UNIVERSITY OF CAPE COAST

MYCOCHEMICAL SCREENING AND ANTIOXIDANT PROPERTIES OF SOME POTENTIAL MEDICINAL MUSHROOMS IN THE CENTRAL REGION OF GHANA

FAUSTINA MENSAH

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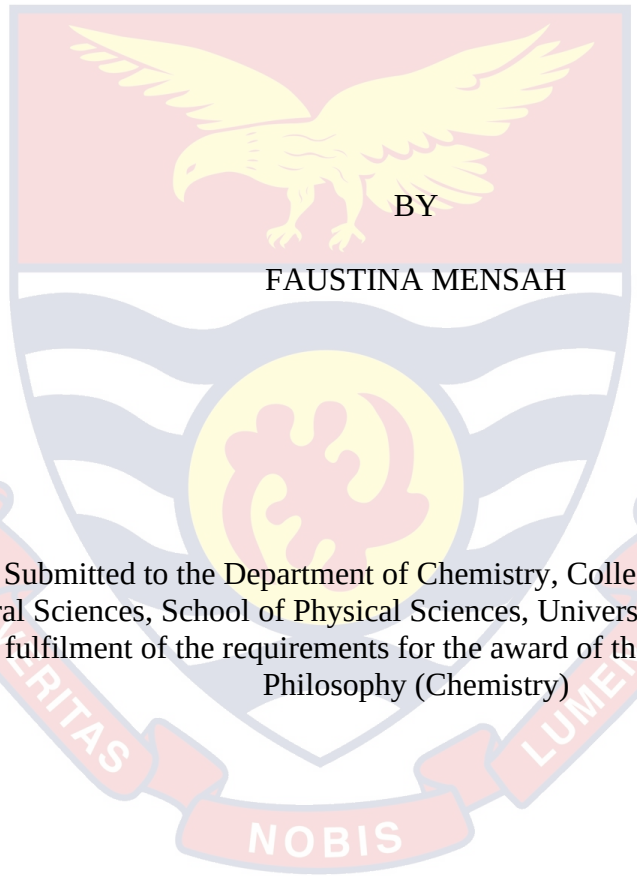

BY

FAUSTINA MENSAH

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DECLARATION

Candidate's Declaration

I hereby declare that this thesis is a result of my own research and that no part of it has been presented for another degree in this University or elsewhere.

Candidate's Signature:…… Date:……

Name: Faustina Mensah

Supervisors' Declaration

We hereby declare that the presentation and preparation of this thesis were supervised in accordance with the guidance of supervision of this thesis laid down by the University of Cape Coast.

Principal Supervisor's Signature:…… Date:……

Name: Dr (Mrs) Genevieve Adukpo

Co-supervisor's Signature:…… Date:……

Name: Mr John P. K. Adotey

ABSTRACT

The methanol extract of eight matured mushroom samples, *Tyromyce chioneus, Polyporous alveolaris, Trametes hirsute, Trametes versicolor, Trametes gibbosa, Chlorophyllum molybdite, Auricularia auricular judae, and Ganoderma spp.,* were mycochemically screened using standard methods for the presence of secondary metabolites. Their total phenolic contents (TPC) using Folin-Ciocalteu method with gallic acid standard and total flavonoid contents (TFC) by aluminium chloride method using quercetin standard were determined. The total antioxidant capacity (TAC) and free radical scavenging activities were also evaluated using phosphomolybdenum method with ascorbic acid as standard and DPPH method with gallic acid standard respectively. The mycochemical screening results revealed the presence of alkaloids, polyphenols, terpenes saponins, and reducing sugars in all the extracts of the samples. Steroids were found to be absent in *Chlorophyllum molybdite, Auricularia auricular judae.* Phlobatannins was found to be present in only *Polyporous alveolaris* and *Trametes hirsute*. The TPC was highest in the extract of *Trametes gibbosa* (8.99 mgGAE/g) followed by that of *Ganoderma spp* (7.44 mg GAE/g). The extract of *Ganoderma spp* had the highest TFC (5052.00 µg QE/g), followed by *Trametes gibbosa* (4318.66 µg QE/g). *Trametes hirsute* (29.91 mg AAE/g), *Polyporous alveolaris* (28.89 mg AAE/g) and *Ganoderma spp* (28.17 mg AAE/g) showed appreciable amount of TAC. Extracts of *Polyporous alveolaris* (80.66 µg/mL) and *Ganoderma spp* (94.37 µg/mL) displayed strong scavenging activity against DPPH as compared with the gallic acid standard (84.01 µg/mL). This study revealed that, most of the investigated mushrooms could be utilized in diet to promote health, taking advantage of the additive and synergistic effects of all the bioactive compounds present.

KEY WORDS

Mushrooms

Mycochemicals

Total phenolic content

Total flavonoid content

Total antioxidant capacity

Antioxidant

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DEDICATION

This work is dedicated to mother.

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LIST OF ABBREVIATIONS

TPC	-	Total Phenolic Content
TFC	-	Total Flavonoid Content
TAC	-	Total Antioxidant Capacity
GAE	-	Gallic Acid Equivalent
AAE	-	Ascorbic Acid Equivalent
QE	-	Quercetin Equivalent
DPPH	-	1, 1-diphenyl-2-picryl-hydrazil

CHAPTER ONE

INTRODUCTION

1.0 Introduction

Traditional systems of medicine which play a critical role in healthcare since the earliest days of mankind are still attracting more and more attention within the context of health care provision and health sector reform (Nahata, 2013). The all-encompassing methodology and conviction towards plant-based medicines make it simple for lion's share of the total populace, particularly in the agricultural nations, to rely upon home grown items for their essential medical care needs. Utilizing mushrooms in traditional medicine throughout the world as medicine and functional foods is now gaining popularity. Mushrooms are very distinct from plants, animals and bacteria and they belong to the Fungi Kingdom (Nagy et al., 2017). They are full scale growth with a particular body which can be epigenous or hypogenous and are sufficiently enormous to be seen and picked by hand (Wandati, Kenji & Onguso, 2013). Mushrooms come in the form of edible, poisonous, wild or medicinal. They can be found in different places like wet climate, rotted plants and animal sites, termites home, palm squanders, leaf litters, under shades and so forth (Udu-Ibiam, Ogbu & Ibiam, 2014). Most of the benefits of mushrooms have been attributed to the pharmaceutical and nutraceutical effects that have been investigated, and studied through scientific methods. There are lots of mushrooms that grow in the wild and they have been reported to be plentiful in minerals and have significant degrees of food supplements (Grangeia, Heleno, Barros, Martins, & Ferreira, 2011).

1.1 Background to the Study

Therapeutic mushrooms have been accounted for to have a few natural properties, for example, antiviral, antibacterial, antifungal and antitumor properties, hostile to parasitic, against diabetic, anticoagulant, calming, insecticidal and cancer prevention agent properties (Appiah, Agyare, & Luo, 2017; Obodai et al., 2017). These properties have been of interest due to the discovery and confirmation of health benefits associated with the intake of these mushrooms (Ferreira, Vas, Vasconcelos, & Martins, 2010). Intensive studies have revealed the substance nature and instruments of activity of the biomedical limit of these medicinal mushrooms (Blagodatski et al., 2018). It was additionally detailed by Bandara et al., 2019 and Obodai et al., 2014 that different species of mushrooms have therapeutic activities because of broad exploration utilizing in vitro measures, in vivo creature models, and, sometimes, human clinical examinations. Numerous mushrooms species have been known and documented to be the sources of many bioactive compounds. These include *Lentinula edodes Hericiumeri naceum, Flammulin avelutipes, Trametes versicolor, Auricularia polytricha* and *Tremella fuciformis, Auricularia spp and Ganoderma spp* amongst others. Studies on these bioactive compounds have attracted considerable interest. Investigation of local medicinal fungi species may yield mycochemicals with a model for the development of drugs. These medications got from fungus are created, exchanged and devoured in huge sums throughout the entire year on the planet (Wang, Xi, Li, Wang, & Yao, 2012).

Recent research on medicinal mushrooms suggested that the mushrooms show fluctuated natural properties, for example, antibacterial,

antimutagenic, antitumoral and antiviral activities basically because of their phytoconstituents (Obodai et al., 2017). Polysaccharides, phenolic compounds, tocopherols and natural acids are the main source of antioxidant properties in mushrooms (Fernandes et al., 2016). Work done *Ganoderma spp.* throughout the years has indicated that the species had an extreme cancer preventive agent, antitumor, mitigating and against nociceptive properties (Sheena, Ajith, Mathew, & Janardhanan, 2003). Active polysaccharides such as β – Glucans isolated from *Ganoderma* species have been found to be responsible for anticancer and antimetastatic activities (Obodai et al., 2017). Phytochemical analysis done on the methanol extract of *Ganoderma lucidum* showed that it contains several terpenes (Sheena et al., 2003). Antibacterial properties were likewise seen from the methanol concentrate of *Ganoderma lucidum* (Sheena et al., 2003). Vitamin D2 which can be obtained from Ergosterol in the presence of light was extracted in appreciable amount from *Ganoderma* species. (Obodai et al., 2017). *Ganoderma lucium* and *Ganoderma frondosa* exhibited potent inhibitory activity against HIV-1 (Rajesh & Dhanasekaran, 2014).

Mycochemical examination on *Tyromyces chioneus* has demonstrated the presence of phenol, triterpenes, steroids, unsaturated fats, sugars, anthraquinones, coumarines, anthrones, tannins, flavonoids and alkaloids (Capistrano et al., 2018). The presence of these bioactive mixtures has been associated with their cell reinforcement, antibacterial, antifungal and antiviral properties. They also cause a reduction of cancer risk and the growth of a tumor. The presence of sugar and fatty acids has a very great health benefit

such as proper functioning of the brain, enhancement of mental and physical performance (Capistrano et al., 2018).

Anthraquinones have also been found in *Tyromyces* which possess anti-cancer and anti-inflammatory properties. The presence of Coumarins also in the *Tyromyces* is very important due to its anti-osteoporosis, anti-arrhythmia, anti-hypertension and anti-tumor prevention of respiration tract diseases. (Capistrano et al., 2018).

1.2 Statement of the Problem

Presently, mushrooms have sparked great interest in different nations because of the presence of bioactive compounds with possible restorative properties (Prasad, Rathore, Sharma, & Yadav, 2015). Edible mushrooms which are known to have high dietary benefit have additionally acquired a lot of significance in their restorative utilization. Numerous non-consumable mushroom species which are known to be therapeutic have gotten considerably more attention in traditional medicine as customary elements for the treatment of different sicknesses and related medical issues. Various chronic health problems worldwide, relate mainly to unhealthy food choices. Poor nutrition and the increasing emergence of infectious diseases represent major threats to human health (Shridhar, Rajendra, Murigendra, & Shridevi, 2015). Also, drug resistance continues to present huge and growing issues in the treatment of diseases. In non-industrial nations, different infirmities and contaminations represent the vast majority of the nation's diseases (Okon et al., 2013). In this manner, there is the requirement for ceaseless hunt and advancement of novel medication from various natural sources to combat these diseases (Padmavathy, Sumathy, Manikandan, & Kumuthakalavalli,

2014). Medicinal mushrooms have become reservoirs of valuable varieties of secondary metabolites with interesting biological actions that can be used as a source for biotherapeutics and nutritional supplements (Prasad et al., 2015).

Regardless of the massive helpful ability of mushrooms, there is basically no investigation and data on local mushrooms as a huge wellspring of naturally powerful substances with therapeutic impetus in a huge part of the African countries.

1.3 Purpose of Study

The main goal of this study was to mycochemically screen sampled potential medicinal mushrooms in the Central Region of Ghana and determines their antioxidant properties. The knowledge of biochemical composition of these mushrooms will help in the creation of model compounds for the improvement of sensible, moderate and compelling drugs to battle complex infections

1.4 Specific Objectives

The specific objectives of this examination were to:

1. Mycochemically screen the sampled mushrooms for the presence of secondary metabolites
2. Determine the total phenolic content, total flavonoid content and total antioxidant capacity of the mushroom samples.
3. Analyse the DPPH free radical scavenging capacity of the mushroom samples.

1.5 Research Questions

The research questions for this study include the following:

1. What kinds of mycochemicals are present in the selected mushrooms that make them potential medicinal mushrooms?
2. What is the total phenolic content, total flavonoid content and total antioxidant capacity in the mushrooms selected?
3. What are the antioxidant properties of these mushrooms that validate their usage as medicines?

1.6 Significant of Study

The essence of the study was to evaluate the sampled mushrooms for their therapeutic qualities. These mushrooms are mostly found in the wild and are not edible. Wild mushrooms are significant characteristic wellsprings of drugs and have been known to have promising cancer prevention agents. Late exploration has demonstrated a wide extent of bioactive compounds present in medicinal mushrooms. Bioactive compounds have been recognized to have cancer prevention agent, antiviral and anticancer activities and are given models to the creation of new medications to battle different infirmities. These exploration discoveries will approve the use of these mushrooms for the treatment of certain sicknesses. Thus, the fruitful development and financially savvy of these therapeutic wild mushrooms in the nation to deliver locally wellbeing enhancements will be supported. As a type of revenue in the country, these can be promoted and even sent out.

1.7 Delimitation

The samples were collected from Abura Dunkwa and Breman Asikuma forest areas due to the raining nature of the area at the time of sampling.

1.8 Limitation

The quantities of mushrooms found at the sites of harvest were not enough for other antioxidant properties to be carried out.

1.8 Organization of study

This study comprises of five chapters. The first chapter contains the background to the study, statement of problem, purpose of study, specific objective, research questions and significant of study. The second chapter covers the literature which is organized under the following subheadings; classification of mushroom, habitat of mushrooms, description of mushrooms, economic importance, mycochemicals as bioactive metabolites in medicinal mushrooms and antioxidant. The third chapter consists of the research methods which include list of chemicals used, sample collection and identification, sample treatment, extraction procedure, mycochemical screening and antioxidant analysis. The fourth chapter contains the results, its interpretation and discussion. The final chapter covers the summary, conclusions and recommendations for further studies.

CHAPTER TWO

LITERATURE REVIEW

2.0 Introduction

Mushrooms are macro fungus with very distinctive fruiting bodies which for centuries have been eaten due to texture and flavour. Most mushrooms come in the form of an umbrella-shaped fruiting body commonly in the family Agaricaceae which bears thin bladelike gills on the under surface of the cap where spores are shed (Ren, 2014). In their regular habitat, mushrooms need antibacterial and antifungal mixtures to endure. Therefore, it comes as no surprise that useful secondary metabolites are isolated not from only plants but mushrooms as well (Gargano et al., 2017).

2.1 Classification of Mushrooms

In the past, mushrooms were delegated lower plant because of the presence of cell wall and their absence of genuine roots, leaves, stem, and products of the soil like typical plants. Recent research has established that mushrooms together with other fungi have a distinctive attribute that was significant enough to classify them under kingdom fungi (Reid, 2019). There are about five divisions of the kingdom namely Ascomycota (sac fungi), Chytridiomycota (chytrids), Basidiomycota (club fungi), Zygomycota (conjugated fungi), and Glomeromycota. Many mushrooms belong mainly to basidiomyces but some species belong to ascomycetes of kingdom fungi. Numerous ascomycetes are of business significance, for example, the yeasts utilized in preparing, blending and wine ageing, in addition to morels, and truffles serving as connoisseur treats. Ascomycota mushrooms can also be harmful to living things including humans. A considerable lot of them cause

tree illnesses, for example, Dutch elm infection and apple curses. Basidiomycota commonly are known as "club fungi, whose members are typically characterized by the presence of a basidium forms a major part of the kingdom fungi. Most of such mushrooms are mostly edible and medicinal Ascomycota and Basidiomycota consist of different classes and orders and these are presented in Table 1.

Table 1: Classification of the Major Divisions of Fungi

Division	Class	Order
Ascomycota	Ascomycetes	Saccharomycetales and Schizosaccharomycetales (yeast)
		Eurotiales
		Sordariales and Xylariales
		Pezizales
		Dothideales
Basidiomycota	Basidiomycetes	Agaricales (mushrooms)
		Lycoperdales, Phallales and Nidulariales (Puffballs)
		Aphyllophorales (Poypores)
		Tremellales, Dacrymycetales and Auriculariales (Jelly fungi)

Source: (Hagen, 2012)

2.2 Habitat of Mushrooms

Mushrooms are found in a variety of places. However, some species are limited to a particular place. Some mushrooms are found in the tropics, subtropics and others in the cooler regions. These can be found in different places like wet climate, decayed plants and creature destinations, termites home, palm squanders, leaf litters, under shades in muggy and cool environments (Udu-Ibiam et al., 2014). Factors such as climate, water, heat,

pH, sunlight and minerals are of great value to the development of mushrooms. Mushrooms also prefer soils rich in organic matter which is a source of essential nutrients and energy (Gezer & Kaygusuz, 2015). *Tyromyces* is mostly found in Asia, Europe and North America. Its common host is birch and other dead hardwood. *Polyporus alveolaris* found mostly in Asia, North America, Australia and Europe. Genus *Trametes* is found also everywhere for instance, *Trametes versicolor* can be found all year round on dead hardwood as well as stumps of dead trees. It is normally found in Britain, Ireland, Asia, China, Africa and Europe. *Ganoderma* species are normally found on the trunks of dead hardwood, palm or living trees.

2.3 Description of Mushrooms

Mushrooms are full-scale growth with a particular body that can be epigenous or hypogenous which is sufficiently enormous to be seen and picked by hand (Wandati et al., 2013). Mushrooms come in many shapes, colour, size and surface structure. Common mushrooms are made up of cap (pileus) which is the most conspicuous characteristics, the stalk (stipe), normally referred to as the mushroom stem and the mycelium which serve as an anchor for the mushroom to connect it to its nutrient and water source. Underneath of the cap is made up of gills, pores or similar variations. These contain spores that are release for reproduction.

A typical image of mushroom is shown in Figure 1 showing the various parts.

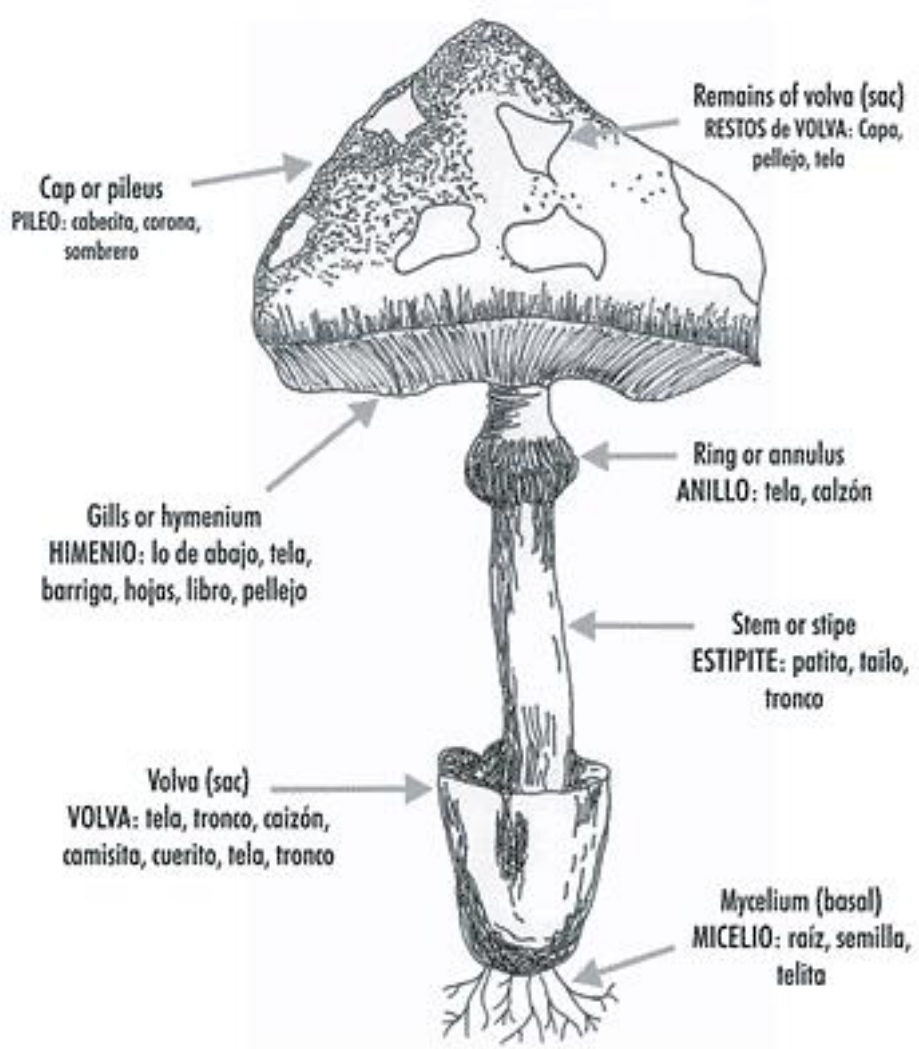


Figure.1 A typical mushroom with its various parts (Image from Food and Agriculture Organization of the United Nations

Lepiota, Pluteus, Agaricus, and Amanita type of mushrooms have free gills that do not stretch out to the stalk. A portion of the mushrooms like the *Omphalotus and Pleurotus* mushrooms have decurrent gills that reach out down the stalk. *Tyromyce chioneus* also known as white cheese polypore has a white fruiting body that turns dark when dried and is short-lived (Chander & Pathania, 2018). The Polyporus mushrooms e.g. *Polyporus alveolaris have* yellowish to orange scaly cap and hexagonal shaped pores. This causes white-rot to dead hardwood and logs. *Trametes* mushrooms have a tough

inedible fruiting body with many colours. They can be flat to wavy; multi-coloured concentric zoning on the upper surface where the zones alternate between silky and smooth. The colours vary between shades of brown, orange, chocolate and grey. Their fruiting bodies differ in thickness and are covered with green algae when matured like in *Trametes versicolor* and *Trametes gibbosa*, *Ganoderma* species otherwise called bracket fungi are described by their enormous, lasting, woody bracket. The fruiting bodies germinate like a fan-like structure on tree trunks either living or dead. *Ganoderma* mushrooms have shorten spores with yellow to brown ornamented inward layers. Bhosle et al., 2010, described the upper surface of *Ganoderma chalceum* to be reddish-brown, with a hard crust margin and yellowish pose surface. The upper surface of *Ganoderma applanatum* is pale grey to dim earthy coloured covered with a layer of chocolate, earthly coloured spore seeming dusty (Bhosle et al., 2010). *Auricularia* mushroom species also known as the '*jelly-ear'* mushrooms have a distinctive fruiting body like the shape of the ear with brown colouration as shown in figure 2.

Name of mushroom: ***Tyromyce chioneus*** **Synonyms:** ***Boletus candidus, Tyromyces albellus***

Name of mushroom: *Trametes hirsute* **Synonyms:** *Boletus hirutus*

Name of mushroom: *Trametes versicolor* **Synonyms:***Coriolus versicolor*

Name of mushroom: *Trametes gibbosa* **Synonyms:** *Agarico-suber scalptum*

Name of mushroom: *Auricularia auricular- judae* **Synonyms:** *Auricularia auricularis*

Name of mushroom: *Polyporus alverolaris* **Synonyms:** *Polyporus tenuiparies laferr &*

Name of mushroom: *Ganoderma species* **Synonyms:** *Ganoderma alba*

Name of mushroom: *Chlorophyllum molybdite* **Synonyms:** *Agaricus molybdite*

Figure 2: A figure showing the selected mushroom samples

2.4 Economic Importance

Mushrooms are regularly known for their delicacy and flavour. They help in dealing with the issues of sickly wellbeing and diseases because of their high food supplements to man and rich restorative properties. Their dietary and useful qualities are viewed as exceptionally high and subsequently considered as nutraceutical food (Valverde, Hernandez-perez, Paredes-lopez, 2014). Mushrooms are incredible wellsprings of supplements and minerals and are diversely utilized by man. Mushrooms are useful to the forest as they are known to be forest decomposers. Their saprophytic property makes them one of the critical experts in giving available food to the virgin forest. The basidiomycetes mushrooms debase proficiently both lignin and cellulose biopolymers which slowly blends in with forest soil and gives food to living trees (Rhodes, 2015).

Mushrooms go about as primary, auxiliary and tertiary decomposers. The fundamental decomposers, for instance, like Shiitake, Oyster and Wine Cap mushroom separate the lignin, cellulose, straw and other plant matter. The substrate is taken over by Auxiliary decomposers after has been partly broken down. Discretionary decomposers usually are created on excrement and merge as White Button mushrooms and Portobello (*Agaricus spp*.). Tertiary decomposers are ordinarily soil inhabitants existing in decreased substrates. The piece of mushroom in excusing fallen timber in the timberland and changing over dead trees and fallen leaves into open food is for the most part huge in managing an organic harmony in the forested areas. Mushrooms have a major task to carry out in mycoremediation. They have the regular capacity to kill harmful substances from their current circumstance and remediate

various kinds of toxins in the dirt (Kulshreshtha et al., 2014). Mycoremediation relies upon the capable proteins, made by the mushroom, for the defilement of various kinds of the substrate and debases (Kulshreshtha et al., 2014). The most suitable mushrooms used in soil remediation are basidiomycetes and, explicitly, the normal social occasions of saprotrophic and biotrophic (Bosco & Mollea, 2019; Treu & Falandysz, 2017). Mushrooms make a natural equilibrium in the forest by causing the decay of fallen wood, barks, and stumps just as dead logs. This causes the breaking down of deadwood into the forest soil to give food to living organic entities in there just as living trees. It moreover partakes in the reusing of dietary segments like nitrogen, phosphorus, potassium, sulphur, iron, calcium which are crucial for the improvement of plants. Complex lignin rich compounds are disintegrated by mushrooms to create natural rich materials prompting a clean climate. A few fundi like *Beauveria bassiana* and *Trichoderma sp*. have been utilized in sustainable disease management for the control of plant illnesses brought about by soil-borne microorganisms and bugs because of their opposing movement. A natural acid is monetarily created from biochemical exercises of moulds. Such acids incorporate gallic acid, oxalic acid, citrus extract, gluconic acid and fumeric acid. The ageing result of *Aspergillus niger* is oxalic acid.

For centuries, mushrooms have been viewed all through the world as both food and prescription due to their sensitive surface and flavour. Mushrooms have been set up to have high health benefit with an obvious measure of protein, nutrients, minerals and some essential micronutrients. In this regards, mushrooms are considered as down to earth food sources and a high elective focal point for the gathering of normal medication and

nutraceuticals (Fernandes et al., 2016). It has been set up those mushrooms contain low calories. Anti-infection agents like penicillin and grisoflavin were acquired from fungi and these are utilized in the assembling of essential nutrients and different medications. Biological active compounds from mushrooms classified under secondary metabolites, glycoprotein and polysaccharides. Secondary metabolites such as polyphenols, triterpenoids, alkaloids, vitamins, sterols and sesquiterpenes have been evaluated for their diverse biological activities such as anticancer, antioxidant, antiviral etc. (Lee et al., 2013; Puri et al., 2006). These bioactive compounds found in various mushrooms such as *Agaricus blazei, A. bisporus, A. subrufescens, Agrocybe aegerita, Ganoderma lucidum* include polysaccharide, pyrogallol, hydroxybenzoic acid derivative, flavonoid, polysaccharide (fucogalactan), fatty acid and triterpene have been found to exhibit significant anti-inflammatory properties. Extracts of mushrooms for decades have been used in immunomodulatory, antiviral, antibacterial, antioxidant, antihypoglycaemic applications and as a dynamic remedy for cardiovascular diseases. (Elsayed et al., 2014). *Boletus aereus, Trichloroma matsutake* and *Lactarius hatsudake* have been a source of food and traditional medicine in China. These mushrooms have an appreciable amount of protein, carbohydrate, essential mineral and low calories which can be compared to other sources like meat, eggs and milk (Valverde et al., 2014). Cultivated mushrooms are known to contain almost no fat, carbohydrates which are edible yet have a higher measure of protein than most vegetable Eatable mushroom *Pholiota adiposa* which is broadly developed in China has been discovered to be plentiful in proteins, fundamental amino acids, trace mineral components, dietary fibre

and carbohydrate (Wang et al., 2014). Polysaccharides especially beta-glucan of varied collection isolated from mushrooms regulate of the immune system. In the pharmacology industry, natural product like mushrooms has been an invaluable source of income due to bioactive compounds been used to manufacture novel medicines to cure various ailments (Valverde et al., 2014).

With the recent cultivation of mushrooms, the socio-economic environment of any farming community has improved through additional revenue by the use of waste from the farm. Wastes from cultivated mushrooms are used in the production of biofuel and biogas, as manures, a potting medium that also provide additional income for the farmer. Cultivation of mushrooms both seasonal and out of season provides income to the farmers. Value-added mushroom products in terms of quality products create another source of income for the farmer.

2.5 Mycochemicals as Bioactive Metabolites in Medicinal Mushrooms

Medicinal mushrooms are beneficial for their medicinal and therapeutic applications because of the presence of diverse bioactive metabolites. These metabolites found in mushrooms are classified as mycochemicals. Mycochemicals have a wide scope of restorative impacts and they can go about as immune-modulatory, anticarcinogenic, antiviral, antioxidant, and mitigating specialists (Elsayed, Enshasy, Wadaan, & Aziz, 2014). These mycochemicals assume huge parts in improving human wellbeing, in various ways. They are liable for the counteraction and treatment of infections to people (Rathee, Rathee, Rathee, Kumar, & Rathee, 2012). Polyphenols, flavonoids, tannins, terpenoids, alkaloids and many others found in mushrooms as secondary metabolites play a very important role as natural

antioxidant and immunodulators (Sudan, Bhagat, Gupta, Singh & Koul, 2014). Bioactive metabolites in mushrooms include polysaccharides, terpenoids, alkaloids, polyphenols, steroids, tannins, proteins, peptides, amino acids, saponins, lignans etc. (Elsayed et al., 2014). Structures of few examples of mycochemical are list in figure 3.

The concentration and adequacy of these metabolites in therapeutic mushrooms differed relying upon the kind of mushroom, development and fruiting conditions, phases of advancement, stockpiling conditions, and cooking handling (Gupta, Summuna, Gupta, & Annepu, 2019).

Côté, Caillet, Doyon, Sylvain, & Lacroix, 2010(Côté et al., 2010) and Archivio, Filesi, Vari, & Scazzocchio, 2010 reported the presence of lignans, tannins, hydroxycinnamic acids, stilbenes, flavonoids, hydroxybenzoic acids, and oxidized polyphenols in some chosen medicinal mushrooms. These compounds display calming properties and they go about as peroxide decomposers, free radical inhibitors, metal deactivators and oxygen scroungers (Gupta, Summuna, Gupta, & Annepu, 2019).

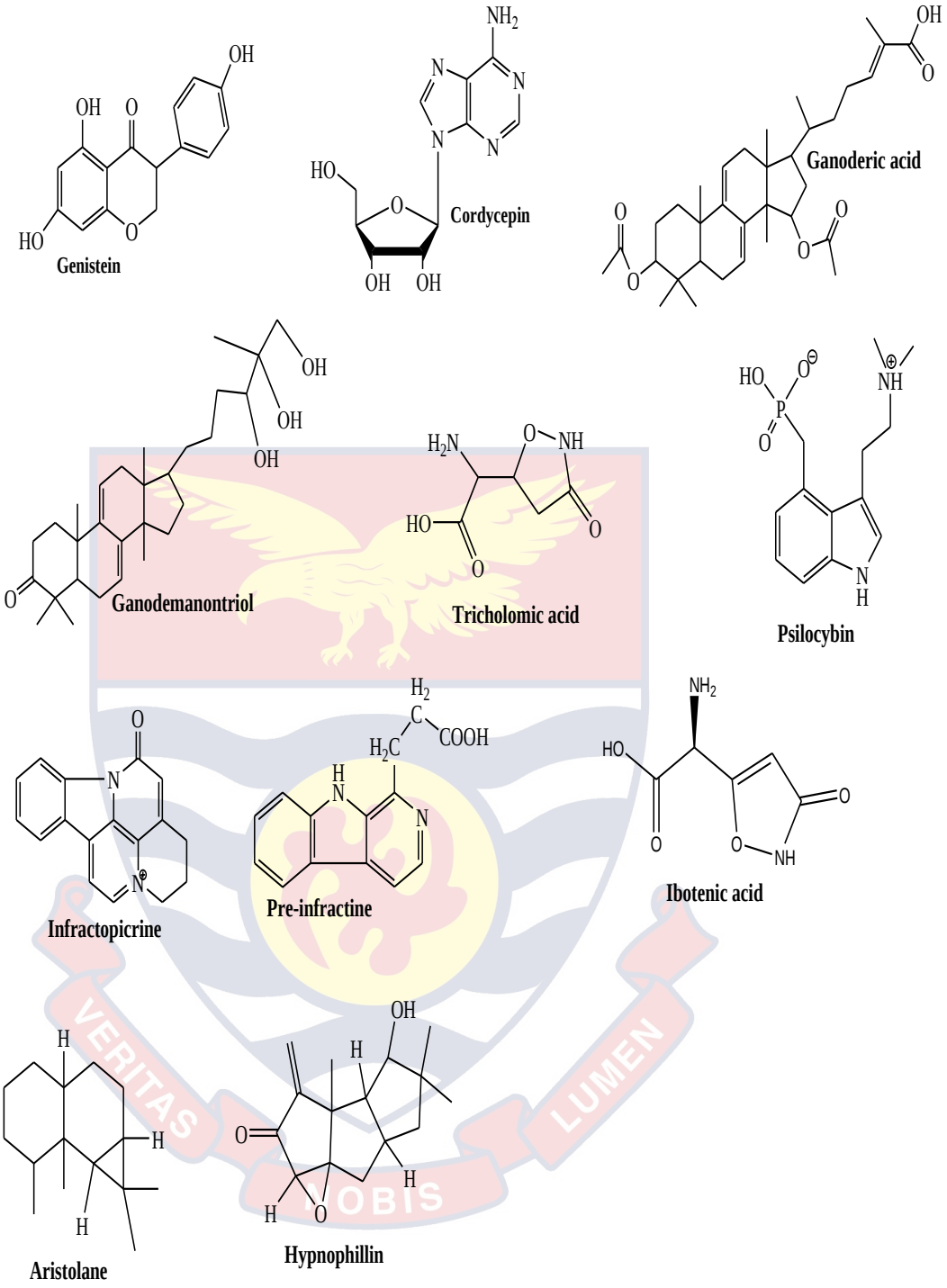


Figure 3: List of some mycochemicals found in medicinal mushrooms

Polysaccharides are significant group of mycochemicals found in medicinal mushrooms and they have remarkable antitumor, cell reinforcement, antibacterial, and antiviral activities (Elsayed et al., 2014). These are available in cell wall with various sorts of glycosidic linkages.

Terpenes are the biggest gathering of anti-inflammatory compounds found in medicinal mushrooms. A few terpenes separated from *Ganoderma lucidum* showed anti-inflammatory property (Gupta et al., 2019). Some triterpenes from *Ganoderma lucidum* (ganoderic acid C and subsidiaries) can repress the biosynthesis of cholesterol while other triterpenes (ganoderic acid F) add to atherosclerosis insurance (Gupta et al., 2019). Different triterpenes and sterols isolated from *Ganoderma lucidum* (i.e., ganoderiol, ganodermanontriol, and ganoderic acid) showed antiviral activity and antioxidative and free radical properties (Kumakura, Kumakura, & Ogura, 2008). Oxygenated lanostane-type of triterpenoid isolated from *Ganoderma lucidum* have been found to exhibit HMG-CoA reductase-inhibiting activity in vitro (Li, Li, & Sun,, 2006).

Alkaloids are very important fungal metabolites and they are known to play a very important role in the defence system of the organism that synthesis them. They represent one of the largest groups of natural products which almost all phyla including higher fungi (mushrooms) are capable to synthesize at any stage of their life cycle (Mahmood, 2013; Wieczorek, Witkowska, & Jasicka-misiak, 2015) analysed the origin, chemistry, biological activity, and the biomedical importance of indoles and isoxazoles alkaloids found in hallucinogenic mushrooms. These are bioactive alkaloids display restorative significance. Psilocin and psilocybin are examples of daydreaming alkaloids which were found in specific mushrooms, strikingly the two Mexican species *Psilocybe mexicana* and *P. cubensis* previously *Stropharia cubensis* (Wieczorek et al., 2015). Psilocin and psilocybin are not utilized in current medication, yet research recommends that they may have possible applications in the treatment of uneasiness and the improvement of personal satisfaction for

critically ill patients (Mahmood, 2013; Wieczorek et al., 2015). Ibotenic acid, an Amanita alkaloid, is a ground-breaking neurotoxin utilized in numerous examinations as a "mind sore" causing agent (Moss & Hendrickson, 2018; Wieczorek et al., 2015).

2.6 Antioxidant

Oxidation occurs in all humans which produce free radicals as end products. These free radicals are chemicals with one or more unpaired electrons, highly reactive, which are responsible for causing biological damage to cells, proteins and DNA causing cancer, heart diseases, ageing and other diseases (Jengg-Leun, Hsiu-Ching, & Chin-Chu, 2002). Free radicals become present in the human body during biological activities in the body and also from the food we eat, medicines we take, the air we breathe and other pollutants in the environment making them a harmful threat to the body (Udu-Ibiam et al., 2014). Evidence has been established that free radicals damages are the cause of many health issues such as cardiovascular diseases, cancer, inflammatory diseases and AIDS (Acquired Immune Deficiency Syndrome) (Wang et al., 2014).

Getting rid of free radicals from the body is very important and this can be achieved by the use of antioxidants. To defeat free radical damage antioxidants are the lifeline of defence and are essential for health. Antioxidants are substances with the ability to defeat free radicals or slow down oxidative stress. Although human bodies naturally produce antioxidants, it is not enough to combat the overwhelming production of free radicals which produce diseases and other health-related issues. Antioxidant intake prevents diseases and improves health (Udu-Ibiam et al., 2014). Antioxidant assumes a

significant part in the avoidance and treatment of an assortment of sicknesses by taking out free radical intermediates and hindering other oxidation responses. The latest interest in the quest for organically bioactive compounds in plants and fungi for their potential medical advantages, for example, antioxidant, antimicrobial, anti-inflammatory has increased because of the significant degree of openness to the causes of free radicals and the requirement for alternative prevention agents for the body to utilize (Udu-Ibiam et al., 2014). Anthraquinone related shades secluded from the mushroom Cortinarius purpurascens, which are rufoolivacin, rufoolivacin C, rufoolivacin D and leucorufoolivacin, were found to display strong DPPH free radical scavenging property (Bai, Wang, Zong, Lei, & Gao, 2013)

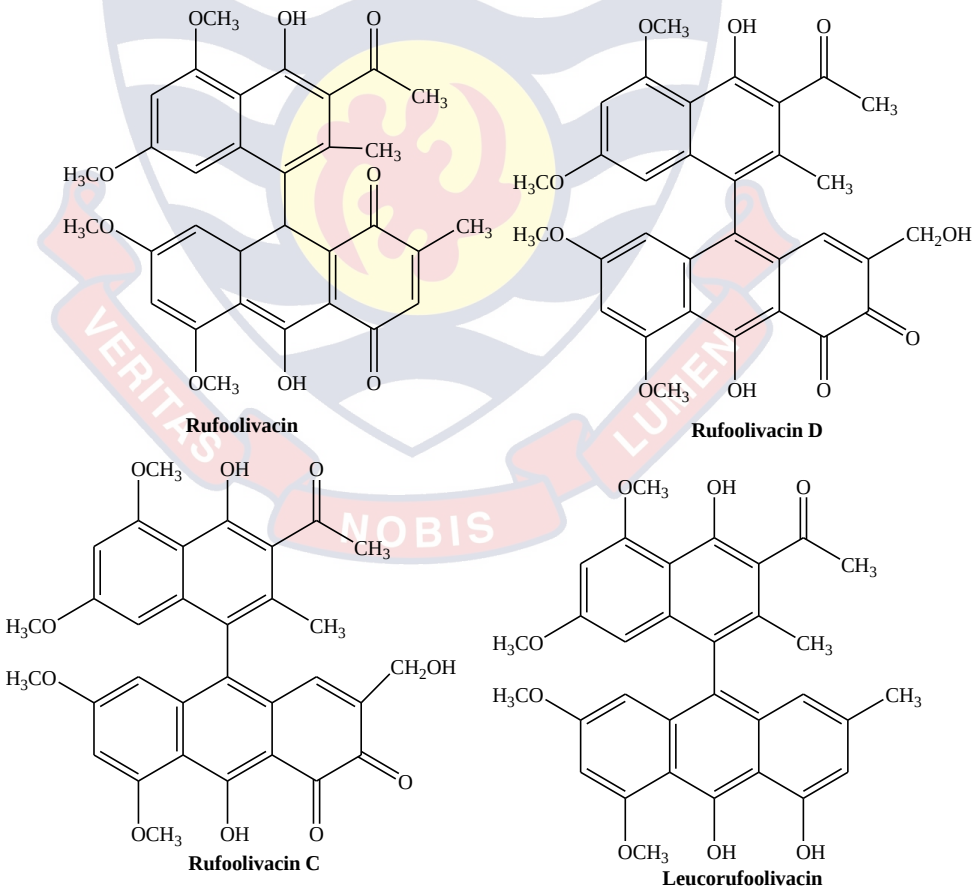


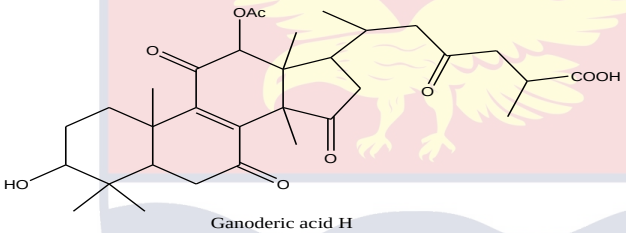
Figure 4: Chemical structures of anthraquinone metabolites isolated from C. purpurascens

Investigation in the past has revealed that some fruits, vegetables, herbs, sprouts and seeds contain phenolic compounds which are major bioactive compounds that are associated with antioxidant properties. Mushrooms additionally need antibacterial and antifungal mixtures to get by in their common natural surroundings henceforth they were likewise examined for optional metabolites including phenolic compounds, polyketides, terpenes and steroids (Wong & Chye, 2009). The initiation and progression of cancer has been altered by Polyphenols and carotenoids isolated from mushrooms and also these bioactive compounds act as an anti-ageing, anti-inflammatory and brain-protective factor as well as protect against cardiovascular diseases. Anthraquinones have also been found in *Tyromyces* which possess anti-cancer and anti-inflammatory properties. Singlet oxygen quenchers and lipid peroxidation chain breakers are enhanced by the presence of Carotenoids. They likewise have been accounted for to diminish prostate malignancy, stomach related tract tumors and constant illnesses (Robaszkiewicz, Bartosz, Lawrynowicz, & Soszy, 2010). Phenols like tocopherols and gallate discovered in mushrooms are known to be powerful antioxidants. Phenolic compounds isolated from mushrooms with phenolic hydroxyl group attached to an ester display antioxidant activity (Wang et al., 2014). Polysaccharides, phenolic compounds, tocopherols and natural acids are the primary wellspring of cancer prevention agent in mushrooms (Fernandes et al., 2016). Antioxidant activity has been seen in *Ganoderma spp.* from the different exploratory systems completed by Obodai et al (Obodai et al., 2017). Documentation over the years on *Pleurotus spp*., which is the most consumed mushrooms in Asian nations, has shown that it contains a considerable number of phenolic

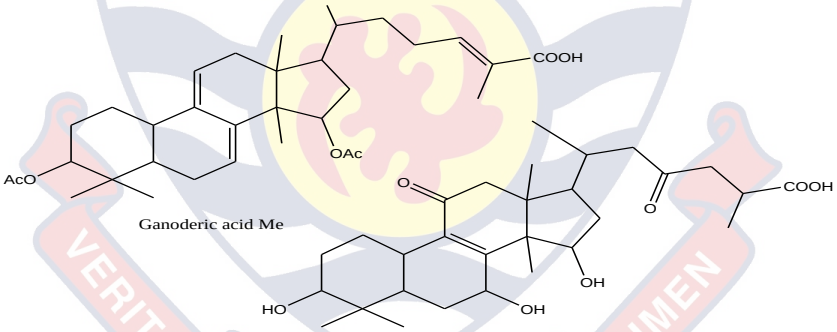
compounds which is the principle bioactive part for antioxidant activity. DPPH test which was completed on the methanolic concentrate of the mushroom showed positive outcomes (Sasidhara & Thirunalasundari, 2014). The methanolic concentrates of *Pleurotus ostreatus, Agaricus bisporus, Boletus badius, polyporus squamosus, Verpa conica, Lepista nuda* and *Russula delica* were taken through different antioxidant mechanisms and they showed critical exercises in vitro because of the different measure of phenolic compounds present in them (Elmastas, Isildak, Turkekul, & Temur, 2006). The presence of phenolic compounds in *Panaeolus antillarium* showed antioxidant action viably at pH 7.0 which implies that phenolic compounds are pH-sensitive in the mushroom (Dulay et al., 2015).

The type of solvent extraction has a significant role to play in the antioxidant activities of mushrooms. The hot water extracts from mature and baby ling zhi showed less antioxidant activities as compared with the methanolic extracts (Mau, Tsai, Tseng, & Huang, 2004). Siangu, Sauda, John, & Njue, (2019), observed that antioxidant activities involving methanolic extract increase with concentration. They observe *G. applanatum* has the highest antioxidant activity due to its relatively high phenolic and flavonoid content. Many extracted polysaccharides from mushrooms have been reported to exhibit various antioxidant activities based on the various in vitro and in vivo assays. However, most fungal polysaccharide tested for antioxidant activities were in their crude form or, to inhibit partially purified fractions with complex or unknown chemical composition and the pure polysaccharide actual activity is uncertain (Siu, Xu, Chen, & Wu, 2015). Polysaccharides (PS), PS-protein and phenolic content in *Ganoderma lucidum* were reported to

possess antioxidant activity, to inhibit lipid peroxidation and scavenge free radicals (Sun, He, & Bi, 2004). β –glucan, active polysaccharides, in *Ganoderma* is a major biologically active compound that has anticancer and antimetastic activities. β(1-3) – glycosidic bonds and side chains linked by β (1-6) glycosidic bonds have been found in fungal polysaccharides β – glucan (Obodai et al., 2017) Ganoderic and Lucidenic acids are two triterpenes isolated from *Ganoderma lucidum* reported to exhibit anticancer, anti-inflammatory, antihistamine and hypotensive activities.

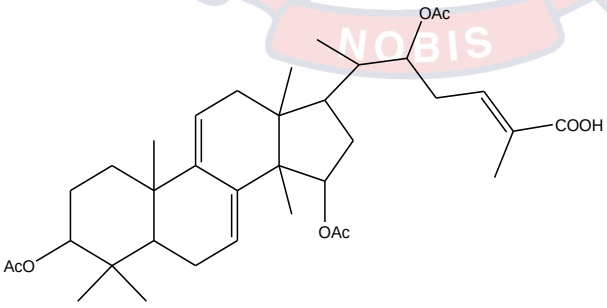


Ganoderic acid H



Ganoderic acid Me

Ganoderic acid C2



Ganoderic acid T

Figure 5: Some triterpenes isolated from Ganoderma spp.

Ganoderic T has been shown to exhibit an inhibitory effect on 95-D cells (Tang, Liu, Zhao, Wei, & Zhong, 2006). Diverse polysaccharides from

mushrooms comprising of β-linked glucans, such as schizophyllan from *Schizophyllum commune*, lentinan from *Lentinus edodes*, ganoderan and ganopoly from *Ganoderma lucidum*, pleuran from *Pleurotus* species, and calocyban from *Calocybe indica*, (Villares, Mateo-Vivaracho, & Guillamon, 2012).

Antioxidant, anti-inflammatory, antitumor and antiviral activities have been shown in extracts or compounds isolated from various fruiting bodies of *Pleurotus* species. Antioxidant activity has been exerted by the aqueous extract of *Pleurotus ostreatus*. The methanolic extract of *Pleurotus abalonus* exhibited higher antioxidant activity and free radical scavenging ability (Li et al., 2012). Extracts from *Pleurotus adiposa* has been found to exhibit antitumor, antimicrobial and antioxidant properties (Wang et al., 2014).

2.7 Summary

The selected mushrooms belong to the family basidiomyces. Mushrooms from this family are mostly edible and medicinal. These mushrooms have been found to contained bioactive secondary metabolites such alkaloids, flavonoid, tannins, polyphenols, steroids, coumarins etc. Previous investigations have revealed that these bioactive compounds play very important and significant role as natural antioxidant, antitumor, anticancer, antiviral, antibacterial, antifungal and anti-inflammatory.

CHAPTER THREE

RESEARCH METHODS

3.0 Introduction

This chapter describes the methods used to determine the presence of secondary metabolites in the sampled potential medicinal mushrooms. The various mushroom samples were evaluated for their antioxidant properties.

3.1 Chemicals

Chemicals utilized for the work were of analytic grade. The accompanying chemical substances were utilized, iron (III) chloride (FeCl3), sodium nitrite (NaNO2), sodium hydroxide (NaOH), hydrochloric acid (HCl), sulphuric acid (H2SO4), acetic acid (CH3COOH), acetic anhydride (CH3CO)2O, Fehling's solution A and B, Benedict Solution, ammonium molybdate (NH4)2MoO4, potassium iodide, iodine, Sodium dihydrogen phosphate (H2NaO4P), Sodium hydrogen phosphate (HNa2O4P), ascorbic acid ((5R)- [(1S)- 1,2-Dihydroxyethyl]-3,4-dihydroxyfuran-2(5H)- one), trichloroacetic acid, Folin-Ciocalteu reagent(Sodium 3,4-dioxo-3,4-dihydronaphthalene-1-sulfonate), gallic acid, hydrogen peroxide (H2O2), sodium carbonate, Potassium hexacyanoferrate (III), methanol, chloroform, and ammonia solution.

3.2 Sample Collection and Identification

The matured mushroom samples were harvested from different dead tree trunks in Abura Dunkwa and Breman Asikuman forest areas in the Central Region of Ghana. The samples were identified at the Department of Botany, University of Ghana, Legon. The samples were labelled as indicated in the Table 2 below

Table 2: The sample ID and their scientific names of the mushroom samples identified

Sample ID	Name of Mushroom
FM 01	*Tyromyces chioneus*
FM 02	*Polyporus alveolaris*
FM 03	*Trametes hirsute*
FM 05	*Trametes versicolor*
FM 06	*Trametes gibbosa*
FM 08	*Chlorophyllum molybdites*
FM 09	*Auricularia auricular judae*
FM 10	*Ganoderma spp.*

Source: Field sampling (2019)

3.3 Sample Treatment

The freshly mushroom samples were thoroughly cleaned by removing the dirt either with a brush or water. The mushroom samples were then cut into smaller pieces and air-dried for about 20 days at room temperature. The dried mushroom samples were broken into much smaller pieces and ground to a fine powder by a hi-speed Magic Bullet blender. The powdered mushroom samples were bagged in airtight zip-lock plastic bags and stored in a dark dry cool place for further analysis.

3.4 Extraction Procedure

Cold maceration was performed on all powdered mushroom samples separately using methanol as solvent. About 50 g of each powdered mushroom sample was soaked and cold macerated with 600 mL methanol for 48 h at room temperature. Extracts were filtered by vacuum filtration and extractions were repeated with 200 mL each of methanol on all the samples. The filtrates were subsequently concentrated using rotary evaporator to get crude extracts

of the samples. The crude extracts were put in evaporation dishes, labelled accordingly and were kept dry in a desiccator for further analysis.

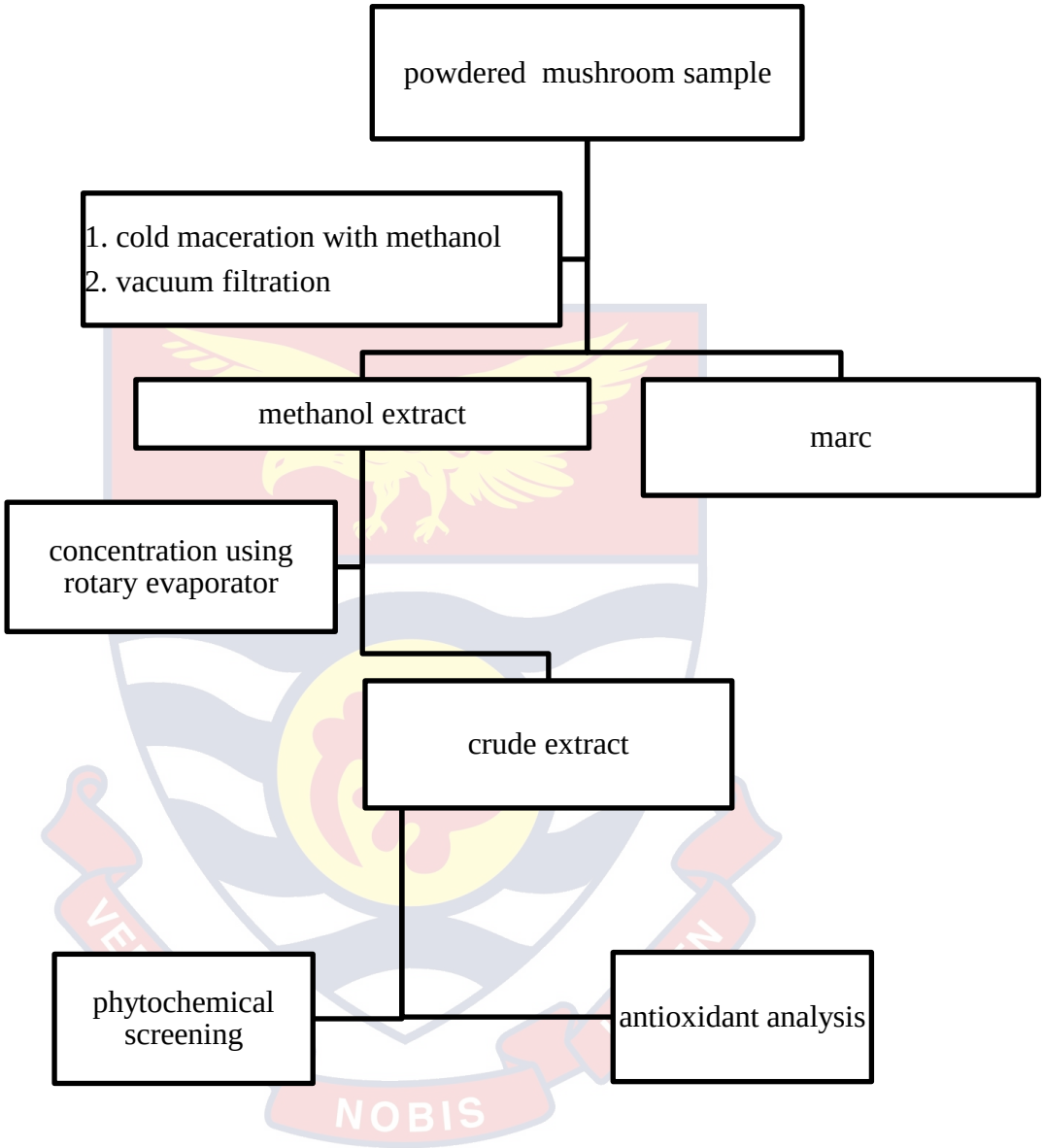


Figure 6: Flow chart diagram for methodology

3.5 Mycochemical Screening

The methanol crude extracts of the mushroom samples were chemically screened for the presence of mycochemicals using standard procedures.

3.5.1 Reducing Sugars

A measure of 0.025 g of every crude methanol extract was dissolved with 25 mL distilled water and filtered. The filtrate samples were kept and utilized for the following tests for reducing sugars:

Benedict test – The strategy utilized was by (Rajesh & Dhanasekaran, 2014). Benedict's reagent (0.5 mL) was added to 0.5 mL of each filtrate test in a test tube. The combination was warmed for 2 minutes on a water bath until a trademark red shaded precipitate at the lower part of the test tube demonstrating the presence of reducing sugars.

Fehling solution test – The test used was by (Jaradat, Hussen, & Ali, 2015). An equivalent volume of 1 mL every one of Fehling solution A and B were added to 2 mL of each filtrate. The blend was incubated for 10 minutes in a boiling water bath. The presence of a brick-red precipitate demonstrates the presence of reducing sugar.

3.5.2 Saponin (Foam Index Test)

The foam index test was performed on the powdered sample as follows: 2 g of each powdered sample was put in a test-tube and 10 mL of distilled was added. The test-tube was agitated vigorously for the test mixture to foam. The time taken for the foam to breakdown completely was noted. A permanent foam formation, which took 1 to 2 hours to break, indicates the presence of saponins.

3.5.3 Phlobatannins

Each powdered mushroom sample (1 g) was into a 50 mL capacity screw-capped bottle with 10 mL of distilled water. The soaked samples were incubated for two hours at room temperature. The mixtures were heated for 2 hours at 100°C, filtered and the aqueous extracts were each boiled with 2 mL of 1% aqueous hydrochloric acid. The presence of phlobatannin is indicated by red precipitate (Rajesh & Dhanasekaran, 2014).

3.5.4 Terpenoid (Salkowski Test)

About 2 mL of chloroform was added to each 5 mL methanol crude extract and 5 drops of concentrated sulphuric acid were added to the mixture which was shaken and allowed to form a layer. The formation of reddish-brown colouration indicates the presence of terpenoid (Rajesh & Dhanasekaran, 2014).

3.5.5 Polyphenol

An amount of 0.001 g of each methanol extract was added to 2 mL of distilled water and shaken, 3 drops of 1% $FeCl_3$ and 3 drops of potassium ferricyanide ($K_3Fe\ [CN]_6$) 1% was added to 1 mL of the extracts. The formation of blue colour indicates the presence of polyphenols (Farhan, Rammal, Hijaza, & Badran, 2011).

3.5.6 Alkaloids (Wagner's Test)

An amount of 0.001 g of the methanolic extract of the sample was treated with 10 drops of diluted hydrochloric acid and shaken well for few minutes and then filtered. 1 mL of Wagner's reagent was added to 2 mL of the filtrate and shaken very well. The presence of alkaloids is indicated by a reddish-brown precipitate.

3.5.7 Anthocyanin

The procedure described by Savithramma, Rao, & Suhrulatha, 2011) was used. An amount each of 1.0 g of the powdered mushroom sample was added to 10 mL of distilled water, shaken and macerated at room temperature for 24 h. The mixture was heated for 2 hours and then filtered to obtain the filtrate. Volumes of 1 mL each of 2 N HCl and ammonia solutions were added to 2 mL of aqueous extract filtrate. A colour change from pink – red to blue violet indicates the presence of anthocyanins.

3.5.8 Tannins

The method used was according to the one described by Rajesh & Dhanasekaran, 2014. An amount of each of 0.5 mg of dried powdered sample was added to 20 mL of distilled water, shaken and boiled in a test tube. The mixture was filtered and a few drops of 0.1% ferric chloride were added to 2 mL of each filtrate. A brownish-green or blue-black colouration indicates the presence of tannins.

3.5.9 Flavonoid

To each 1.0 g of the powdered mushroom sample, was added to 10 mL of distilled water, shaken and allowed to stand for 24 hours at room temperature. The mixture was heated for 2 h at 100°C and the mixture was then filtered to produce the aqueous extract. Diluted ammonia solution of about 5 mL was added to each aqueous extract followed by addition of few drops of concentrated sulphuric acid. The appearance of yellowish colouration indicates the presence of flavonoids (Rajesh & Dhanasekaran, 2014)

3.5.10 Coumarins

A volume of 10 mL of distilled water was added to 1 g of each powdered mushroom test, shaken and permitted to stand for 24 h at room temperature. The combination was then warmed for 2 h and separated to get the watery concentrate. About 3 mL of 10% NaOH was added to 2 mL of each aqueous extract. The appearance of yellow tone demonstrates the presence of coumarins (Savithramma et al., 2011).

3.5.11 **Steroid**

Steroids was tested by adding 2ml of chloroform to crude methanol extract followed by addition of 2ml of conc. H_2SO_4 slowly at sides of the test tube Formation of red lower layer indicates the presence of steroids.

3.5.12 Glycosides

Glycoside was tested by adding 2ml of acetic acid and 2ml of chloroform to an aqueous solution of the methanol extract. Few drops of concentrated sulphuric acid were added along the sides of the test tube. Formation of green colouration indicates the presence of glycosides

3.6 Antioxidant Analysis

3.6.1 Total Phenolic Content

Total phenolic content in each mushroom crude concentrate was resolved to utilize the Folin – Ciocalteu (FC) reagent strategy with Gallic acid as the standard. Every crude concentrate was dissolved to a concentration of 1mg/ml in methanol. about 0.5 mL of each concentrate arrangement was blended in with 2.5 mL of Folin – Ciocalteu reagent (10% or dissolved 10-folds with distilled water) and 2 mL of Na_2CO_3 (7.5%). The test solution was

blended well and incubated for 15 minutes at 45°C. The absorbance was estimated at a frequency of 765 nm utilizing T70 UV/VIS Spectrometer. The test samples were rehashed on two additional occasions and the mean was determined. A similar technique was rehashed for the standard solution of Gallic acid (Stankovic, Niciforovic, Topuzovic, & Solujic, 2011). All determinations were carried out in three-fold. The total phenolic content was expressed as milligrams of Gallic acid equivalent (GAE) per g of concentrate.

Figure. 7: Total phenolic content determination after 15 minutes

3.6.2 Total Flavonoid Content

The total flavonoid content of the mushroom methanol extract was determined spectrophotometrically using Aluminium chloride solution according to the method used by (Fattahi et al., 2014). The crude extract was prepared to a concentration of 1 mg/mL. The reaction mixture was prepared by mixing 1 mL of the crude extract solution with 60 µL of sodium nitrite and this was incubated for 5 minutes. After which 60 µL of 10% Aluminium chloride was added. After 6 minutes 400 µL of sodium hydroxide was added

to the mixture. The absorbance was determined using the spectrophotometer at a wavelength of 510 nm. The samples were prepared in triplicates for analysis. The same procedure was repeated for the standard solution Quercetin of different concentration from 0 to 1000 µg/mL.

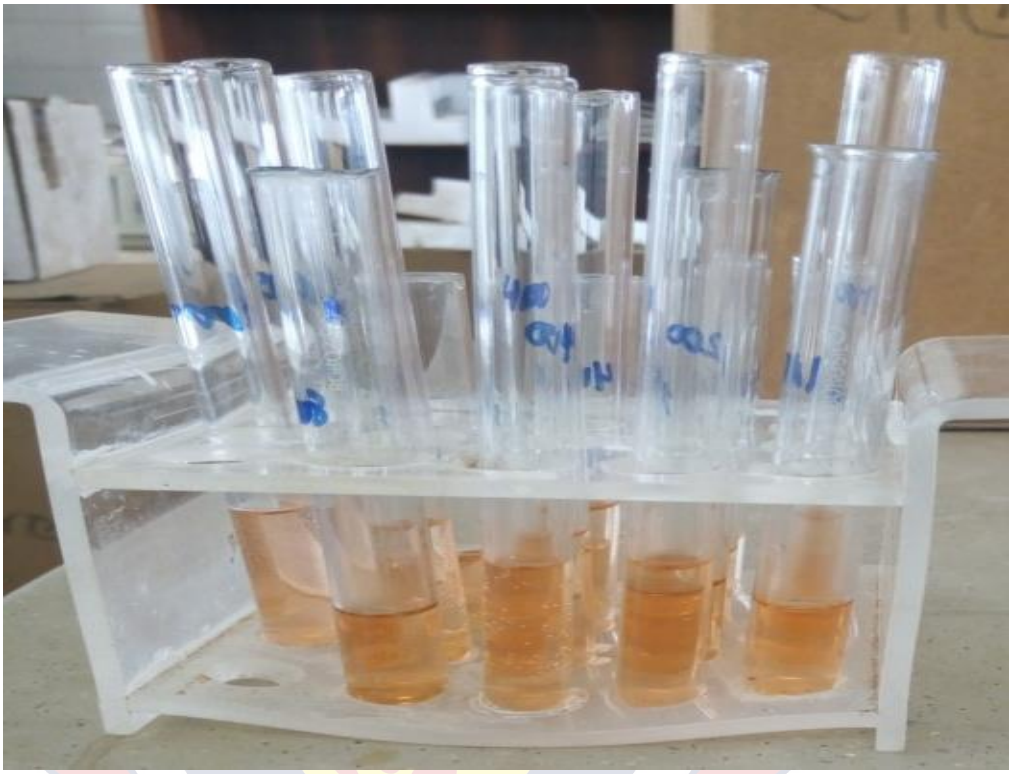


Figure 8: Total flavonoid content determination after incubation

3.6.3 Total Antioxidant Capacity (Activity)

Total antioxidant capacity of the mushroom extract was assessed by the phosphomolybdate method utilizing ascorbic acid as a standard. The test depends on the decrease of Mo (VI) to Mo (V) by the concentrate and afterwards the formation of a green phosphate/Mo (V) complex at an acidic pH. A concentration of 1 mg/mL in methanol of each mushroom extract was made. An aliquot of 0.3 mL extract solution of each sample was blended in with 3 mL of the reagent solution (4 mM ammonium molybdate, 28 mM sodium phosphate and 0.6 M sulphuric corrosive, all blended in 1:1:1

proportion individually). The subsequent blend was incubated for 90 minutes at 95°C after which the absorbance of the green phosphomolybdenum complex was estimated at 695 nm against a blank (containing 0.3 mL methanol and 3 mL of reagent solution). The experiment was led in sets of three and values communicated as equivalent of ascorbic acid in mg per g of concentrate (Jan, Khan, Rashid, & Bokhari, 2013).

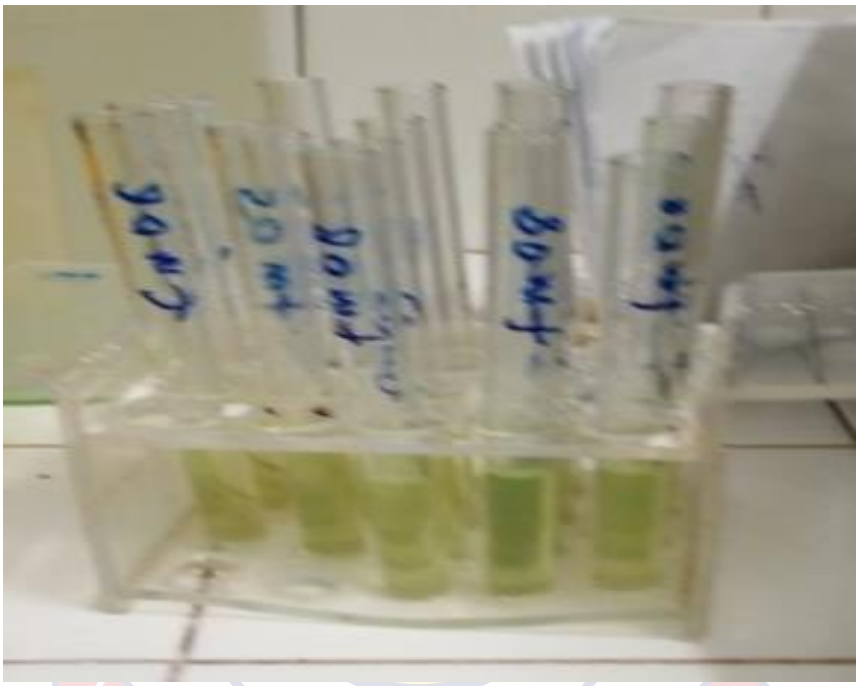


Figure 9: Total antioxidant capacity determination after incubation

3.6.7 DPPH Scavenging Activity

The free radical scavenging activity of the mushroom extract was assessed by the technique as per Stankovic et al., 2011 and Khatoon et al., 2013 utilizing 1, 1-diphenyl-2-picryl-hydrazil (DPPH). 0.1 mM of DPPH was set up by dissolving 8 mg in 200 mL of methanol. The crude methanol extract was diluted to a concentration of 1 mg/mL in methanol. Crude sample extract with various concentration (50 – 500 µg/mL) and Gallic acid standard was readied. Several different concentrations (50 – 500 µg/mL) of 1 mL of each

sample extract were placed into a test tube and 1 mL of the test reagent of 0.1 mM DPPH were consolidated together. The reaction solution was shaken energetically and left in obscurity at room temperature for 30 minutes to brood. The absorbance was estimated spectrophotometrically at 517 nm against a methanol blank. The control test contained just methanol and the test reagent. All examinations were conducted in sets of three. The percentage scavenging activity of the DPPH free radical was determined from the equation.

% Scavenging activity = ((Abs control-Abs sample)/ (Abs control))x 100

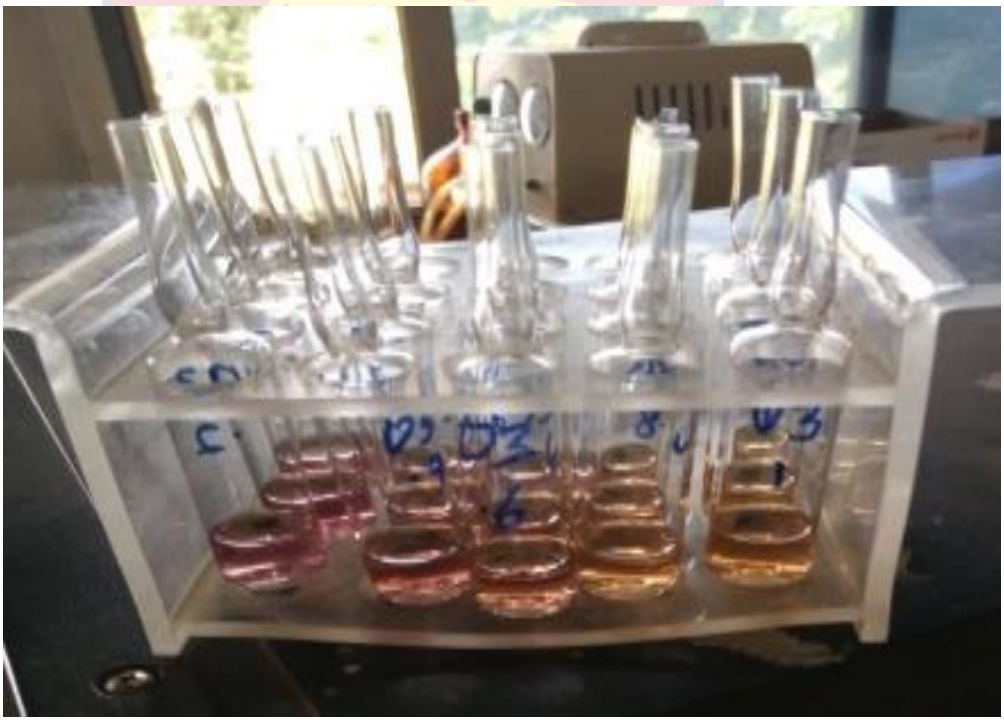


Figure 10: DPPH radical Scavenging after incubation

3.7 Summary

Eight mushrooms were harvested from the forest areas of Abura Dunkwa and Breman Asikuma in the Central Region of Ghana. The samples were air dried and milled into powder. Methanol was used to cold macerate each of the samples. The crude methanol extract of each sample was chemically screened for the presence of mycochemicals. Total phenolic content, total flavonoid content and total antioxidant capacity were determined. DPPH free radical scavenging activity was also carried out on the extracts of the mushrooms.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.0 Introduction

The purpose of this study was to mycochemically screen eight selected potential medicinal mushrooms for their secondary metabolites, determine their total phenolic, total flavonoid content and total antioxidant capacity as well as their scavenging activities. This chapter presents the results of the preliminary chemical screening of the methanol extracts of mushroom samples for the bioactive compounds present. It also discusses the results of the antioxidant analysis performed. The samples worked on were: *Tyromyces chioneus, Polyporus alveolaris, Trametes hirsute, Trametes versicolor, Trametes gibbosa, Ganoderma spp., Auricularia auricular judae, and Chlorophyllum molybdites*.

The statistical tool used was SPSS and Graph pad prism for the results analysis

4.1 Mycochemical Screening of Methanol Extract of Mushroom Samples

Mycochemicals are chemicals produced by mushrooms and other fungi species. These mycochemicals perform metabolic functions in mushrooms and protect them. Methanol extracts of the mushroom samples were screened using standard methods of (Evans, 2009) and result is presented in Table 3. The following mycochemicals were tested for: alkaloids, anthocyanins, tannins, flavonoids, coumarins, polyphenols, terpenes, phlobatannins, saponins, reducing sugars, steroids and glycosides were done. (See Table 3)

Table 3: Preliminary mycochemical screening of methanol extracts of eight mushrooms samples

Test	FM 01	FM 02	FM 03	FM 05	FM 06	FM 08	FM 09	FM 10
Alkaloids	+	+	+	+	+	+	+	+
Anthocyanin	-	-	-	-	-	-	-	-
Tannins	-	-	-	-	-	-	-	-
Flavonoids	-	-	-	-	-	-	-	-
Coumarins	-	-	-	-	-	-	-	-
Polyphenols	+	+	+	+	+	+	+	+
Terpenes	+	+	+	+	+	+	+	+
Phlobatannins	-	+	+	-	-	-	-	-
Saponins	+	+	+	+	+	+	+	+
Reducing sugars	+	+	+	+	+	+	+	+
Glycosides	-	-	-	-	-	-	-	-
Steroids	+	+	+	+	+	-	-	+

+ = Positive; - = Negative

FM 01- *Tyromyce chioneus*; FM 02-*Polyporous alveolaris*; FM 03-*Trametes hirsute*; FM 05 *Trametes versicolor*; FM 06-*Trametes gibbosa*; FM 08-*Chlorophyllum molybdite*; FM 09-*Auricularia auricular judae;* FM 10-*Ganoderma spp*

The presence of alkaloids, polyphenols, terpenes, saponins, and reducing sugars in the methanol extract of all the eight mushroom samples was revealed. Anthocyanin, tannins, flavonoids, coumarins, and glycosides were

also absent in all the extract samples. Steroid was present in all mushroom samples except *Auricularia auricular judae* and *Chlorophyllum molybdite*. Phlobatannins was positive for *Polyporous alveolaris* and *Trametes hirsute* but negative for all the other mushrooms. The presence of alkaloids, tannins, saponins, terpenes polyphenols and steroid have been confirmed and reported in *Trametes* species. (Appiah et al., 2018; Fagbohungbe & Oyetayo, 2014; Leliebre-lara et al., 2015; Oluwafemi, 2018) According to (Knezˇević et al., 2018), extracts of *Trametes gibbosa*, *Trametes hirsute* and *Trametes versicolor* have significant medicinal potentials based on the biologically active compounds. Chemical analysis indicated strong synergistic action of triterpenes; sugars and polyphenols present in the *Trametes* species (Knezˇević et al., 2018). Steroid isolation from *Trametes gibbosa* exhibited broad-spectrum antifungal and antibacterial activities as reported by Lin et al., 2010 and Appiah et al., 2018.

Methanol extract of the *Ganoderma spp.* tested positive for alkaloids, polyphenols, terpenoids, saponins, reducing sugar and steroid but tested negative to tannins, flavonoids, coumarins, and glycosides. According to (Imtiyaz et al., 2015), *Ganoderma lucidium* showed a positive result to alkaloids, polyphenol, terpenoids, saponins, reducing sugars, and steroids. Terpenoids isolated from *G. lucidum* have attracted extensive consideration attributable to their notable pharmacological exercises (Ahmad, 2019). *Tyromyce chioneus* showed the presence of alkaloids, polyphenol, terpenoids, reducing sugars, saponins, and steroids. However, it tested negative to anthocyanins, tannins, flavonoids, coumarins, phlobatannins, and glycosides. An epic cadinane sesquiterpene (named 4β,14-dihydroxy-6a,7β H-

1(10)- cadinene) was segregated from cultures of *Tyromyces chioneus*. This molecule appeared to have critical anti-HIV-1 action with EC50 =3.0 µg/mL (SI=25.4) (Liu, Wang, Yang, Zheng, & Liu, 2007). *Chlorophyllum molybdite* showed the presence of alkaloid, polyphenol, terpenoids, saponins reducing sugar, but the result was negative for steroids, phlobatannins, tannins, flavonoids coumarins, anthocyanins, and glycosides. Methanol extract of *Auricularia auricular judae* was positive for alkaloid, polyphenol, terpenoids, saponins, and reducing sugar but negative for anthocyanins, tannins, flavonoids, coumarins, phlobatannins, and steroids.

Mushrooms are grounded wellspring of bioactive compounds, and much work has been focused on the separation of mushroom-derived natural products (Homer & Sperry, 2017). They gather an assortment of secondary metabolites which are liable for their medicinal properties. The capability of medicinal mushrooms as a wellspring of bioactive compounds has forestalled the appearance of some diseases (Ogidi & Oyetayo, 2016). It is beneficial to screen the concentrates of these mushroom samples in view of their antimicrobial, antioxidants, anticancer and mitigating significance. The presence of polyphenols in the mushrooms samples proposes the solid antioxidant properties which forestall oxidative harm to biomolecules like DNA, lipids and proteins (Capistrano et al., 2018; Manach et al., 2004). Polyphenols assume a part in the anticipation of sicknesses identified with oxidative pressure like cancer, cardiovascular illnesses, diabetes, and neurodegenerative. Polyphenol compounds unquestionably meddle with phases of the disease interaction, conceivably bringing about a decrease of cancer risk (Capistrano et al., 2018)

4. 2 Quantitative Analysis

The quantitative analysis of polyphenols and flavonoids were determined in the methanol extracts of the various mushroom samples. The quantitative analysis carried out were total phenolic content and total flavonoid content using standard procedures.

4.2.1 Total Flavonoid Content (TFC)

The total flavonoid content of the diverse mushroom separates was determined using the aluminium chloride colorimetric procedure. This relies upon the plan of a complex between the aluminium particle and the carbonyl and hydroxyl groups of flavonoid auxiliaries to convey a yellow colour (Bhaigyabati, Devi, & Bag, 2014; Pontis, Antonia, Alves, Jose, & Flach, 2014). The TFC in the methanol concentrates of mushroom tests were resolved (Table 4) from the standard curve R^2 = 0.9784 using quercetin as standard (Figure 11) and the results are imparted as mg of quercetin comparable per gram of concentrate (µg QE/g). The standard curve was procured using different concentration (0-1000 µg/ml) of quercetin with their absorbance as shown in Figure 11.

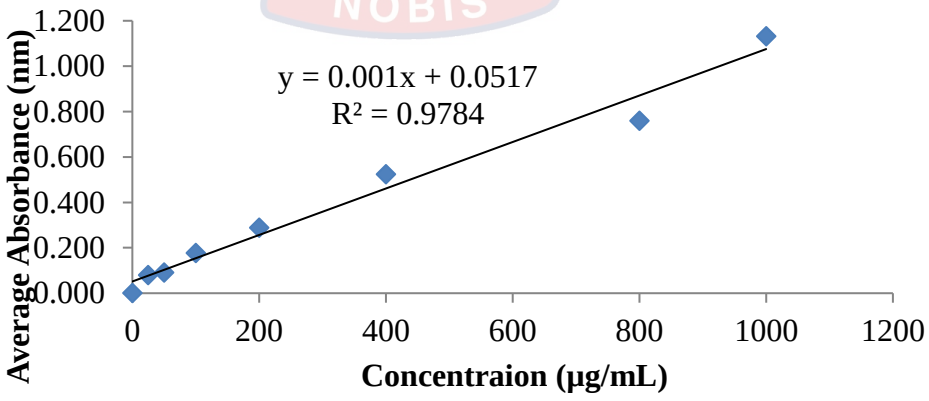


Figure 11: Standard curve of quercetin

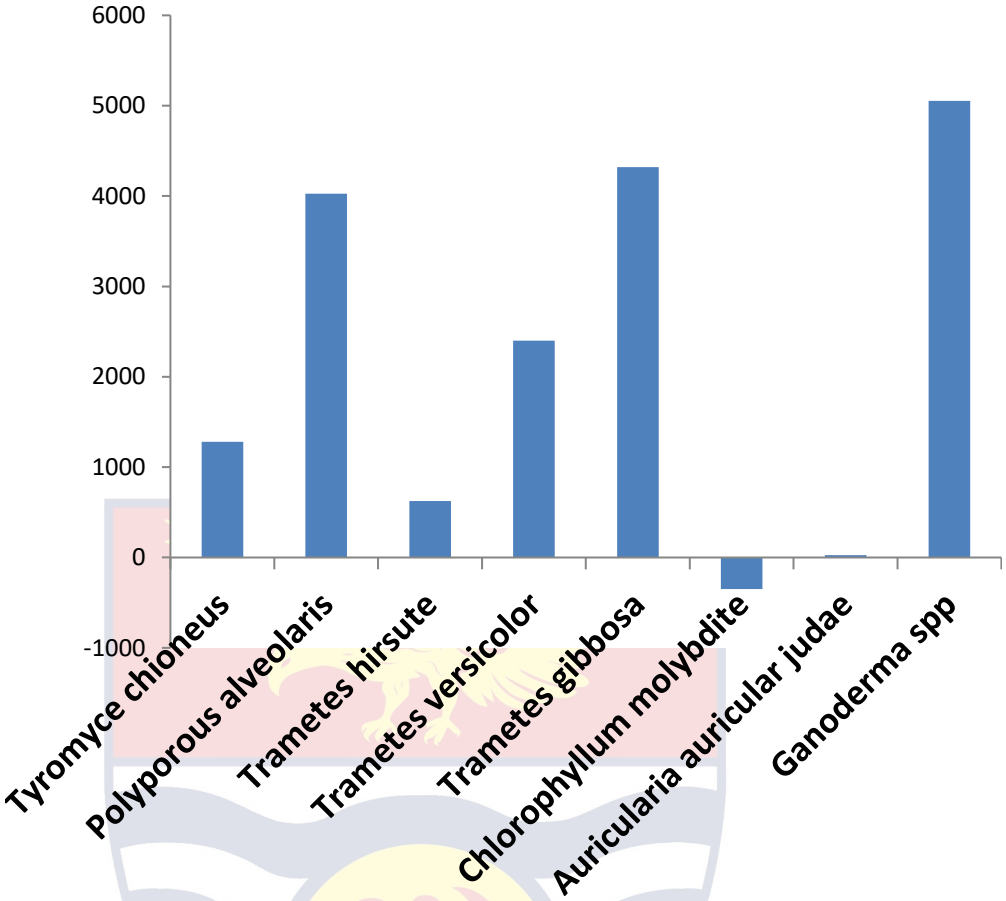


Figure 12: Total flavonoid content in Quercetin equivalent

The total flavonoid content for the various mushroom samples ranged from -348.00 ± 277.12 to 5052.00 ± 2983.42 µg QE/g (table 4). Variations were observed in the various mushroom samples, the highest flavonoid amount was found in the *Ganoderma spp* with TFC value of 5052.00 ± 2983.42 µg QE/g, followed by *Trametes gibbosa* (4318.66 ± 1763.78 µg QE/g) and *Polyporous alveolaris* 4025.33 ± 622.68 µg QE/g. *Chlorophyllum molybdite* (-348.00 ± 277.12 µg QE/g) *and Auricularia auricular judae* (25.33 ± 161.5 µg QE/g) have appreciably very low TFC than the rest of the mushroom test samples and therefore are significantly different from the others. TFC for *Trametes versicolor* was low as compared to data obtained by Abugri & Mcelhenney, 2013, which is 30.58 mg GAE/mL and *Ganoderma applanatum* is 84.49 mg GAE/mL. According to Zengin et al., 2016, the TFC

of the methanol extract of *Trametes gibbosa* (2140 µg RE/g) was lower and *Trametes hirsute* (12370 µg RE/g) was higher than what was observed in this study (4318.66 µg QE/g and 625.33 µg QE/g respectively) These differences perhaps could be attributed to genetic differences, type of extraction method and time, moisture content in the mushroom, particle size of the mushroom, and the difference in solvents. Furthermore, flavonoid content in *Trametes versicolor* methanol extract was not detected as well as the acetone extract. According to Pop et al., 2018 *Trametes gibbosa* also gave a total flavonoid content of 11.65± 0.40 in the methanol extract while the water extract gave 2.83±0.30 mg QE/100 g. The presence of flavonoid is essential in the body to help regulate and fight off free radical activities. It is reported by (Kozarski et al., 2015), that, flavonoid contribute to the antioxidant effects in edible and wild mushrooms.

4.2.2 Total Phenolic Content (TPC)

Phenolic compounds are attributed to being the main source of antioxidant properties in any natural product (Fernandes et al., 2016). Phenolic compounds are made up of aromatic ring(s) having hydroxyl group directly attached to a phenyl or other aryl groups and are able to defeat any free radicals by forming resonance-stabilised phenoxyl radical (Rice-Evans et al., 1997). The total phenolic content of the methanol extract of the mushroom samples was evaluated using the principle of the transfer of electrons from phenolic compounds to the Folin-Ciocalteu reagent in alkaline medium. Formation of a blue colour complex results from the reaction between Folin-Ciocalteu reagent and phenolic compounds which absorbs at 765 nm (Pontis et al., 2014). The standard curve was plotted using the various absorbance of the

standard gallic acid at 765 nm at different concentrations from 0.2 – 1 mg/mL of the standard solution as shown in Figure 13.

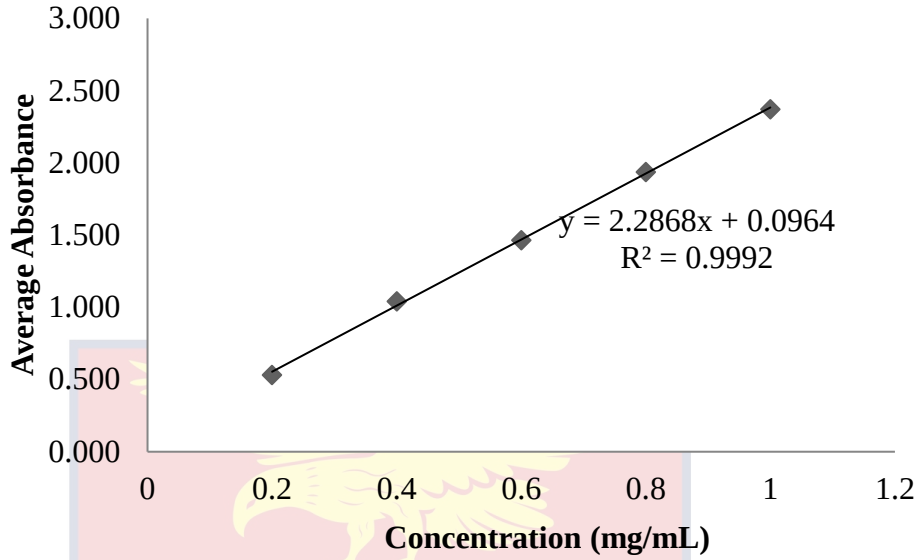


Figure 13: Standard Gallic acid curve for phenolic content determination

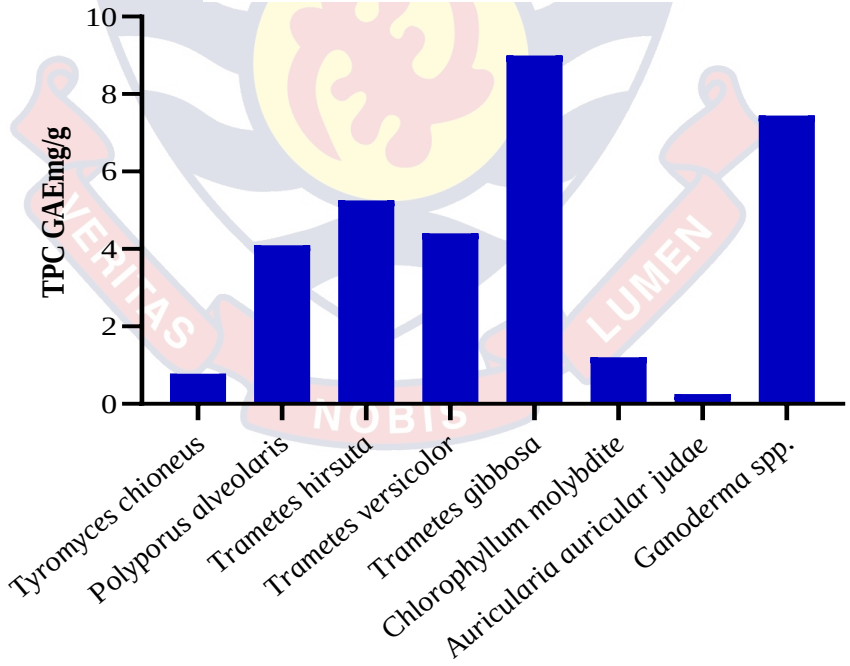


Figure 14: Total phenolic content in Gallic acid e quivalent (GAE)

Table 4: Analysed Data for Total phenolic content (mg GAE/g), total flavonoid content (µg QE/g) and total antioxidant capacity (mg AAE/g)

Sample ID	Total Phenolic Content (mg GAE/g)	Total Flavonoid Content (µg QE/g)	Total Antioxidant Capacity (mg AAE/g)
Tyromyce chioneus	0.77 ± 0.05^{ab}	1278.66 ± 514.32^{abc}	6.12 ± 0.42^{a}
Polyporous alveolaris	4.09 ± 0.11^{c}	4025.33 ± 622.68^{bcd}	28.89 ± 3.27^{c}
Trametes hirsute	5.26 ± 0.16^{d}	625.33 ± 166.53^{ab}	29.91 ± 2.25^{c}
Trametes versicolor	4.40 ± 0.04^{c}	$2398.66 \pm 483.87^{abcd}$	22.92 ± 2.69^{b}
Trametes gibbosa	8.99 ± 0.56^{f}	4318.66 ± 1763.78^{cd}	26.42 ± 4.78^{bc}
Chlorophyllum molybdite	1.20 ± 0.08^{b}	-348.00 ± 277.12^{a}	7.86 ± 0.53^{a}
Auricularia auricular judae	0.25 ± 0.07^{a}	25.33 ± 161.5^{a}	15.36 ± 10.06^{ab}
Ganoderma spp	7.44 ± 0.02^{e}	5052.00 ± 2983.42^{d}	28.17 ± 1.87^{c}

All results have been expressed as means ± standard deviation (n = 3). Mean values followed by different letters in the same column are significantly different ($p \leq 0.005$)

Total phenolic content (TPC) for the mushroom samples were calculated from the standard equation (y=2.2868x+0.0964, R^2=0.9992) and expressed in mg gallic acid equivalent per gram (mg GAE/g). The calculated values of TPC for the various mushroom samples are presented in table 4. *Trametes gibbosa* has the highest TPC value of 8.99 ± 0.56 mg GAE/g followed by Ganoderma spp (7.44 ± 0.02 mg GAE/g). *Auricularia auricular judae* has the lowest TPC value of 0.25 ± 0.07 mg GAE/g. *Tyromyces chioneus* (77 ± 0.05 mg GAE/g) and *Chlorophyllum molybdite* (1.20 ± 0.08 mg GAE/g) also registered very low TPC. The TPC of *Polyporous alveolaris (*4.09 ± 0.11 mg GAE/g) and *Trametes versicolor* (4.40 ± 0.04 mg GAE/g) are not significantly different from each other. The total phenolic content obtained in this study for *Ganoderma spp.* and *Trametes hirsute* were higher as compared to what was obtained by Imtiyaz et al., 2015. The results obtained for the total phenolic content *Trametes gibbosa* and *Trametes hirsute were* lower than that obtained by Zengin et al., 2016, The TPC of *Auricularia auricular judea* was also lower as compared to that of Kho et al., 2009. The total phenolic content obtained from this study for *Tramestes versicolor* and *Trametes gibbosa* were higher than what was obtained by Pop et al., 2018. *Ganoderma spp* and *Trametes gibbosa* have significant amount of phenolic and flavonoid contents relative to their antioxidant activity which are high. They may contribute directly to antioxidative action and inhibitory effects on mutagenesis and carcinogenesis in humans (Johnsy & Kaviyarasana, 2011).

4. 3Antioxidant Analysis

The antioxidant activity of the methanol concentrates of the mushrooms samples were set up by their absolute antioxidant limit utilizing phosphomolybdenum strategy and antiradical searching movement utilizing 1,1-Diphenyl-2-Picrylhydrazyl (DPPH).

4.3.1 Total Antioxidant Content (TAC)

The total antioxidant activity (TAC) of the different mushroom extract was resolved to utilize the phosphomolybdenum technique. The presence of antioxidant in the extracts is required to decrease the Mo (VI) to Mo (V) and green phosphate/Mo (V) compounds in acid medium absorbs at 695nm. Ascorbic acid was utilized as the standard and its calibration curve is showed in Figure 14. The total antioxidant activity of all the test mushroom samples was determined utilizing the condition, y=0.7522x-0.0391 with $R^2 = 0.998$ and communicated quantitatively in ascorbic acid equivalent mg/g of concentrates (AAE). The qualities are introduced in Table 4.

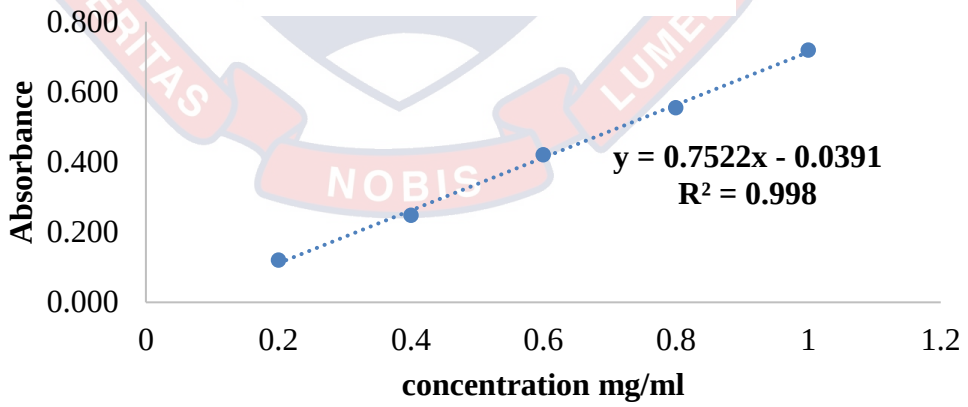


Figure 15: The calibration curve for ascorbic acid in total antioxidant capacity

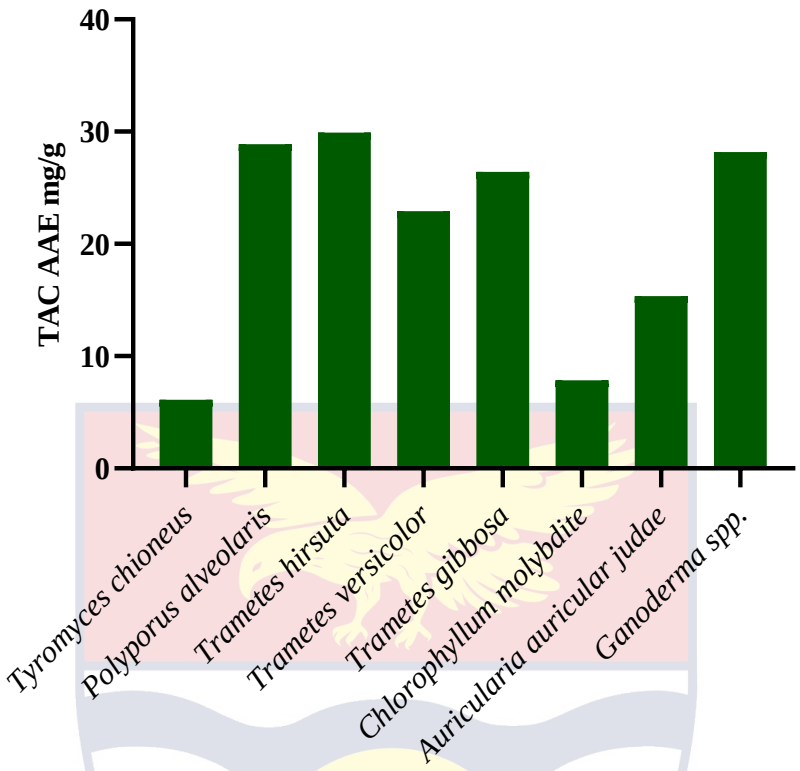


Figure 16: Total antioxidant content in mgAAE/g

Trametes hirsute had the highest TAC, 29.91 ± 2.25 mg AAE/g and Tyromyces chioneus with the smallest value of 6.12 ± 0.42 mg AAE/g. From the result, *Trametes hirsute* (29.91 ± 2.25 mg AAE/g), *Polyporous alveolaris* (28.89 ± 3.27 mg AAE/g) and *Ganoderma spp* (28.17 ± 1.87 mg AAE/g) show appreciable amount of TAC and they are significantly not different from each other. The TPC and TFC of these three mushrooms were appreciable, indicating that their antioxidant activity may be due to the polyphenolic compounds present. Phenolic compounds found in mushrooms play a very important role in neutralising free radicals which cause oxidative stress. The neutralisation of the free radicals is an indication of antioxidant potential of the mushroom. *Trametes gibbosa* also showed a good amount of TAC, 26.42 ± 4.78 mg AAE/g which is also not significantly different from *Trametes*

versicolor (22.92 ±2.69 mg AAE/g), see Table 4 and Figure 16. The three *Trametes* specie (*Trametes versicolor, Trametes hirsute and Trametes gibbosa*) among the samples have attested to the result obtained by Zengin et al., 2016, Johnsy & Kaviyarasana, (2011) and Pop et al., (2018). They reported very good amount of total phenolic and flavonoid content and antioxidant capacity of *Trametes* species which is in agreement with the result obtained.

4.3.2 DPPH Free Radical Scavenging Activity

Free radical scavenging activity has been reported in fruits, spices, mushrooms and vegetables. DPPH molecule has delocalised free electrons on the molecule as a whole which makes it stable. DPPH molecule accepts hydrogen from extracts with potential antioxidant property. DPPH free radical scavenging activity is established on the ability of those potential antioxidants found in these spices, mushrooms, fruits and vegetables to scavenge the DPPH free radicals. Gallic acid was used as the standard. The free radical scavenging activities of the various mushroom extracts were determined in vitro by DPPH method and the results are presented in Table 5. Table 5 shows the percentage scavenging of the mushroom extracts of *T. chioneus, P. alveolaris, T. hirsute and T. versicolor* increased gradually with increasing concentration. *T. gibbosa* increased inhibition up to (200 µg/ml) and dropped at 300 µg/ml but gradually increase inhibition thereafter. The percentage scavenging of *Ganoderma spp.* increased with increased concentration. *Chlorophyllum molybdite* showed an irregular percentage inhibition with the various concentrations.

From the results obtained *Ganoderma sp.* and *Polyporus alverolaris* had the highest scavenging activity of 94.37 ± 0.92 and 80. 66 ± 1.93 respectively at 500 µg/mL. Several studies have been carried out on these mushrooms. According to lkay Koca & Gençcelep, 2011 the methanolic extract of *Agaricus blazei* had a higher scavenging ability of 97.1% at 2.5 mg/mL and the DPPH scavenging activity of *Agrocybe cylindracea* to be 93.8% at 5 mg/mL. DPPH scavenging activity of *T. versicolor* was found to be higher than that of A. auricular (Akgul, Sevindik, Coban, Alli, & Selamoglu, 2017) which is in agreement with the results obtained. DPPH scavenging activity carried out by Siangu et al., 2019 showed that *G. applanatum* had the highest scavenging ability of 95.56%. Scavenging ability of *Ganoderma sp. (tsugae, lucidium and lucidium antler*) at 0.64 mg/mL ranged from 67.6 – 74.4 % (Jengg-Leun et al., 2002).

Antioxidants play an important role in their interactions with oxidative free radical. The importance of the DPPH method is that antioxidants react with the stable free radical (1,1 – diphenyl-2-picrylhydrazyl (deep violet colour) and convert it to (1,1 – diphenyl-2-picrylhydrazine with discolouration.

Table 5: Results Showing Percentages of DPPH in Different Concentrations;

Sample ID	%DPPHScavenging					
	50 µg/mL	100 µg/mL	200 µg/mL	300 µg/mL	400 µg/mL	500 µg/mL
Tyromyce chioneus	33.95 ± 0.90^{d}	36.69 ± 2.03^{c}	38.48 ± 1.34^{bcd}	44.93 ± 0.36^{d}	46.29 ± 2.12^{d}	58.82 ± 1.34^{e}
Polyporous alveolaris	38.90 ± 1.29^{e}	47.48 ± 2.65^{de}	58.05 ± 0.83^{e}	72.60 ± 1.03^{f}	78.52 ± 0.34^{f}	80.66 ± 1.93^{f}
Trametes hirsute	32.57 ± 1.22^{d}	35.05 ± 7.99^{c}	39.14 ± 1.06^{cd}	44.99 ± 1.15^{d}	45.97 ± 1.49^{d}	47.97 ± 0.29^{d}
Trametes versicolor	20.94 ± 1.51^{bc}	22.90 ± 1.05^{b}	30.95 ± 2.52^{bc}	33.12 ± 1.61^{c}	36.63 ± 1.94^{c}	35.86 ± 1.79^{c}
Trametes gibbosa	18.66 ± 2.80^{b}	23.56 ± 1.47^{b}	35.13 ± 3.06^{bcd}	28.14 ± 2.90^{b}	33.82 ± 3.07^{c}	32.63 ± 1.44^{c}
Chlorophyllum molybdite	7.63 ± 1.67^{a}	5.84 ± 1.21^{a}	5.25 ± 1.67^{a}	1.06 ± 1.69^{a}	5.55 ± 0.50^{a}	8.36 ± 0.61^{a}
Auricularia auricular judae	23.03 ± 0.94^{bc}	22.36 ± 3.46^{b}	24.64 ± 1.36^{b}	26.56 ± 1.82^{b}	26.66 ± 2.55^{b}	27.25 ± 2.53^{b}
Ganoderma spp	32.08 ± 6.39^{c}	50.03 ± 0.63^{e}	56.01 ± 13.91^{e}	72.41 ± 1.84^{f}	74.81 ± 0.27^{f}	94.37 ± 0.92^{g}
GA	27.72 ± 0.75^{cd}	38.44 ± 1.63^{bc}	45.69 ± 1.36^{de}	50.30 ± 0.89^{e}	67.84 ± 0.54^{e}	84.01 ± 0.80^{f}

All results have been expressed as means ± standard deviation (n = 3). Mean values followed by different letters in the same column are significantly different ($p \leq 0.005$)

4.4 Summary

Mycochemical screening tests done on the methanol extracts of the mushroom samples revealed the presence alkaloids, polyphenol, terpenes, saponins and reducing sugars in the entire samples. Anthocyanin, tannins, flavonoids, coumarins and glycosides were found to be absent in all samples. Steroids were also present for all the samples except *Chlorophyllum molybdite* and *Auricularia auricular judae*. The results for TPC and TFC showed very good efficient antioxidative activity of *Trametes gibbosa* and *Ganoderma spp*. they also showed good radical scavenging potential which is indicative of the antioxidant activity. Extracts of *Chlorophyllum molybdite* and *Auricularia auricular judae* had the least TFC and TPC.

CHAPTER FIVE

SUMMARY, CONCLUSION AND RECOMMENDATION

5.0 Introduction

This chapter presents the summary of the whole work, analysis and conclusion derived from the research study as well as recommendations from the studies.

5.1 Summary

Many mushrooms in the world over are known for their antioxidant properties due to the presence of secondary metabolites in them. Antioxidants are substances with the ability to defeat free radicals or slow down oxidative stress. From the preliminary screening carried out on the *Tyromyces chioneus, Polyporus alveolaris, Trametes hirsute, Trametes versicolor, Trametes gibbosa, Ganoderma spp., Auricularia auricular judae, and Chlorophyllum molybdites* mushroom samples, alkaloids, polyphenols, terpenes, saponins and reducing sugars were found to be present in all the samples. However, phlobatannins and steroids for all the samples analysed tested negative except *Trametes hirsute* and *polyporus alveolaris* which tested positive. The presence of flavonoid, anthocyanin, tannins, coumarins and glycoside were not determined in all the samples (*Tyromyces chioneus, Polyporus alveolaris, Trametes hirsute, Trametes versicolor, Trametes gibbosa, Ganoderma spp., Auricularia auricular judae, and Chlorophyllum molybdites*) analysed.

Antioxidant capacity was also carried out on the various samples. From the analysed results it was observed that total phenolic content for various samples were significantly different ($p \leq 0.005$) from each other. Similarly, the same observation was observed for the total antioxidant content and total flavonoid

content. DPPH scavenging was also carried on the various samples. From the results analysed, the % DPPH scavenging activity of the various samples were seen to increase with increasing concentration of sample extracts which were similar for all the samples except *Chlorophyllum molybdite* which showed variations in % DPPH scavenging.

5.2 Conclusions

Mushrooms are known to contain several metabolites which possess antibacterial, anti-fungal, anti-inflammary as well as antiviral activities. The different types of secondary metabolites mostly found in mushrooms include alkaloid, triterpenoids, steroids, polyphenol, flavonoid etc. The mycochemical screening test performed on the methanol crude extract of the various mushroom samples showed the presence of alkaloids, terpenes, polyphenols, saponins and reducing sugars. Phlobatannins and steroids were found only in *Trametes hirsute* and *polyporus alveolaris.* Flavonoid, anthocyanins, tannins, coumarins and glycoside were also tested for in the extract of all the samples but the result did not detect their presence.

All the mushroom samples showed variation of total phenolic content, total antioxidant capacity, total flavonoid and radical scavenging activity. For the total phenolic content, *Trametes gibbosa* had the highest value of 8.99 GAE with *Auricularia auricular judae* giving the lowest value of 0.25 GAE. *Trametes hirsute* had the highest total antioxidant capacity with *Tyromyces chioneus* with the lowest antioxidant capacity of 29.91 AAE and 6.12 AAE respectively. From the preliminary screening, it was deduced that flavonoids were not present in all the samples, however, from the quantitative analysis of the total flavonoid content, *Ganoderma spp.* had the highest content with

Chlorophyllum molybdite showing a negative value of 5052 QE and -348 QE respectively. At concentration of 500 µg/mL the strongest radical scavenging activity was demonstrated by *Ganoderma spp.* with *Chlorophyllum molybdite* showing the lowest radical scavenging activity of 94.37% and 8.36% respectively.

From the analysis carried out, it is clear that *Polyporus alveolaris Trametes gibbosa*, *Trametes hirsute* and *Ganoderma spp* showed very promising antioxidant capacity and radical scavenging activity.

5.3 Recommendations

It is recommended that further work should be carried out to isolate compounds from the various mushroom samples since much work has not been done in the country. Also, an in vitro study on the various mushroom samples be carried out to find their antibacterial, anti-inflammatory etc. properties. It is also recommended that the Department of Chemistry, UCC should collaborate with other institutions to enable it set up a very good organic research laboratory with modern analytical instruments. Furthermore, the department should have sponsored research work which will address the needs of the nation for future Mphil students.

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APPENDIX

SOLUTION PREPARATION

Dilute ammonia solution

A 42.5ml of concentrated ammonia solution taken into a 100ml volumetric flask and this is topped to the mark with distilled water.

2N Hydrochloric acid

16.6ml of concentrated hydrochloric acid taken into a 100ml volumetric flask and this is topped to the mark with distilled water.

10% Ferric chloride

10g of ferric chloride is dissolved in 100ml of distilled water

10% trichloroacetic acid

10ml of TCA taken into a 100ml volumetric flask and this is topped to the mark with distilled water.

0.6M sulphuric acid

3.14ml dissolved in 100ml distilled water

28mM Sodium phosphate

0.397g in 100ml distilled water

4mM Ammonium molybdate

0.494g in 100ml distilled water

0.5mM Ascorbic acid

0.022g of ascorbic acid in 250ml distilled water

2% Alummium chloride

2g of alummium chloride dissolved in 100ml of distilled water

7.5% Sodium carbonate

7.5g of Na_2CO_3 dissolved in 100ml of distilled water

Gallic acid

0.1g of gallic acid dissolved in 100ml methanol

10% Follin Cioalteu (F.C)

10ml of F.C taken into a 100ml volumetric flask and this is topped to the mark with distilled water

Quercetin

0.01g of quercetin was dissolved in 100ml of methanol