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Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing *Escherichia coli* in the Calgary Health Region: emergence of CTX-M-15-producing isolates. *Antimicrob. Agents Chemother.* 51: 1281-1286.

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Full Length Research Paper

Evaluation of the effects of the combination of NPK fertilizer, cow dung, humus soil and poultry droppings with sawdust on the number of days to primordia formation, maturity and harvest of *Pleurotus ostreatus*

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This study on the effects of combination of NPK fertilizer, cowdung, humus soil and poultry droppings with sawdust on the number of days to primordia formation, maturity and harvest of *P. ostreatus* was carried out in the Department of Plant Science and Biotechnology, Imo State University, Owerri. The objectives of the study were to identify the most suitable substrate combination in the determination of the number of days to the formation of primordia, maturity and harvest of *P. ostreatus*. Seventeen (17) treatments were laid out in completely randomized design (CRD). The data collected were subjected to analysis of variance (ANOVA) and means were separated by Fisher's least significant difference (LSD). From the experiment, treatments T6, T0, T8, T1, T3, T4, T7 and T2 formed primordia in 49, 37.67, 35.67, 34.67, 34, 28, 26.33 and 17 days, respectively. The result further revealed the mean days for maturity and harvesting of *P. ostreatus* which were 52 days in T6 (400g sawdust + 100 g humus soil) followed by 37.67 days in T0 (sawdust alone), 35.67 days in T8 (300g sawdust + 200 g humus soil), 34.67 days in T1 (450 g sawdust + 50 g cow dung), 34 days in T3 (300g sawdust + 200 g cow dung), 28 days in T4 (300 g sawdust + 200 g cow dung), 26.33 days in T7 (350 g sawdust + 150 g humus soil) and 17 days in treatment two (T2) (400g sawdust + 100 g cow dung). The study further reveals a significant difference between the treatments and the control at 5% probability ($P = 0.05$).

Key words: *Pleurotus ostreatus*, primordia formation, maturity, harvest, substrates.

INTRODUCTION

Mushroom biology is the branch of mycology that deals with mushrooms. Etymologically, we say that mycology is the study of mushrooms, since mushrooms are fungi. Fungi are a large group of simple thallus-like achlorophyllous and heterotrophic thallophytes, made up of hyphae, which together constitute mycelium. They are

majorly cosmopolitan in distribution and found in all available habitats on earth where organic materials are present. Some species occur in fresh or marine water, others are terrestrial while some are airborne, with majority preferring to grow in dark, dim lighted moist habitat.

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Mushroom is defined as a macrofungus with a distinctive fruiting body which can either be epigeous (growing on or close to the ground) or hypogeous (growing under the ground) (Chang and Miles, 1991). The fruiting bodies are large enough to be seen with naked eyes, and to be picked up by hand, and appear in different shapes and sizes.

Mushrooms are heterotrophs as they lack chlorophyll and cannot photosynthesize, but instead they take nutrients from outer sources. They are often found growing on dead wood and other decaying organic matter, and are rich in protein, vitamins and mineral salts (Singh et al., 2009; Sinha and Vashishta, 2005; Oei, 2003). Some mushrooms are edible while some are inedible. Edible mushrooms are a low calorie food usually eaten cooked or raw and as garnish to a meal. They are a good source of B vitamins, such as riboflavin, niacin and pantothenic acid, and the essential minerals (selenium, copper, potassium etc). Fat, carbohydrate and calorie contents of mushroom are low, with absence of vitamin C and sodium.

Pleurotus ostreatus is of great importance in Nigeria and many parts of the world including America, India, Asia, Australia, Japan, Cameroon, etc, because of its high nutritional value and high level of vitamins and proteins (Onuoha, 2007; Shah et al., 2004). It is cultivated worldwide for food, as they are often used in preparing continental dishes, stews, soups, sauces, sandwiches, burgers and salads (Chang and Chiu, 1992). The medicinal values of mushrooms have long been recognized in China, Korea, Japan, India and partly in Nigeria. The mushroom, *P. ostreatus* not only serve as vegetables (food), but also produces several bioactive compounds that are usually associated with the cell wall and are of medicinal value including being used as complementary medicine/dietary supplements for anticancer, antiviral, antitumor, anti-hypertensive, antimicrobial, immunopotentiating, hypocholesterolaemic and hepatoprotective agents (Chang and Buswell, 1996; Gregori et al., 2007). *P. ostreatus* contains lovastatin that helps in reduction of cholesterol in man, thus making it suitable for patients with high blood pressure, heart diseases and diabetes (Gunde and Cimerman, 1995; Eger et al., 1976). It also contains lectins which are glycoproteins and have been shown to have anti-tumor and immunomodulatory activities (Wang et al., 1996).

Mushrooms in recent years are used as bioconversion of agricultural and industrial wastes into food has attracted the world attention. Mushrooms like *P. ostreatus* are either harvested wildy or cultivated by individuals in the laboratory, homes or farmland, as such mushroom cultivation is environment friendly, having beneficial impacts such as reduction of environmental pollution, biogas and biofertilizer production, bioremediation/mycoremediation, mycofiltration, mycopesticides and mycoforestry. This study is therefore designed to assess the efforts of nutrient combination on the numbers of days to

primordia formation, maturity and harvest of *P. ostreatus*.

MATERIALS AND METHODS

Source of materials

The spawn used in this work was obtained from the Department of Plant Science and Biotechnology of the University of Port Harcourt in Rivers State. Fresh hard wood sawdust was gotten from the Njoku and Sons Wood Mill in Owerri, Imo State, two months old cow dung was gotten from the relief veterinary centre along Egbu road in Owerri, two months old poultry droppings was collected from the Onyekwere's poultry farm, Egbu in Owerri North Local government Area of Imo State. NPK fertilizer was bought at the Eke Ukwu market, Owerri while the humus soil was gotten from the university farm.

Preparation and sterilization of substrates

To a heap of sawdust on a cement platform, water was added in the ratio of 1:2 (v/v) and mixed thoroughly, the substrate was then piled up into a heap of 11:3 m high by 1.2 m diameter, covered with a black plastic polyethylene sheet to undergo fermentation for four (4) weeks, with regular turning. After four weeks, the fermented sawdust was measured, mixed with 1% lime dust and the other substrates were added indigenously, due to the presence of the animal droppings, the mixed substrates were given another two (2) weeks to reach a uniform temperature of 28°C. The treatments include: T0: 500 g fermented sawdust (Control); T1: Mixture of 450 g fermented sawdust and 50 g of cow dung; T2: Mixture of 400 g fermented sawdust and 100 g of cow dung; T3: Mixture of 350 g fermented sawdust and 150 g of cow dung; T4: Mixture of 300 g fermented sawdust and 200 g of cow dung; T5: Mixture of 450 g fermented sawdust and 50 g of humus soil; T6: Mixture of 400 g fermented sawdust and 100 g of humus soil; T7: Mixture of 350 g fermented sawdust and 150 g of humus soil; T8: Mixture of 300 g fermented sawdust and 200 g of humus soil; T9: Mixture of 450 g fermented sawdust and 50 g of NPK; T10: Mixture of 400 g fermented sawdust and 100 g of NPK; T11: Mixture of 350 g fermented sawdust and 150 g of NPK; T12: Mixture of 300 g fermented sawdust and 200 g of NPK; T13: Mixture of 450 g fermented sawdust and 50 g poultry droppings; T14: Mixture of 400 g fermented sawdust and 100 g poultry droppings; T15: Mixture of 350 g fermented sawdust and 150 g poultry droppings; T16: Mixture of 300 g fermented sawdust and 200 g poultry droppings.

Bagging and pasteurisation

Five hundred grams (500 g) of the treatments were measured into polypropylene plastic bags (8 cm high x 18 cm width). The concentrations of the treatments used are 50, 100, 150, 200 g, produced in three replicates. Each bag was watered thoroughly, properly sealed, and sterilized at 100°C at 121psi for five (5) hours, using a pressure pot. After the sterilization, the substrates were left to cool for 48 h to enable them cool to an ambient temperature (30°C), so that the substrates containing the animal droppings could stabilize and be uniform with the other substrates.

Inoculation and incubation

Having reached a uniform temperature of 28°C, the bags were inoculated with the spawns of the *P. ostreatus* mushroom, at the rate of twenty grams (20 g) per bag, plugged with cotton wool and



Plate 1. a and b showing primordia of *P. ostreatus* on cowdung substrate.

banded to keep it sealed and air proof), all processes were done under aseptic condition, then the bags were kept in a dark room at a temperature of $(25 \pm 2^\circ\text{C})$ and watered very properly and regularly, to create a very humid environment for mycelium colonization. After about 30 days, the bags were transferred to the main laboratory (the cropping area) and opened to monitor the rate at which the mycelia had colonized the bags, and then they were for fructification (sporulation). The growing area and the substrate bags were slightly watered daily to keep them always relatively humid (RH) in the morning and evening during cropping.

Experimental layout

All the treatments for the experiment were laid out, and arranged in a completely randomized block (CRD) design, and each treatment was set up in three replicates. For each treatment, there were 3 replicate bags, making up a total of sixty (60) bags in the experimental block. The total number of days to primordia appearance was calculated and noted. Also, calculated is the number of days for maturity and harvesting of the *P. ostreatus*.

RESULTS

The outcome of the experiment showed that all the treatments had mycelial colonization after 30 days in varied proportions, except those of NPK and poultry droppings lost with mycelia before maturity. Furthermore, it showed that the highest mean number of days to primordia formation was recorded in 400 g of sawdust and 100 g of humus soil (49 days) and it is significantly different from all other treatments while the least mean number of days recorded in 400 g of sawdust and 100 g of cowdung (16.33 days) is significantly different from the

highest and all other treatments. 32.67 days was gotten for 450 g of sawdust and 50 g of cowdung, 32 days for 350 g of sawdust and 150 g of cowdung, while 36 days were gotten for sawdust alone which acted as the control. Furthermore, 26.33 days were gotten for 300 g of sawdust plus 200 g of cowdung, 24 days for 350 g of sawdust and 150 g of humus soil and 27 days for 300 g of sawdust and 200 g of humus soil. Other treatments did not form any primordia as shown in Plate 1, Table 1 and Figure 1.

The result further shows the highest mean number of days reached for maturity and harvest is 52 days recorded in 400 g of sawdust plus 100 g of humus soil is significantly different from the mean number of days in 400 g of sawdust plus 100 g cow dung which is 17 days, and from the other treatment with mean number of days as 500 g of sawdust (37.67 days) of equal significance, which in turn are significantly different from that of 300 g of sawdust + 200 g of cow dung (28 days) at $P < 0.05$. Also, 35.67 days were gotten for 300 g sawdust + 200 g of humus soil while 34 days was recorded for treatment three (T3) which is 350 g of sawdust + 150 g of cow dung as shown in Table 2, Plate 2 and Figure 2.

According to the results, treatment T5 did not produce fruiting bodies, this development could be attributed to environmental factor, dryness, imbalance of the mixtures used (Ayodele and Okhunya, 2007).

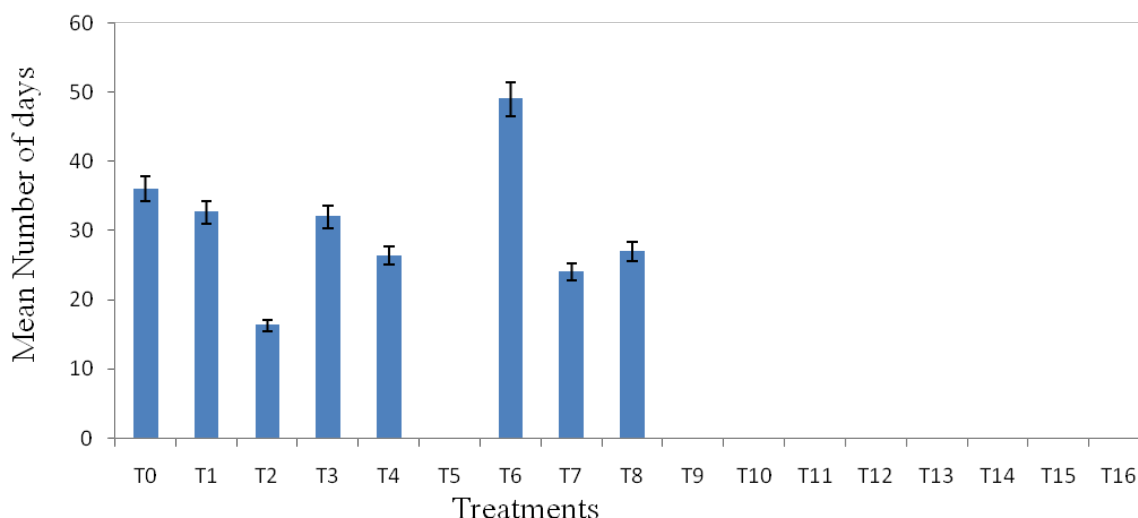
DISCUSSION

The result of this research work has revealed that growth

Table 1. Effect of combination of NPK, cow dung, humus soil and poultry droppings on the number of days to primordia formation.

Treatment (T)	Mean number of days to primordia formation
500 g sawdust (control) (T0)	36.00 ^{ab}
450 g Sawdust + 50 g Cow dung (T1)	32.67 ^{ab}
400 g Sawdust + 100 g Cow dung (T2)	16.33 ^{bc}
350 g Sawdust + 150 g Cow dung (T3)	32.00 ^{ab}
300 g Sawdust + 200 g Cow dung (T4)	26.33 ^{abc}
450 g Sawdust + 50 g Humus soil (T5)	0.00 ^c
400 g Sawdust + 100 g Humus soil (T6)	49.00 ^a
350 g Sawdust + 150 g Humus soil (T7)	24.00 ^{abc}
300 g Sawdust + 200 g Humus soil (T8)	27.00 ^{abc}
450 g Sawdust + 50 g N.P.K (T9)	0.00 ^c
400 g Sawdust + 100 g N.P.K (T10)	0.00 ^c
350 g Sawdust + 150 g N.P.K (T11)	0.00 ^c
300 g Sawdust + 200 g N.P.K (T12)	0.00 ^c
450 g Sawdust + 50 g Poultry dropping (T13)	0.00 ^c
400 g Sawdust + 100 g Poultry dropping (T14)	0.00 ^c
350 g Sawdust + 150 g Poultry dropping (T15)	0.00 ^c
300 g Sawdust + 200 g Poultry dropping (T16)	0.00 ^c
LSD Value	28.96

Each value is a mean of 3 replicates. Means in the same column, with the same letter (s) are not significantly different at $P < 0.05$.

**Figure 1.** Effect of combination of NPK, cow dung, humus soil and poultry droppings on the number of days to Primordia formation.

of *P. ostreatus* was supported by 500 g of sawdust, 450 g sawdust + 50 g cowdung, 400 g sawdust + 100 g cow dung, 350 g sawdust + 150 g cowdung, 300 g sawdust + 200 g cowdung, 400 g sawdust + 100 g humus soil, 350 g sawdust + 150 g humus soil and 300 g sawdust + 200 g humus soil. This can be attributed to the fact that

cowdung contained the essential nutrients needed by the mushroom to grow properly. This is in line with the works of Zadrazil (1980) who reported that the growth of *Pleurotus* species is favoured on substrates low in nitrogen content and this also is the reason why all the combinations containing NPK fertilizer did not support the

Table 2. Effect of combination OF NPK, cow dung, humus soil and poultry droppings on the number of days to maturity and harvest.

Treatment (T)	Mean Number of days to Maturity & Harvest
500 g sawdust (control) (T0)	37.00 ^{ab}
450 g Sawdust + 50 g cow dung (T1)	34.67 ^{ab}
400 g Sawdust + 100 g cow dung (T2)	17.00 ^{bc}
350 g Sawdust + 150 g cow dung (T3)	34.00 ^{ab}
300 g Sawdust + 200 g cow dung (T4)	28.00 ^{abc}
450 g Sawdust + 50 g humus soil (T5)	0.00 ^c
400 g Sawdust + 100 g humus soil (T6)	52.00 ^a
350 g Sawdust + 150 g humus soil (T7)	26.33 ^{abc}
300 g Sawdust + 200 g humus soil (T8)	35.67 ^{ab}
450 g Sawdust + 50 g N.P.K (T9)	0.00 ^c
400 g Sawdust + 100 g N.P.K (T10)	0.00 ^c
350 g Sawdust + 150 g N.P.K (T11)	0.00 ^c
300 g Sawdust + 200 g N.P.K (T12)	0.00 ^c
450 g Sawdust + 50 g poultry dropping (T13)	0.00 ^c
400 g Sawdust + 100 g poultry dropping (T14)	0.00 ^c
350 g Sawdust + 150 g poultry dropping (T15)	0.00 ^c
300 g Sawdust + 200 g poultry dropping (T16)	0.00 ^c
LSD VALUE	31.56

Each value is a mean of 3 replicates. Means in the same column, with the same letter (s) are not significantly different at $P < 0.05$.

**Plate 2.** Matured *P. ostreatus* ready for harvest.

growth of *P. ostreatus*. Shah et al. (2004) reported that *P. ostreatus* gave maximum biological efficiency on saw-

dust. This also supported the result of this study as sawdust equally supported the growth of the mushroom

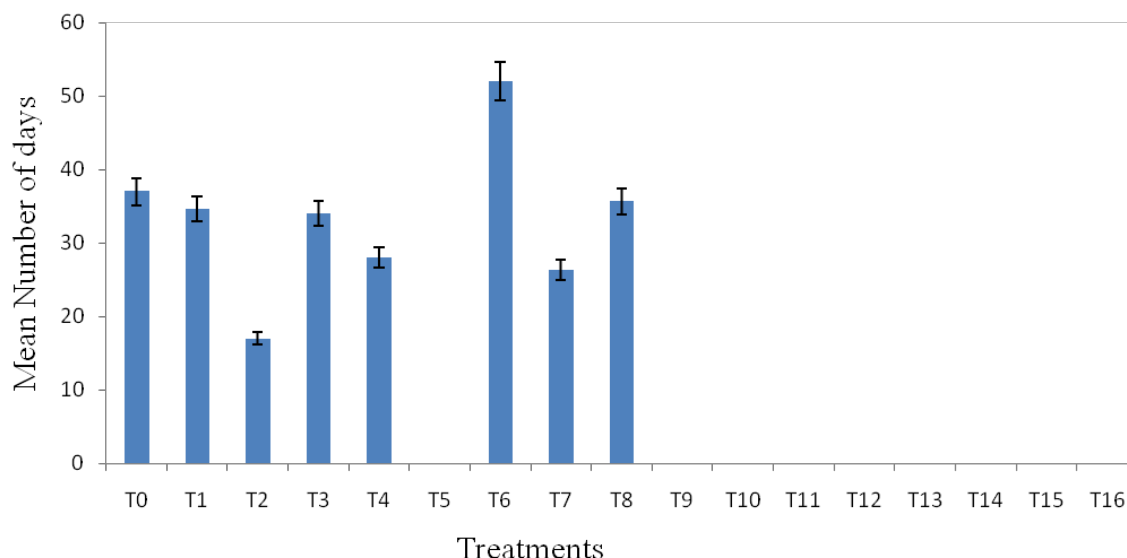


Figure 2. Effect of combination of NPK, cowdung, humus soil and poultry droppings on the number of days to maturity and harvest.

when used alone. This confirmed the works of Candy (1990) who grew mushrooms on different pure sawdust types and obtained the best result from Eucalyptus sawdust.

The results further showed that all possible combinations with poultry droppings and NPK fertilizer did not show any growth signs. This could be attributed to the fact that poultry droppings produced heat and there is every likelihood that the heat produced by the treatment might have destroyed the nutrients needed by the *P. ostreatus*. Also, NPK fertilizer combinations yielded no growth as the NPK on its own may have burnt up the spawn due to its corrosive nature or probably that the concentrations of the treatments were too much or too little for the normal growth of the mushroom. Their combinations might change the sequence of decomposition of the substrate components as reported by (Ayodele and Okhunya, 2007).

Shah et al. (2004) and Ponmurugan et al. (2007) reported that full colonization in *P. ostreatus* takes 17-20 days on different substrates thus supporting the result gotten from 400 g sawdust + 100 g cow dung which took 16.33 days to attain full colonization, while other results gotten from 500 g sawdust, 450 g sawdust + 50 g cow dung, 350 g sawdust + 150 g cow dung, 300 g sawdust + 200 g cow dung etc took longer days for full colonization. This is in line with the works of Royse et al. (2004), Oseni et al. (2012), Khare et al. (2010) and Mane et al. (2007). The production of enzymes such as cellulases, hemicellulases and lignases by fungal mycelium, is a crucial part of the colonization process and thus this is important in determining mushroom growth and maturity (Buswell et al., 1996). This might be the reason for the longer days for maturity of *P. ostreatus* as shown by the

results. From the results, it was revealed that NPK fertilizer and poultry droppings are not good substrate combination for the growth and maturity of *P. ostreatus* as they showed no growth of the mushroom.

Conclusion

The study reveals that the best substrate combination which have least number of days for the formation of primordia, maturity and harvest is 400 g sawdust + 100 g cow dung followed by 350 g sawdust + 150 g humus soil as they produced in the least number of days, thereby reducing the longer days it takes for the maturity of the mushroom. Furthermore, mushroom growers should utilize more of the cow dung and humus soil in combination with sawdust, than cultivation with only sawdust since they are economically feasible and available year round.

Conflict of Interests

The author(s) have not declared any conflict of interest.

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Review

Bioactive potential of wild edible mushrooms and need for their conservation

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The field of natural products and their use in the development of medicinal or other health-related products vis-à-vis their conservation needs special focus. The challenges for fungal conservation are daunting. As nature's recyclers, fungi are like municipal refuse collectors employed to take away our rubbish. We do not notice them until they go on strike. The well-being of fungi is necessary for sustainable life on this planet. Mushrooms have long been used as a valuable food source, widely appreciated for their unique taste and flavor and as traditional medicines around the world since ancient times, especially in Japan and China. In recent years, the interest in mushrooms as a dietary fiber or healthy food has increased. Scientists have known for over 100 years that, like animals and plants, fungi too are affected by the destructive activities of mankind. The impact of air pollution on lichen forming fungi is one particularly well documented example. Although there is still insufficient information about the conservation status of fungi, there is no reason to suppose that fungi are any less vulnerable than other groups of organisms to habitat loss and climate change. The topic is far too important to ignore. Knowledge gap is one of the challenges which should be solved to strengthen connections between the pharmaceutical industry and conservation biology. Public awareness of their importance is, however, very low, not least because biodiversity - the full and wonderful diversity of life - is still widely portrayed as "flora and fauna" or "animals and plants".

Key words: Mushrooms, medicinal value, conservation.

INTRODUCTION

Without fungi we would not have bread, beer, wine or antibiotics, but more importantly without the nutrient recycling and plant nutrition provided by fungi, we probably could not survive at all. Mushrooms being neither plant nor animal have been placed in a kingdom, called Myceteae (Miles and Chang, 1997). The word mushroom may mean different things to different people and countries. In a broad sense "mushroom is a macro

fungus with a distinctive fruiting body, which can be either epigeous or hypogeous and large enough to be seen with naked eye and to be picked by hand" (Chang and Miles, 1992). Mushrooms belong to only two subdivisions of fungi; the vast majority belongs to Basidiomycetes, and a few belong to Ascomycetes. It is estimated that about 140,000 different species of mushrooms exist in the planet; however, only about 10% are known

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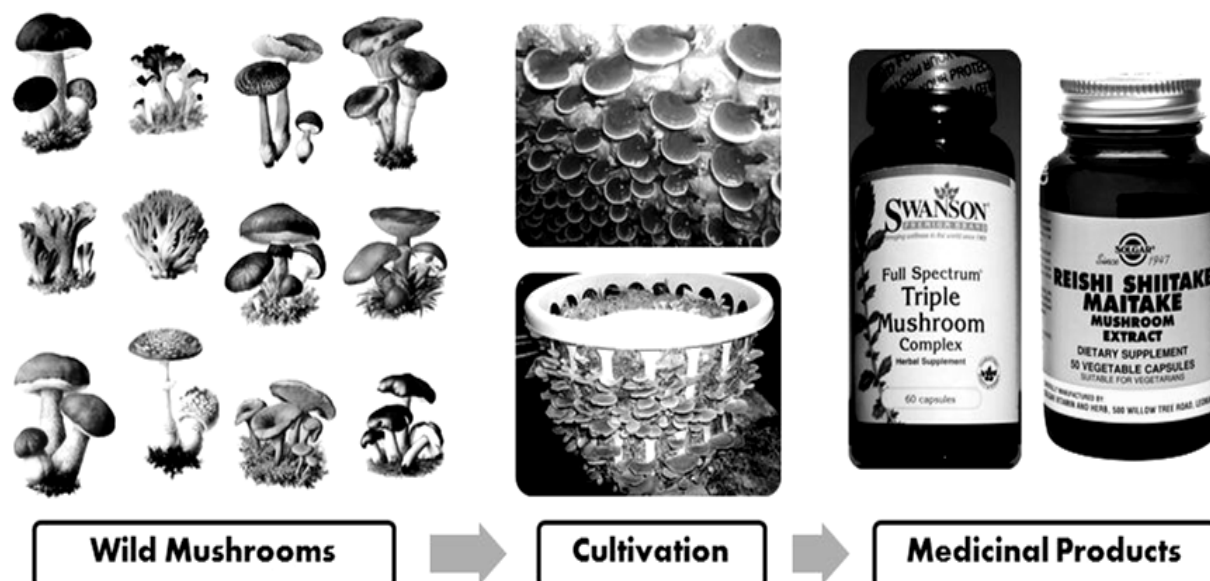


Figure 1. Wild mushrooms, their cultivation and final products used as dietary supplements or medicine.

(Hawksworth, 2001). Half of them present nutritious properties. About 2,000 species of mushrooms are safe and approximately, 700 species are known for presenting some pharmacological properties. Edible mushrooms are attractive because of their flavor, taste, and delicacy (Diyabalanage et al., 2008).

Although many species of edible mushrooms exist in the nature, less than 20 species are used as food and only 8 to 10 species are regularly cultivated in significant extent (Figure 1). Geologically, mushrooms existed on the earth even before man appeared on it, as evidenced from the fossil records of the lower cretaceous period.

Thus anthropologically speaking, there is every possibility that man used the mushrooms as food when he was still a food gatherer and hunter on the chronology of cultural evolution. It has been known that macro fungi are used as a valuable food source and traditional medicines since Greek and Roman antiquity (Anke, 1989). Dioscorides, first century Greek physician, knew that *Laricifomes (Fomitopsis) officinalis* (Vill.) Kotl. & Pouzar (*Fomitopsidaceae*) can be used for treatment of "consumption", a disease now known as tuberculosis (Stamets, 2002).

Mushrooms offer tremendous applications as they can be used as food and medicines besides their key ecological roles. They represent as one of the world's greatest untapped resources of nutrition and palatable food of the future. Mushrooms have been found effective against cancer, cholesterol reduction, stress, insomnia, asthma, allergies and diabetes (Bahl, 1994). Due to high amount of proteins, they can be used to bridge the protein malnutrition gap. Edible mushrooms have been widely utilized as human foods for centuries and have been appreciated for texture and flavor as well as some medicinal and tonic attributes (Manzi et al., 1999).

However, the awareness of mushrooms as a healthy food and as an important source of biological active substances with medicinal value has only recently emerged (Cheung et al., 2003). Mushrooms are considered as healthy food because they are low in calories and fat but rich in proteins and dietary fibers (Manzi et al., 2001). The mushroom protein contains all the nine essential amino acids required by humans. In addition to their good protein content, mushrooms are a relatively good source of the nutrients like phosphorus, iron and vitamins, including thiamine, riboflavin, ascorbic acid, ergo sterol, and niacin (Barros et al., 2008).

Mushrooms need antibacterial and antifungal compounds to survive in their natural environment. It is therefore not surprising that antimicrobial compounds with more or less strong activities could be isolated from many mushroom species and some proved to be of benefit for humans (Lindequist et al., 2005).

In early studies performed by Anchel et al. (1941), diverse antibiotic activity was detected in basidiocarp or mycelia culture extracts of more than 2000 fungal species (Rosa et al., 2003).

In recent *in vitro* studies, screening for the antimicrobial activity of basidiomycetes, some studies were done both in basidiocarp and in submerged culture. Antimicrobial activities of basidiomycetes from different countries were screened in submerged culture (Rosa et al., 2003; Wasser and Weis, 1999; Ezeronye et al., 2005). In this context 14 mushroom isolates were detected with significant activity against one or more of the target microorganisms (Rosa et al., 2003).

Observations were also made that 75% of polypore fungi that have been tested show strong antimicrobial activity (Zjawiony, 2004). Although discovery and application of antibiotics has been an important medical success

of the 20th century, but still the pharmacological screening of wild mushrooms is wanting so as to combat the emergence and spreading of antibiotic resistance.

FUNGAL CONSERVATION

Practical conservation of biodiversity depends on reliable information on what kind, how much and where the diversity is. In many cases the basic level of biodiversity information is observations on species occurrences over time and space. Biodiversity information databases and platforms have seen considerable progress in recent years. They have a high potential in conservation science in general, but may be even more revolutionary in relation to poorly known species groups such as fungi, whose practical conservation work has been jeopardized by scattered and poorly controlled information and this may seriously undermine conservation priorities and scientific conclusions (Molina et al., 2011; Jetz et al., 2012). Accurate recording of species occurrence over large geographical areas is time consuming and therefore costly if performed by proficient specialists. There is need to put emphasis on the importance of information on collection effort, including the use of GPS based tracking data, along with the observations. In practice, funding for surveying species is very unfairly distributed, and targeted towards organism groups that are generally considered spectacular, attractive or intelligent. Fungal communities are notoriously difficult to fully characterize for ecological and biodiversity studies as well as for conservation purposes. Recently there has been lot of emphasis on DNA Metabarcoding to fungal conservation (Geml et al., 2009). Fungal identification which is the basic requisite in conservation is largely done by molecular approaches, yet the high throughput sequencing methods are still in their infancy and are not currently used in monitoring programmes.

MUSHROOMS AS A SOURCE OF BIOACTIVE COMPOUNDS

Mushrooms are rich a source of natural bioactive metabolites, which can be low molecular weight (LMW), and high molecular weight (HMW), compounds respectively. LMW compounds are mainly secondary metabolites such as sesquiterpenes and other terpenes, steroids, anthraquinone and benzoic acid derivatives, and quinolines, but also primary metabolites. Screening of these wild mushrooms has bestowed us with many potent anti-tumor compounds (Figure 2). Medical usage of mushrooms with regard to antimicrobial and antitumor activity is very diverse. A number of medicinal mushrooms, such as *Aleurodiscus*, *Coprinus*, *Clitocybe*, *Daedalea*, *Marasmius*, *Merulius*, *Pleurotus*, *Polyporus*,

Poria, *Psathyrella*, and *Tricholoma* spp. are rich sources of β -glucan, proteoglucan, lectin, phenolic compounds, flavonoids, polysaccharides, triterpenoids, dietary fibre, lentinan, schizophyllan, lovastatin, pleuran, steroids, glycopeptides, terpenes, saponins, xanthenes, coumarins, alkaloid, purin, purimidine, kinon, fenil propanoid, kalvasin, volvotoksin, flammotoksin, porisin, AHCC, maitake D-fraction, ribonucleas, eryngeolysin, and also have been used extensively in traditional medicine for curing various ailments. Mushrooms are known to show antimicrobial, antiviral, antiparasitic, anticancerous, antitumor, antiinflammatory, cardiovascular, immunomodulating activities etc. This mini review highlights many such activities studied in past by various researchers.

In this context Ishikawa and co-workers evaluated the antibacterial activity of 35 isolates of *Lentinula edodes*, a shiitake mushroom against *Bacillus subtilis* by diffusion technique in agar with a semi-solid overlay. The result shows that all isolates inhibited *B. subtilis* and the isolate Le1 promoted the formation of the largest inhibition zone. *L. edodes* Le1 also presented antibacterial activity against food borne pathogens and food contaminant bacteria, particularly Gram positive species (Ishikawa et al., 2001). Further biologically active natural products isolated from Aphyllophorales, many of which are known as polypores were studied (Zjawiony, 2004). The result showed that 75% of polypore fungi that have been tested show strong antimicrobial activity, and these may constitute a good source for developing new antibiotics. Numerous compounds from these fungi also display antiviral, cytotoxic, and/or antineoplastic activities. Some of the protein bound polysaccharides from polypores and other basidiomycetes have found their way to the market in Japan as anticancer drugs.

Further isolates of four polypores; *Ganoderma colossum*, *Ganoderma lucidum*, *Trametes cingulata* and *Daedalea quercina* were compared using the High performance liquid chromatographic profiles of their triterpenoids (Ofodile et al., 2008). A higher abundance of colossolactone E was found in *Ganoderma colossum* isolate (FC 876) when compared with FC 872 obtained at different periods and dried differently and 23-hydroxycolossolactone E found in FC 876 was not observed in FC 872.

Equal abundance of constituents was also found in *Ganoderma lucidum* isolates (FC 871 and FC 875) collected from different hosts and geographical locations. The isolates of *Trametes cingulata* that were of different ages showed predominance of the major constituents in FC 873 and FC 885 isolates when compared with FC 870. The abundance of the triterpenoid in the isolates of *Daedalea quercina* was almost doubled in FC 882 when compared with that of FC 878. These conform with the chemical spot test results on these polypores in a previous work. The ability of the polypores to produce triterpenoids

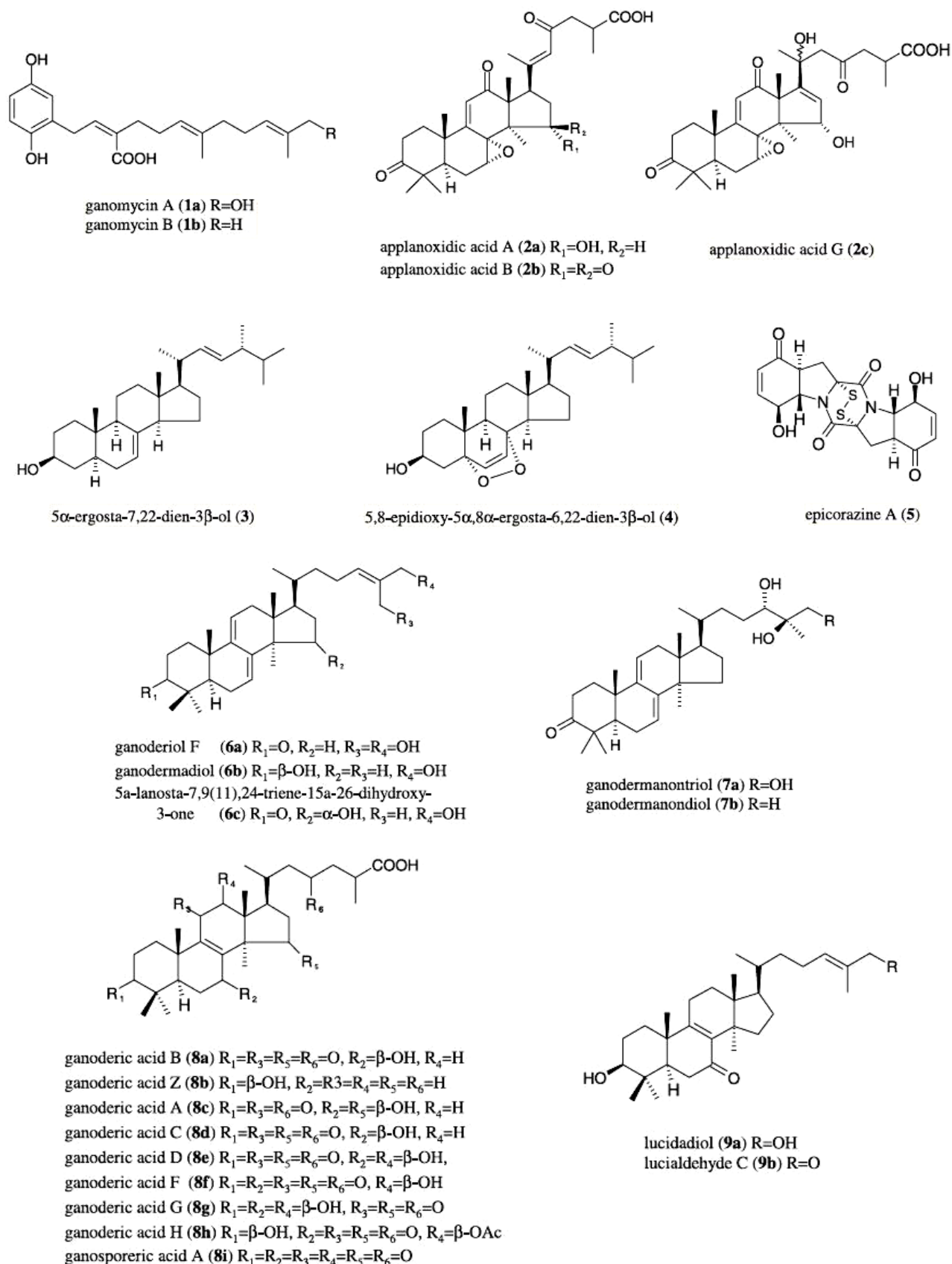


Figure 2. Anticancerous compounds obtained from mushrooms.

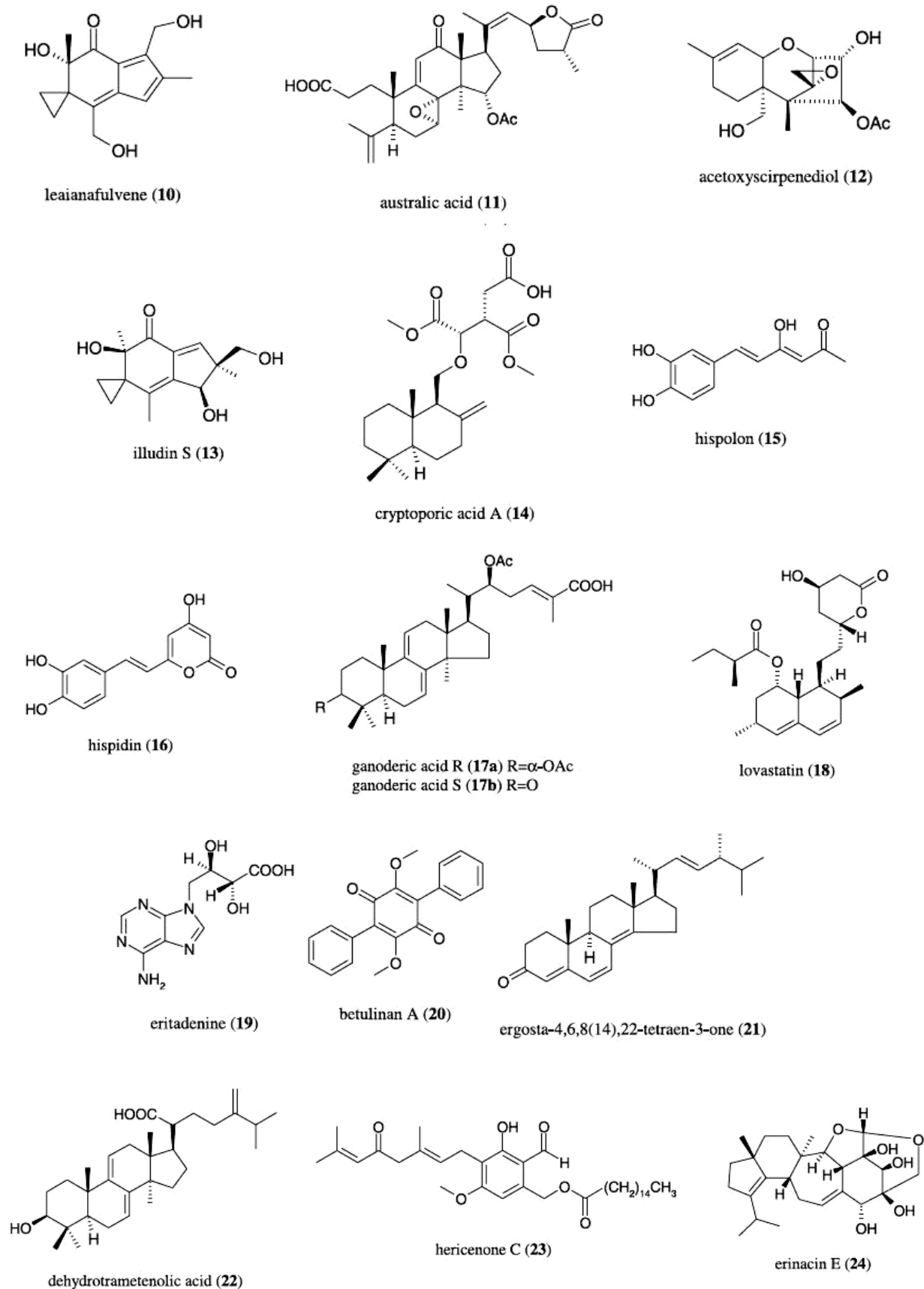


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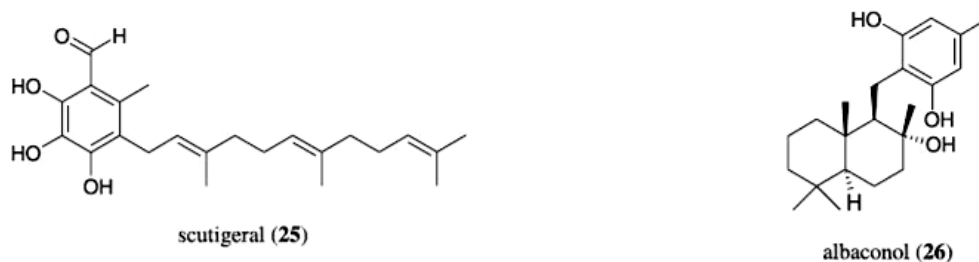


Figure 2. Contd.

is affected by their age, period of collection, geographical location and method of drying, which also affected the High Performance Liquid Chromatography characteristics of their secondary metabolites. Finally, numerous compounds with cardiovascular, phytotoxic, immunomodulatory, analgesic, antidiabetic, antioxidant, insecticidal, and nematocidal activities, isolated from polypores, are also presented. Antimicrobial activities of Methanolic extracts of five Nigerian mushrooms *Auricularia polytricha*, *Corilopsis occidentalis*, *Daldinia concentrica*, *Daedalea elegans* and *Tricholoma lobayensis* were investigated using filter paper disc and hole diffusion methods (Gbolagade and Fasidi, 2005). The result showed that all the mushrooms used in this study were found to exhibit various degrees of antagonistic effects against the tested microorganisms. This was evidenced by the clear zone of inhibition produced by the bacteria and fungi around the tested mushroom extracts. Further investigations regarding the antimicrobial properties of phenolic extracts of Portuguese wild edible mushroom species (*Lactarius deliciosus*, *Sarcodon imbricatus* and *Tricholoma portentosum*) against pathogens was carried out (Barros et al., 2006). The minimal inhibitory concentrations (MICs) were evaluated for the entire mushroom, the cap and the stipe, separately; the portion of the mushroom used proved to be influenced in the results obtained, which are directly correlated with the content of total phenols and flavonoids in the extracts. The study on the antifungal effect of these mushrooms revealed that *Candida albicans* and *Cryptococcus neoformans* were differently inhibited for the mushrooms used. Further two species of basidiomycetes, *Lentinula boryana* and *Lentinula edodes*, were evaluated for their antibacterial activities, biomass production and growth in two different culture media (Carvalho et al., 2007).

Mycelia from each species were incubated in liquid media for 28 days and vacuum-filtered. *L. boryana* showed the largest biomass production in both culture media when compared to *L. edodes*, which presented significant differences in growth when cultivated in different culture media. Antibacterial activity of the two species was evaluated against 10 bacterial species, six of them being of clinical importance. Both Basidiomycetes *L. boryana* and *L. edodes* showed

antibacterial activity against *Bacillus cereus* and *Staphylococcus aureus*, although only *L. edodes* was active against *S. mutans*. Antimicrobial activity from the extract of *P. eryngii* var. *ferulae* which was obtained from various culture media was done by disk diffusion method using *Bacillus megaterium* DSM 32, *Staphylococcus aureus* COWAN 1, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* FMC 5, *Candida albicans* FMC 17, *Candida glabrata* ATCC 66032, *Trichophyton* spp., and *Epidermophyton* spp (Akyuz, and Kirbag, 2009). At the end of the experimental studies, the methyl alcohol extracts of *P. eryngii* var. *ferulae* were shown to inhibit to different degrees the growth of microorganisms to (7.7-10.3 mm) also; mushroom extracts have a lower antimicrobial activity as to a comparison antibiotic (13.0-18.0 mm). Further investigations were done to know the proximate composition, antioxidant, anthelmintic and insecticidal efficacy of methanolic extract of a macrolichen *Ramalina conduplicans* Vain. (Ramalinaceae) (Vinayaka et al., 2009). The results show that methanol extract exhibited marked antioxidant activity by scavenging DPPH* (free radical) and converting into DPPHH in a dose dependent manner. In anthelmintic study conducted using adult Indian earthworms, the methanol extract exhibited a dose-dependent inhibition of spontaneous motility. *In-vitro* antimicrobial properties of *Lentinus tuberregium* were tested using four different solvent systems (hexane, dichloromethane, chloroform and ethyl acetate) (Manjunathan and Kaviyaran, 2010). The activity was evaluated by well diffusion tests using bacteria and yeasts. Vancomycin and fluconazole were used as positive controls for bacteria and yeasts, respectively. The crude extracts of *L. tuberregium* have relatively high antimicrobial activity. Among the four organic extracts ethyl acetate extract was more effective and inhibited the growth of human pathogenic bacteria and yeast. Macrolichen *Parmotrema pseudotinctorum* (des. Abb.) Hale (Parmeliaceae) collected from forest area of Bhadra wildlife sanctuary was studied for its antibacterial, anthelmintic and antioxidant activity of activities (Kumar et al., 2010). The extract exhibited marked antibacterial activity. The minimum inhibitory concentration of the extract was found to be lesser in case of Gram negative bacteria than Gram positive

bacteria. The lichen extract exhibited a dose dependent inhibition of spontaneous motility. Antibacterial and antifungal activities of aqueous and methanolic extracts of fruit bodies of *Trametes hirsuta* against five pathogenic fungi like *Penicillium* sps., *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus flavus* and *Mucor indicus* and five bacterial strains such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus*, and *Streptococcus mutans* were tested by well diffusion assay (Sivaprakasam et al., 2011). The result shows that the maximum antibacterial activity of aqueous extract of whole fruit bodies of *Trametes hirsuta* was found 33 mm at 200 mg against *Staphylococcus aureus* than that of methanol extract. The significant antifungal activity of aqueous extract was found 46 mm at 200 mg against *Aspergillus flavus* than that of methanol extract. The antimicrobial activity was showed at concentration dependent. Phytochemical and antimicrobial activity of common cultivated mushroom, *Agaricus Bisporus* was studied (Tammina and Hariprasad, 2011). The different fractions of methanolic extracts of the whole mushroom of *Agaricus bisporus* was subjected to preliminary phytochemical and *in-vitro* anti-microbial studies. The result shows that the fraction II of the methanolic extract inhibited the growth of all the test bacterial species whereas fraction III and fraction IV have shown weak antibacterial activity. Evaluation of antibacterial and cytotoxic activity from basidiocarp extracts of the edible mushroom *Lactarius indigo* was done against diarrheagenic *Escherichia coli* strains (EIEC, EPEC, ETEC-LT and ETEC-ST), *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Staphylococcus aureus* and *Salmonella enteric* (Zarzosa et al., 2011). Results showed that *L. indigo* basidiocarps contain substances with antibacterial and cytotoxic activities. Recently quantitative determination of polysaccharides in *Ganoderma lucidum* fruit bodies from different sawdust cultivation substrates and their antibacterial activity was done (Skalicka-Woźniak et al., 2012). Thirty six samples were analyzed. Four strains of *Ganoderma lucidum* (GL01, GL02, GL03 and GL04) were cultivated on the growth substrates of three different sawdust types: birch (Bo), maple (KI) or alder (Ol) amended with wheat bran in three different concentrations: 10, 20 and 30% (w/w). Even though the richest in polysaccharides was GL01 strain, the highest yields of the polysaccharides were determined in GL04KI3 sample and was 112.82 mg/g of dry weight. The antibacterial activity of polysaccharides was determined *in vitro* using micro-dilution broth method. The panel of eight reference bacterial strains was used. All the polysaccharide samples tested showed the broad spectrum and the moderate antibacterial activity. *Micrococcus luteus* ATCC 10240 strain was the most sensitive with minimal inhibitory concentration (MIC) = 0.63 – 1.25 mg/mL. Most recently the phytochemical, antioxidant and antimicrobial *in vitro* assay of *Pleurotus pulmonarius*-LAU09 (JF736658) was evaluated (Adebayo

et al., 2012). The metabolites obtained from *Pleurotus pulmonarius* was characterized by IR analysis. It revealed the absorption of O-H, C-H, C-O bonds and hydrated water peaks (1650.6 cm^{-1}), without no absorption at uronic acid peak (1730 cm^{-1}). ^1H NMR spectrum analysis of the metabolite has anomeric carbon peaks of 5.10 and 4.51 ppm, characterized as α and β linkages of glucan compound. The phytochemical screening of the mushroom extract revealed the presence of alkaloids, saponins, steroids, phlobatannins, flavonoids and anthraquinones. The metabolite was active against all tested pathogens except *P. aeruginosa* with percentage activity of 85.75%. The highest zone of inhibition was obtained against *Staphylococcus aureus* (30 mm), while the lowest zone size obtained was against *E. coli* (7 mm). The antioxidant activity of evaluated mushroom extracts gave positive results with free radical scavenging activity found to be higher in all used *in vitro* methods. The result obtained from this study has showed the potential of mushroom extract as a potent therapeutic agent and a food supplement.

Recently *in vivo* anticoccidial effects of aqueous extract of wild mushroom *Fomes fomentarius* were evaluated (Ahad et al., 2013). The study showed that treatment with *F. fomentarius* resulted in a marked reduction in the number of coccidian oocysts shed in the faeces, leading to improved weight gain and better feed conversion ratio. Besides Willis and his co-workers (Willis et al., 2013) conducted an experiment to evaluate the feeding of four medicinal mushrooms: Shiitake (*Lentinus edodes*), Reishi (*Ganoderma lucidum*), Oyster (*Pleurotus ostreatus*) and Cordyceps (*Cordyceps sinensis*) on performance, blood Parameters and natural coccidiosis infection in floor-reared broilers. The results from this study indicate that different fungi and levels of their inclusion into the basal feed can impact production performance responses significantly and enhance the overall health of broiler chickens. The study revealed that there are ample advantages to using natural medicinal mushrooms as immunonutrition verses antibiotics to enhance health and production performance of broiler chickens.

CONCLUSION

Medicinal mushrooms are gifts from nature that contain biologically active metabolites which can be used as support remedies for cancer treatments and many other ailments. Additional studies of the activities and mechanisms of action of these metabolites coupled with new methods and techniques are desired so to develop potent drugs from them. There is further need to develop technology for domestication, large scale production and subsequent use as a source of natural nutrients and nutraceuticals. Lazy and misleading descriptions about mushrooms have been portrayed. Those responsible include a range of major biological institutions and

learned societies which should and do know better. The five kingdom classification of life, which recognizes fungi in a kingdom of their own, has been generally accepted by scientists since at least 1970. The broader conservation movement, as a result, remains largely unaware of the need to conserve fungi. Priority habitats for conservation, such as biodiversity hotspots, are almost always defined on the basis of bird, mammal and flowering plant diversity. This means that habitats rich in fungal diversity are missed and remain unprotected. Most nature reserve management plans do not take fungi into account. Fungi (for example host-specific species known only on rare endemic plants) are often treated as part of the problem (a threat to the plant) rather than recognized as themselves being in need of protection and in many countries there is no explicit legal protection for fungi.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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