µg/ml)	1550.998	623.300

Values are represented as mean ± S.E.M. where n = 3.

This indicate a low shelf life; it means that both mushrooms can deteriorate easily when not used immediately or properly preserved; also, it was observed that *Pleurotus tuberregium* has a higher moisture content than *Ganoderma lucidum*. The protein content of *Ganoderma lucidum* and *Pleurotus tuberregium* mushroom samples were 20.588 ± 0.800% and 21.200 ± 0.700%; from the result obtained as indicated in table 1 above, it can be clearly stated that *Pleurotus tuberregium* is more proteinous than *Ganoderma lucidum.* Onuegbu *et al*. (2020) working on *Pleurotus tuberregium* reported the protein content to be at the range of (12.35-19.83%) and they equally reported the fat content of same mushroom to be at the range of (1.95-3.96%) which is relative to the result obtained in this study as the determined amount fell within the percentage range provided by Onuegbu *et al*. (2020) which is (2.455±0.8%). *Ganoderma lucidum* just like *Pleurotus tuberregium* has a low fat content of (3.051 ± 0.200%), but a little bit higher than the later. The low fat content is important for those who prefer a low fat diet/supplement. *Ganoderma lucidum* and *Pleurotus tuberregium* mushrooms have high carbohydrate content 63.906% and 58.621%; which comprised mainly of dietary fibre. Onuegbu *et al.* (2020) reported also that the carbohydrate content of sclerotia of *Pleurotus tuberregium* gotten from different market to be within the range of (67.42 – 74.03). Dietary fibres are also another nutrients of interest in foods that are considered healthy. This is because of the enormous roles they play in maintaining good bowel movement, low cholesterol levels etc. Dietary fibers are high molecular weight materials which are resistant to digestion and absorption in human beings but it can move along with food through the digestive system absorbing water. Chemically, dietary fiber consists of plant components such as cellulose, lignin, waxes, chitins, pectins and β-glucans. Edible mushroom is considered as a novel source of dietary fiber (Kaushik, 2016).

Mineral Composition

Table 2: Mineral compositions of the two medicinal mushrooms *Ganoderma lucidum* and *Pleurotus tuberregium*.

Parameters	*Ganoderma lucidum*	*Pleurotus tuberregium*
Sodium (g/kg)	0.800	0.500
Potassium (g/kg)	92.300	83.000
Calcium (g/kg)	1.200	0.200
Magnesium (g/kg)	2.320	2.460
Manganese (g/kg)	0.610	0.162
Copper (g/kg)	0.100	0.100
Zinc (g/kg)	0.490	0.690
Iron (g/kg)	1.400	1.900

Phytochemical Content

Several phytochemical compounds were found in *Pleurotus tuberregium* and *Ganoderma lucidum* (Table 3), such as phenolic, flavonoid, saponin, alkaloid, and tannin.

Table 3: Phytonutrient composition of the two medicinal mushrooms *Ganoderma lucidum* and *Pleurotus tuberregium*.

Parameters	*Ganoderma lucidum*	*Pleurotus tuberregium*
Total Alkaloids (%)	0.360	0.220
Total Phenolics (µg/mL)	38.500	20.400
Total Flavonoid (µg/mL)	25.000	5.000
Total Saponin (µg/mL)	20.462	2.605
Total Tannin (µg/mL)	23.091	6.810

The amount of alkaloid, flavonoid, tannin content of *pleurotus tuberregium* are 0.220%, 5.000%, and 6.81%. While that of *Ganoderma lucidum* are 0.36%, 25.00%, and 23.091 respectively. In comparison of the both; *Ganoderma lucidum* have high contents of alkaloid, flavonoid and tannin. The results of the flavonoid and tannin contents of the *pleurotus tuberregium* samples were comparatively a little higher than the results given by Onuegbu *et al.* (2020). But the alkaloid contents in the former, ie *Pleurotus tuberregium* sample was below the range of alkaloid result reported by Onuegbu *et al.* (2020); (1.660 – 2.137%). The flavonoid compositions of *Pleurotus tuberregium* and *Ganoderma lucidum* mushrooms are significantly lower than the tolerable limit (52.020mg/100g) by World Health organization (2005), indicating that the mushrooms are equally safe and could be good sources of antioxidants that boosts body immunity. The value of saponin content of *Pleurotus tuberregium* and *Ganoderma lucidum* mushrooms obtained in this study were 2.603 and 20.462% respectively which are within the WHO maximum permissible limit (48.500 mg/10g). The results suggest that the mushrooms could be safe for consumption. Therefore, *Pleurotus tuberregium* with very low saponin content clearly shows its edibility; its use as food. For *Ganoderma lucidum*, its edibility is minute as a result of its average amount of saponin contents; which shows that it is not poisonous though it might not be used as food but can be used in medicine and supplements. The alkaloid results of this study show *Ganoderma lucidum* to be 0.360% and *Pleurotus tuberregium* to be 0.220%.However, Wood *et al.* (2021) who worked on four *Ganoderma* species: *G. resinaceum*, *G. applanarum*, *G. praelongum* and *G. lucidum* reported them to have their alkaloid ranges from 1.11 – 7.26% with an average value of 4.71 ± 0.10% which is higher than the result obtained in this study. Also, Wood *et al.* (2021) using *Pleurotus plumonarus* as control reported it to have the highest value; but the WHO safe limit is 61.00 mg/100 g. The values obtained indicated that both mushrooms *Pleurotus tuberregium* and *Ganoderma lucidum* are safe for consumption in large quantity and can help take care of various ailments. Research scientist has opined that *Pleurotus* spp. are carriers of alkaloids that can be extracted for pharmaceutical purpose due to its antioxidant properties.

Antimicrobial activity

The antimicrobial susceptibility of the ethanol extract of *Pleurotus tuberregium* and *Ganoderma lucidum* shows that all test microorganisms which include *Staphylococcus aureus*, *Pseudomonas areuginosa*, *E. coli*, *Micrococcus* sp, Enterococcus sp, *Klebsiella* sp, *Bacillus* sp, *Arthrobacter* sp, *Acinetrobacter* sp, *Providencia* sp, where inhibited by the ethanol extracts of both mushrooms at various levels. *Ganoderma lucidum* ethanol extract inhibited all the bacteria at all concentration: 3.125%, 6.250%, 12.500%, and 25%; however, 6.250% shows a better zone of inhibition against *Staphylococcus aureus*, *Pseudomonas areuginosa*, *Micrococcus* sp, and *Klebsiella* sp (figure 2). *Pleurotus tuberregium* ethanol extract on the other hand against *Staphylococcus aureus*, shows that antimicrobial activity increase with increase in the extract concentration; against *Providencia* sp, and *Bacillus* sp, it was noted that they are only susceptible at higher concentration (25%). However better zone of inhibition was exhibited at 3.125% against *E. coli* (figure 1). When *Pleurotus tuberregium* and *Ganoderma lucidum* ethanol extracts effect against bacteria are compared the following are observed: at 3.125% concentration *Bacillus* sp, is not susceptible to *Pleurotus tuberregium* ethanol extract but susceptible to *Ganoderma lucidum* ethanol extract. Against *E. coli*, *Pleurotus tuberregium* has more significant zone of inhibition than *Ganoderma lucidum*. *Ganoderma lucidum* on the other hand has more significant zone of inhibition against *Klebsella* and *Providencia* than *Plurotus tuberregium*. The other concentration levels show that *Ganoderma lucidum* ethanol extract has a better and more significance zones of inhibition against the test bacteria.

Antifungal Activity

The antifungal susceptibility of the ethanol extract of *Pleurotus tuberregium* and *Ganoderma lucidum* shows that all test microorganisms which include *Candida albican Mucor* sp, *Penicillium* sp, *Trichoderma* sp, *Aspergilus temare* and *Aspergilus niger*. *Ganoderma lucidum* ethanol extract inhibited all fungi; however, a significant/better zone of inhibitions were shown at 6.250% against mucor, at 3.125% against *Penicillium* and *Aspergillus niger* while at 25% concentration *Aspergilus niger* also shows a significant zone of inhibition (figure 3). At all concentration levels there were zone of inhibition for *Pleurotus tuberregium* ethanol extract. It inhibited all fungi; however a significant and better zone of inhibition was record against *candida* sp and *mucor* sp; at 12.5% and 25% concentration respectively (figure 4). In comparison of both extracts at different concentration; *Pleurotus tuberregium* ethanol extract At 3.125% concentration has susceptibe effect against *Bacillus* while *Ganoderma lucidum* ethanol extract does. Against *E. coli*, *Pleurotus tuberregium* ethanol extract has more significant zone of inhibition than *Ganoderma lucidum*. *Ganoderma lucidum* ethanol extract on the other hand have more significant zones inhibition against *Klebsella* and *Providencia* than *Plurotus tuberregium*. For other concentration levels *Ganoderma lucidum* ethanol extract have a more appreciatable zones of inhibition more than *Pleurotus tuberregium* ethanol extract.

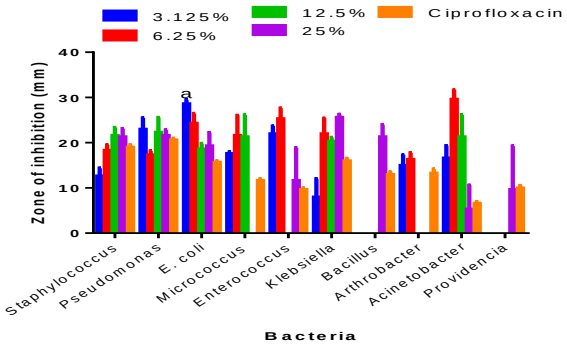

Figure 1: Inhibition of various concentration of ethanol extracts of *Pleurotus tuberregium* and ciprofloxacin against bacteria. At 3.125% concentration there was a better zone of inhibition of *E. coli* however in other concentration also there was zone of inhibition against different bacteria. Values are represented as mean ± S.E.M. Where n= 4 ($^{a}p<0.01$).

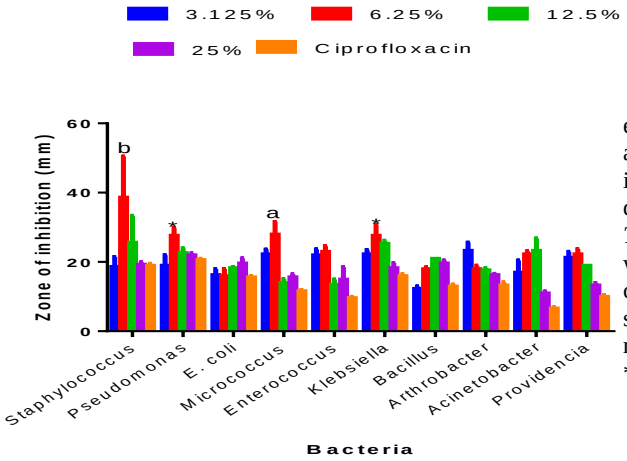

Figure 2: Inhibition of various concentration of ethanol extracts of *Ganoderma lucidum* and Ciprofloxacin antibiotics against bacteria. At the concentrations there was zone of inhibition; *Ganoderma lucidum* inhibit all; however 6.25% concentration shows a better zone of inhibition for *staphylococcus aureus*, *pseudomonas*, *micrococcus* and Klebsaiella. Values are represented as mean ± S.E.M. Where n= 3. ($^{b}P<0.0001$, $^{a}p<0.01$, $*p<0.05$).

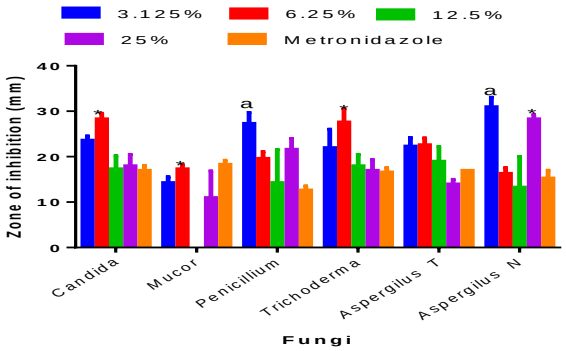

Figure 3: Inhibition of various concentration of ethanol extracts of *Ganoderma lucidum* and metronidazole against fungi. *Ganoderma lucidum* at all concentration inhibited the test fungi but better zones of inhibition were displayed at 6.25% conc against *Candida*, *Mucor* and *Trichoderma*. While *Penicillium* and *Aspergilus niger* were more susceptible at 3.125% and at 25% concentration also *Aspergilus niger* was significantly susceptible to *Ganoderma lucidum*. Values are represented as mean ± S.E.M. Where n= 3. (, $^{a}p<0.01$, $*p<0.05$).

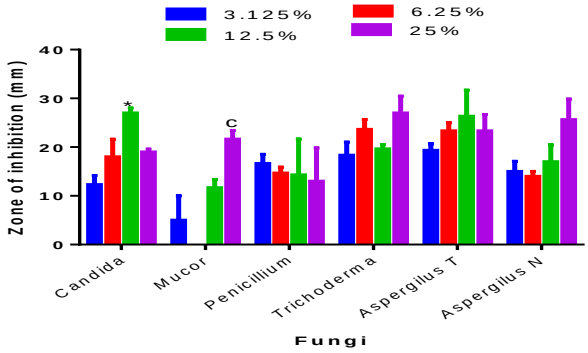

Figure 4: Inhibition of various concentrations of *Pleurotus tuberregium* against fungi. At all concentration levels there were zone of inhibition, pleurotus tuberregium inhibited all fungi; however a significant zone of inhibition was record on candida an mucor where 12.5% conc. Shows a better zone of inhibition among other concentration so also Mucor at 25% concentrations.

Values are represented as mean ± S.E.M. Where n= 3. ($^{c}p<0.001$, $^{*}p<0.05$).

Conclusion

The phytochemical compositions observed have shown the presence of some vital bioactive compounds of the two mushrooms: *Ganoderma lucidum* and *Pleurotus tuberregium*. The observed levels suggest that these mushrooms would be a good source of some natural antibiotics and antioxidants. Therefore, consumption of these mushrooms in large quantity has no toxic effect and need to be domesticated owning to its nutritional and pharmacological essence. More refined studies could be envisaged in order to accurately identify and isolate the active compounds of the crude extracts that inhibit fungi and bacteria, with the ultimate goal being to manufacture natural or new pharmaceutical products potentially more efficient against diseases caused by the tested human pathogenic bacteria and fungi; on the basis of these rather promising results, more refined studies could be envisaged in order to accurately identify and isolate the active compounds of the crude extracts that inhibit fungi and bacteria, with the ultimate goal being to manufacture natural or new pharmaceutical products potentially more

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APPENDIX

- The pictorial display of the procedures/outcomes of the cultivated *Pleurotus Tuberregium* Mushroom species:

Plate 1: Sclerotia of *Pleurotus tuberregium* (Photo Credit).

Plate 2: Shade provided for planted sclerotia of *Pleurotus tuberregium* (Photo Credit).

Plate 3: Mushroom growth at its early stage (Photo credit).

Plate 4: Fully matured *Pleurotus tuberregium* ready to harvest (Photo Credit).

Plate 5: Fully matured *Pleurotus tuberregium* ready to harvest (Photo Credit).

Plate 6: Fresh fruiting body of *Ganoderma lucidium*