PLEUROTUS TUBER-REGIUM (FR.) SING TEA FORMULATIONS WITH SOME LOCAL HERBS SUCH AS MORINGA, BITTER LEAF AND SCENT LEAF

BY

Israel Obokhai EGERUAN

LSC1708985

DEPARTMENT OF PLANT BIOLOGY AND BIOTECHNOLOGY

FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

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A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF PLANT BIOLOGY AND BIOTECHNOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE (B.Sc.) DEGREE HONOURS in PLANT BIOLOGY AND BIOTECHNOLOGY

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CERTIFICATION

We certify that this research work was carried out by Israel Obokhai Egeruan of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria.

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Prof. J. A. Okhuoya Date

Project Supervisor

_____ _____

Prof. E. O. Akpaja Date

Head of Department

Plant Biology and Biotechnology

DEDICATION

This project is dedicated to God Almighty, the Father and Lord of Jesus Christ, the Saviour of the world.

ACKNOWLEDGEMENT

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I want to appreciate my father, my mother and my siblings, for their love, prayers and financial and moral support. I love you all.

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God bless you all!

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ABSTRACT

The mushroom Pleurotus tuber-regium formed tea formulation with some local herbs such as Moringa, Bitter leaf and Scent leaf. P. tuber-regium formed three (3) different combinations with each of the three (3) herbs. That is, the tea formulations were of nine (9) different ratio combinations, which are: MU/MO 5:45, MU/MO 10:40, MU/MO 20:30; MU/SL 5:45, MU/SL 10:40, MU/SL 10:40; MU/BL 5:45, MU/BL 10:40, MU/BL 20:30. The combinations were each made into infusions by adding water into a cup with the tea mixture. The infusions were given to fifty (50) participants to consume at different time and location. After consuming the tea, the participants then gave their responses based on two (2) categories, taste and acceptability. Generally, the responses from the participants were positive and encouraging, and a few not so, which can be tackled by organizing programs/seminars for creating public awareness about the health benefits of mushrooms, through individual and government involvement in mushroom commercialization, through the development of adaptable mushroom cultivation, among others. It is worthy of note that none of the participants had complaints of a significant side effects like stomach upset, dizziness and allergies. This shows that this tea formulation is safe for consumption and that it fits the description of an ideal component of a healthy dietary habit that can be used to promote health.

CHAPTER ONE

1.0 INTRODUCTION

1.1 BRIEF HISTORY

Before the origin of man, mushroom lamellae have been observed to leave imprints on wood. Galen in 130-200AD recommended only picking mushrooms from fields, grasslands, and meadows, whereas Hippocrates in 470-400BC wrote on the medicinal benefits of mushrooms. Mushroom consumption most likely started during the hunting and gathering era of prehistory. Unlike plants, it could not be cultivated initially and was only harvested for a long period before cultivation began. Even today, only a small number of mushroom species can be grown.

Mushrooms have played a role in human culture since time immemorial. They were thought to have a unique and supernatural origin. Egyptians believed mushrooms were "plants of immortality" 4,600 years ago, and their Pharaohs commanded that only they could eat mushrooms. The Ancient Greeks believed that mushrooms gave warriors strength in war. The Romans considered them "food of the gods" and their sale was governed by law throughout the Roman Empire. They were revered as an "elixir of life" by the Chinese and Japanese people. Many myths and misconceptions about mushrooms still exist today.

For thousands of years, the Chinese and the Japanese have used mushrooms as medicines. Even before their production began in America and Europe, the former have been cultivating them for a very long time. It is unknown when cultivation first began in Asia, particular China and Japan. However, the cultivation of the fungus *Agaricus bisporus* was first documented in the Western world in France in 1630. The mushroom also known as white button mushroom, was grown outdoors on ridges of compost gotten from horse manure. The most widely grown mushroom in the world today is the white button mushroom.

1.2 DEFINITION

The term "mushroom" can mean different things to different individuals in different places, depending on the culture or perspective of the individual. Like in the western part of the world, some people only refer to mushrooms as the "button" or "white" mushroom, scientifically known as *Agaricus bisporus,* while considering all other cultivated species to be "exotic." But according to Chang and Miles (1992), mushroom is “a macro fungus with a distinctive fruiting body which can be either epigeous or hypogenous and large enough to be seen with the naked eye and to be picked by hand. Thus, mushrooms need not be basidiomycetes, nor aerial, nor fleshy, nor edible. Mushrooms can be ascomycetes, grow underground, have a non-fleshy texture and need not be edible.”

The word "mushroom" is most often applied to those fungi (Basidiomycetes, Agaricomycetes) that possess a stem, a cap (pileus) and gills (lamellae) on the underside of the cap. The gills produce microscopic spores that help for their dispersal across the surface of the ground or on its occupant surface (Chang and Miles, 2004). But some species of mushroom do not have gills e.g., *Ganoderma sp*. Unlike green plants, mushrooms are heterotrophs, because they lack chlorophyll, and so cannot undergo photosynthesis to produce nutrients, rather they derive their nutrients from outer sources. The name "mushroom" is also used to designate a wide range of gilled or gillless fungus, with or without stems, and is thus used to describe the fleshy fruiting bodies of some Ascomycota. Mushrooms have been known as a non-timber forest products and are commonly found growing as a saprophyte on soil, open fields, wood and roadside areas.

Mushroom can be divided roughly into four groups, viz.:

i. **Edible mushrooms**: Those that are fleshy and edible, e.g., *Pleurotus sp*., *Agaricus sp*., *Volvariella sp.*, etc

ii. **Medicinal mushrooms**: Those considered to have medicinal applications, e.g., *Ganoderma lucidum*, *Cordyceps sinensis*, etc.

iii. **Poisonous mushrooms**: Those known or suspected to be poisonous, e.g., *Amanita phalloides.*

iv. **Miscellaneous mushrooms**: Also called "other mushrooms". They include a large number of mushrooms whose properties are not well-defined.

1.3 BIODIVERSITY

Hawksworth (2001) calculated that 140,000 of the 1.5 million estimated fungal species produced fruiting bodies large enough and with the right structure to be classified as macrofungi, which, by Chang and Miles' definition, can be referred to as mushrooms (1992). About 70,000 of these have been described, of which 14,000 have been specifically identified as mushrooms. Out of the 14,000 species, 5,000 are thought to have varied degrees of edible qualities, and more than 3,000 species from 30 genera are considered to be the finest edible mushrooms. 100 species are grown for research purposes, 60 are grown economically, and 30 for commercially. 15 species are produced industrially, with 7 being the most widely produced in various regions of the world (Jibrin *et al.*, 2007). They are:

i. *Agaricus bisporus* (White button mushroom)

ii. *Lentinula edodes* (Shiitake mushroom)

iii. *Pleurotus spp*. (Oyster mushroom)

iv. *Flammulina velutipes* (Golden needle mushroom)

v. *Volvariella volvacea* (Paddy straw mushroom)

vi. *Aricularia auricular* (Jewish ear mushroom)

vii. *Calocybe indica* (Milky mushroom)

Additionally, it has been estimated that 1,800 species have therapeutic potential, of which 400 species have known medicinal properties. 30 species, which is a relatively small number, are regarded as harmful (Jib\rin *et al.,* 2007).

1.4 MUSHROOM CULTIVATION

The necessity to plant mushrooms—edible and medicinal, in Nigeria cannot be overstated given their potential to increase agricultural production and serve as a significant source of protein (Onouha, 2007). By producing a quick-yielding, nutrient-rich food supply and a steady source of income, mushroom farming can help people live more comfortably and minimize their vulnerability to poverty. Activities related to mushroom production can significantly help the local economy by enhancing agriculture, food security, nutrition, and medicine; providing new jobs and income through local, regional, and international trade; and creating business opportunities for processing companies (Marshall and Nair, 2009).

Mushroom farming is a very effective way to manage and dispose waste in an environmentally friendly manner (Chinda and Chinda, 2007). According to Pathak *et al*., (2009), mushroom cultivation is the second most commercial microbial technology after yeast. It is now recognized as a food and is grown commercially in many countries, including Nigeria (Onouha, 2007). Nowadays, mushroom production methods are becoming more and more popular worldwide because they are easy to cultivate and don't require any technical expertise. Mushrooms can be grown using a variety of agricultural wastes, which act as a substrate for their growth. According to reports, mushrooms can be cultivated on several lignocellulose wastes, including sawdust, banana leaves, cereal straw, paper scraps, and poultry droppings (Onouha, 2007), while additional nutrients sources such as wheat bran, oil palm fibre, local cassava starch, Bambara groundnut flour, etc., can be used to supplement these substrates. There are numerous tasks

involved in mushroom production, all of which must be done meticulously. The cultivation of a particular mushroom species will affect the conditions for substrate preparation, inoculation, incubation, and production.

1.5 NUTRITIONAL VALUE

Mushroom is regarded as a complete health food that is suited for all age groups, from children to elderly people, as it contains all the nutrient elements a person need in the right amount. The species, variety, developmental stage, and environmental conditions are only a few of the many variables that influence the nutritional content of mushrooms. Mushrooms are widely distributed in nature, and are common and vital foods because they have minimal calories, carbohydrate, fat, and sodium content as well as being cholesterol-free. They supply vital nutrients such as proteins, fibre, riboflavin, selenium, potassium, niacin, and vitamin D. (Maria *et al*., 2015).

• **Protein**

Mushrooms typically provide 19–35% protein by dry weight. The type of mushroom, size of the pileus, time of harvest, and composition of the substrate all affect the protein content of a mushroom. Edible mushrooms contain proteins that are rich in theronine and valine but very low in sulphur-containing amino acids (ethionine and cysteine). All of the essential amino acids needed by an adult are found in mushrooms.

• **Carbohydrate**

Mushrooms contain 50–65% carbohydrates by dry weight, however the majority is made up of nutritive and fermentable fibres; they do not contain starch (have insignificant proportion of sugars). In mushrooms, there is 11% free sugar (mono and disaccharides), of which mannitol makes up 80%. The diet of diabetic patients contains mannitol as a sweetener.

• **Fat**

Mushrooms have an extremely low fat content that is primarily made up of unsaturated fatty acids. Linoleic acid, an important fatty acid, is abundant in mushrooms. Another benefit of mushrooms is their low lipid content, which is completely free of cholesterol, and their higher percentage of polyunsaturated fatty acids. Ergosterol, which is found in mushroom, is a precursor for the body's production of vitamin D.

• **Vitamins**

Mushrooms are one of the richest sources of vitamins, particularly vitamin B (Folic acid, Thiamine, Riboflavin and Niacin). Additionally, it has very little vitamin C and is deficient in A, D, and E.

• **Minerals**

The mineral proportions of mushrooms vary depending on the species, age, fruiting body diameter, and substratum type. Magnesium (which makes up 56-70% of the total ash content), calcium, sodium, potassium and phosphorus are the main mineral components found in mushrooms. Minor component parts of mushrooms include iron, zinc, copper, cadmium and molybdenum. It has been discovered that mushrooms contain heavy metals such as copper, lead, mercury, silver, nickel, arsenic, chromium, and cadmium. Wild mushrooms have been found to have a higher mineral content than cultivated ones. Most importantly, mushrooms contain a variety of compounds that aid in growth, including enzymes, alkaloids, sterols, antioxidants, and other unidentified organic complexes.

1.6 MEDICINAL VALUE

Edible mushrooms are important for their medicinal as well as nutritional properties, as they have beneficial effects for health and treatment of some diseases and ailments. They have been reported to have a lot of nutraceutical properties, such as prevention/treatment of cardiovascular diseases, diabetes, cancer, asthma, among others. They have been found to be effective against stress, insomnia, and high cholesterol levels. The mushroom components that gives mushrooms their nutraceutical properties are proteins, lipids, vitamins, fibres, phenolic compounds and minerals. These biomolecules have captured the interest of researchers all over the world due to their proven health attributes. Mushrooms contain both primary and secondary metabolites. The primary metabolites provide energy while the secondary metabolite exhibits therapeutic effects, which constitutes the plenitude of nutraceutical properties of mushrooms, including: immunomodulatory, antiviral, antibacterial, antifungal, antioxidant, anti-inflammatory, antitumor, anticancer, anti-HIV, antidiabetic, anticholesterolic and antiarthritic activities (Singh *et al.,* 2021).

The hypocholesterolemic action of edible mushrooms has been observed in some mushrooms species from Basidiomycota taxon: *Lentinus edodes* (Shitake mushroom), *Auricularria polytricha* (Mun mushroom), *Flammulina velutipes* (Golden needle mushroom) and *Agaricus bisporus* (White button mushroom) (Fan *et al.,* 1989, Mau *et al*., 2001, Hossain *et al.,* 2003, Bernaz *et al*., 2006, Regula *et al.,* 2007, Du *et al.,* 2011, and Reczynki *et al.,* 2013). Some mushrooms have shown hypotensive effect when blood pressure is high. On the other hand, the presence of antioxidant and anti-inflammatory compounds in mushrooms may be clinically relevant in the management of heart and circulation health complications. There are some mushroom components such as proteins, lipids, vitamins, fibres, phenolic compounds and minerals, involved in the present or treatment of cardiovascular diseases. They are also used to reduce the likelihood of cancer invasion and metastasis due to antihumoral attributes (Maria *et al.,* 2015).

1.7 THE KING TUBER MUSHROOM *(Pleurotus tuber-regium)*

• Taxonomy/Scientific classification

Kingdom: Fungi

Division: Basidiomycota

Class: Agaricomycetes

Order: Agaricales

Family: Pleurotaceae

Genus: Pleurotus

Species: *P. tuber-regium*

• Description

Pleurotus tuber-regium, the king tuber mushroom, is an edible mushroom and a tuberous wild specie of white rot Basidiomycota which produces fruiting bodies from a unique globose sclerotium that resembles a giant truffle (Nwokolo, 1987). It occurs in both tropical and subtropical region of the world (Okhouya and Okogbo, 1991), including Africa, Asia, and Australasia (Osu, 1977). *Pleurotus* species are marked by a white spot print attached to the decurrent gills, often accompanied by an eccentric (off-centre) stipe or no stipe at all. Naturally, they are saprotrophs found growing typically on dead standing trees or fallen logs made of wood, including *Daniellia* trees in Africa (Okhuoya and Okogbo, 1990). In addition to being saprotrophic, P. *tuber-regium* is also nematophagous, capturing nematodes by paralyzing them with a toxin. (Hibbett and Thorn, 1994). The king tuber mushroom differs from other *Pleurotus* species in that it consumes and infects the dead decaying wood, where it produces a true sclerotium or storage tuber usually

buried within the wood tissues, or between the wood and bark, or in the underlying soil (Iwagwu and Onyekweli, 2002). Also, it does not have a pleurotoid habit and produces additional nutritive foods (Ishikhuemhen and Nerud, 1999).

The sclerotium is an ovoid-shaped structure that can be fairly enormous, with a weight of about 5kg and a diameter up to 30 cm, or even more (Iwagwu and Onyekweli, 2002). It is dark brown on the outside and white on the inside. Food reserves, in the form of a tightly packed mass of hardened fungal mycelium, are stored in the sclerotia of *Pleurotus tuber-regium*, which helps the fungi in withstanding harsh environmental conditions (Thorn *et al*., 2000). Locals in Nigeria collect the sclerotium of the fungus from the forest, bury it in warm, humid soil to encourage the formation of fruiting bodies, which appear in a relatively short amount of time (Okhuoya and Efugo, 1993). With the rapid erosion of their natural environment brought about by deforestation, this ensures a ready and steady supply of fresh mushroom. The sclerotium is called "usu, erousu or ikeusu" in Igbo "katala" in Hausa, "amu" in Igala, "umoho" in Igede, all in Nigeria (Catherine Ikewuchi and Jude Ikewuchi, 2008).

• Economic Importance

P. *tuber-regium* is utilized both as food and medicine in Nigeria. It has a history of economic importance in Africa as food and as a medicinal mushroom (Oso, 1977) and (Isikhuemhen and Okhuoya, 1996). The t\uber is very nutritious (particularly rich in proteins), expensive and regarded as a delicacy (Okhuoya and O\kogbo, 1990). The hard sclerotium is skinned and pulverized while the fruiting body is diced and uti\lized in vegetable soup (Oso, 1997). So the sclerotium and the fruiting bodies are both edible. When making sauces and soups traditionally, the tuberous sclerotium can be used to partially replace groundnut cake *(Arachis hypogea)* and melon seed *(Citrullus lonatus).* Since the sclerotium is heavy in proteins, the nutritional value of mushroom can be comparable to that of meat, milk, and eggs (Oei, 2003). The fungus

also contains vitamins and an abundance of essential amino acids (Sanchez, 2004). P. *tuber-regium* has a long history in some Ghanaian communities as traditional medicine used by herbalists to treat ailments like asthma, high pressure and underweight in children among other conditions. (Dzomeku, 2009).

Although the cultivation of P. *tuber-regium* for industrial purposes is not yet prevalent, research have shown that it can grow on organic wastes like sawdust, corn, cardboard, and other materials (Okhuoya and Okogbo, 1990; Isikhuemhen and Okhuoya, 1996 and Isikhuemhen and LeBauer, 2004). Mycelial growth takes place between 15 °C and 40 °C, with an maximum growth rate at 35 °C (Oso, 1977). Fresh mushroom species and their polysaccharide extract have been proven to have antihyperglyceric and antioxidant properties, and it has also been established that these polysaccharides act as effective antidiabetic and antioxidant compounds and promote immunity (Wong *et al*., 2011). Mushrooms have generally been proven to be useful in lowering cholesterol as well as in fighting cancer, diabetes, asthma, stress, and insomnia. Yongabi (2004) confirmed that the sclerotia of P. *tuber-regium* is a good coagulant and disinfectant which can be utilized to purify natural and waste water. It was shown that polysaccharides of P. *tuber-regium* were effective at delaying the development of diabetes and its related problems in rats with insulin inhibition (Huang, 2012). *Pleurotus tuber-regium* can degrade polyethylene film (Nwogu, 2012).

1.8 HERBAL TEA

Aside from water, tea is one of the most popular drinks worldwide. Presently, almost 80% of the world's population relies on indigenous or traditional medicines for their basic health needs, and the majority of this therapy uses plant extracts, frequently in the form of aqueous solutions (Zhang, 2002). In essence,

herbal tea is a blend of herbs brewed from various plants' leaves, seeds, or roots. Many people believe that since herbal tea looks like tea and is made similarly to tea, it should be classified as a tea, but actually, it isn't a tea at all. This is because, they are not derived from the *Camellia sinensis* plant, which is the source of all teas (Kumar, 2005). The term "tisanes" is more appropriate for herbal teas, which are actually blends of numerous components (Ravikumar, 2014).

Tisanes are prepared by combining dried leaves, seeds, grasses, nuts, barks, fruits, flowers, or other botanical components, that give them their ingredients and offer the advantages of herbal teas. Green tea, black tea, cinnamon tea, chamomile tea, peppermint tea, ginger tea, ginseng tea, oolong tea, and others, are some of the more well-known herbal beverages. They are locally gathered and traditionally prepared at home or at herbalists' shops for immediate use, without going through any additional manufacturing processes. Nevertheless, there is an increasing trend toward their conversion into industrial products, which are then sold in markets or in conventional herbal stores, either in the form of labeled bags containing the ingredient mixture or in individual bags in which the ingredients have been ground to powder. Due to recent advancements on a global scale, there are now more products in the market than ever before, many of which are only known from the perspective of traditional medicine (Kumar *et al.*, 2005).

In assessing the modulatory effects of herbal teas, the interaction with the intestinal environment and bioavailability of major components are crucial. Herbal teas have positive impacts on human health both directly and indirectly by altering the gut microbiota, though this has not yet been fully understood. The bioavailability of essential components varies depending on the type of tea—hot or cold, or how frequently it is consumed—both in terms of volume or habit (Naithani *et al*., 2006).

In the last decade, numerous studies have detailed the health benefits of tea drinking, including its antioxidative, anti-inflammatory, antibacterial, antiviral, antiarthritic, and neuroprotective qualities. Medicinal herbal teas have been used to treat diseases for ages, because of their long history of use in the treatment of certain health problems like diabetes, stomach ailments, liver disease, heart disorders, etc. Additionally, it is now well-known that the active ingredients of tea can also help in lowering cholesterol levels and in preventing cancer and cardiovascular disease. Due to its multiple health benefits as well as their popularity as a daily routine, herbal teas rank among the most popular drinks in the world (Aoshima *et al.*, 2007).

Several herbal mixtures have been used for ages and have been proven to promote health. Although very complicated, the study of these mixtures holds great potential. Therefore, understanding the composition of these conventional blends will help create new blends that can enhance a population's health. (Ndhlala *et al.,* 2011; Barros *et al.*, 2012; Obón *et al.*, 2014). Most herbal teas may have one main herbal component or a combination of herbal compounds, each of which is designed to achieve a certain goal, such as relaxation, rejuvenation, or relief from a particular condition, among others. However, some general benefits can be gotten from most herbal teas, they are:

- achieving a more calm and relaxed state of mind
- supporting heart health
- aiding with stomach and digestive problems
- providing cleansing properties for the body
- promoting energy and wellness
- nourishing the nervous system
- strengthening the immune system
- providing antioxidants to the body

- boosting energy levels and invigorating the body
- relieving stress
- helping to avoid colds
- \stimulating the internal organs
- promoting a good night's sleep
- caffeine free and tastes great.

(Ravikumar, 2014).

1.9 AIM AND OBJECTIVES

The aim of the study is to prepare *Pleurotus tuber-regium* tea formulations with some local herbs such as *Moringa oleifera* (Moringa), *Vernonia amygdalina* (Bitter leaf) and *Ocimum gratissimum* (African basil or Scent leaf) in combinations 5:45, 10:40 and 20:30 for each herbs.

Specific objectives are:

1. to determine the taste and acceptability of each combination
2. to analyze for antioxidants.

\

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 EXPERIMENT ONE

• Study Area

The study was done at Mycofarms and Allied Synergy Ltd (simply called Mycofarms) at Isihor in Benin City, Edo State.

• Materials Used

Sclerotia of *Pleurotus tuber-regium*, *Moringa oleifera* (Moringa), *Vernonia amygdalina* (Bitter leaf) and *Ocimum gratissimum* (African basil or Scent leaf), sandy loam soil, shovel, wheelbarrow, blender/blending machine, aluminum tray, knife, plastic plate, bowl and bucket, measuring scale.

• Collection of Materials

Three sclerotia were purchased from Ekiuwa market in Benin City. The three local herbs, *Moringa oleifera* (Moringa), *Vernonia amygdalina* (Bitter leaf) and *Ocimum gratissimum* (African basil or Scent leaf) were also purchased, but at Uselu market in Benin. City. Trips of sandy loam soil was collected from Mycofarms.

• Procedures

Firstly, the leaves of the three herbs are separated from the sticks. Then, the leaves are placed in separate metal trays and taken outdoors or in a room void of moist air. If placed outdoors, it must be observed and removed when it rains. The leaves are left outside until they dry. Leaves are dried if they crack when rubbed with the hands. The dried leaves are ground separately into powdered form with a blender. The herbs powder are then kept separately in polythene bag or inside airtight container which is then placed in a cool and dry room. The herbs powder are collected when needed, which is usually when the mushroom powder has been gotten and the tea mixture is to be made.

The following are the step-by-step procedure of the tea formulation:

a. **Soaking of sclerotia**: The three sclerotia were soaked separately in warm water inside three plastic buckets. The buckets were covered with flat metal lids and a big stone placed over them. This was to ensure the lids do not fall off and the covering remain intact. The soaking was done so that the sclerotia would soften thereby reducing the time it will take for pinheads to sprout after planting. The sclerotia were left in their respective bowls for a week.

b. **Burying of sclerotia:** A portion of a fairly large land at Mycofarms used for farming, was chosen. Six spots were marked and clayey loam soils (a mixture of clay and loamy soil) were made out of them. The clay soil was bought. The sclerotia were then cut into equal halves (6 halves). Six shallow holes were dug from those spots, and each of the six sclerotia were placed separately in them. The holes were covered with the clayey loam soil, to form a heap. So six heaps of soil were made in the process. There was space of few centimetres between each heap.

c. **Watering of soil heaps:** The soil heaps were drenched immediately with buckets of water. Watering was done continuously every day, early in the morning and late in the evening. Since the experiment was carried out at the start of rainy season, watering was done only in days when there was very little or no rainfall. This is because the soil heaps could become waterlogged as a result of excess water.

d. **Harvesting of mushroom:** Pinheads started appearing 1 week after planting the sclerotia. Fully mature mushroom with fruiting body was seen 1 week after pinheads appeared. Meaning, it took about 7 days for germination and another 7 days for maturation to occur. So the fruiting body of *Pleurotus tuber-regium* is seen 2 weeks after planting sclerotium. The mushroom is harvested by using a sharp object e.g., razor blade, knife, to cut gently from the point where the stipe just extends from the soil heap.

e. **Washing of mushroom:** The mushrooms are first washed with clean water and then rinsed. This is to remove dirt stains on the mushroom. The whole fruiting body including the stipe is shredded into bits.

f. **Drying of mushroom:** The shredded mushrooms are then dried in a food dehydrator, where they are kept for 1 to 2 hours. Care must be taken not to leave the mushrooms beyond the duration to avoid them getting burnt. Normally the duration depends on the number of mushroom and the thickness of the fruiting body. Using a dehydrator is more effective than drying under the sun, because the mushroom loses more of its nutrients when under the former than the latter. But generally, drying normally reduces the nutrients in food substance.

g. Blending of mushroom: The dried mushrooms are ground into powder with a blender to get mushroom powder. The mushroom powder is then stored in the same condition as the herbs powder until when needed.

h. Mixing of mushroom and herbs powder: The herbs and mushroom powder are mixed to form tea formulation by ratios. The ratios are the masses (in grams) of the mushroom and herbs powder weighed with a measuring scale.

The tea mixture is then stored in cool dry room. For commercial purposes, it could be packaged into small tea bags called sachet, or into sealed containers, and sold to the public.

Plate 1: Picture showing sclerotium

Plate 2: Soaking of sclerotium

Plate 3: Making of soil heaps for the cultivation of P. *tuber-regium*

Plate 4: A mature P. *tuber-regium* mushroom

Plate 5: A harvested P. *tuber-regium* mushroom

Plate 6: Shredded P. *tuber-regium* mushroom after washing

Plate 7: Mushroom-moringa tea formulation for the three ratio combination

Plate 8: Mushroom-bitter leaf tea formulation for the three ratio combinations

Plate 9: Mushroom-scent leaf formulation for the three ratio combinations

2.2 EXPERIMENT TWO

• Study Area

The study was carried out at the laboratory unit of Mycofarms and Allied Synergy Ltd at Isihor in Benin City, Edo State.

• Materials Used

Aluminum foil, masking tape, filter paper, petri-dish, test-tube, beaker, measuring scale, cuvette, test-tube and test-tube rack, micropipette and tip, measuring cylinder, test-tube bath, centrifuge machine, spectrophotometer.

• **Reagents**

Ascorbic acid, sulphuric acid, ammonium molybdate, disodium hydrogen peroxide, when distilled water**.**

• Collection of materials

All the materials needed to carry out this experiment, are provided for at Mycofarms and Allied Synergy Ltd.

• Procedures

Total Antioxidant Capacity

Total antioxidant activity was estimated by phosphomolybdenum assay (Prieto *et al.,* 1999). The method is based on the reduction of molybdenum (IV) to molybdenum (V) by the extract and the subsequent formation of a green phosphate/molybdenum (V) complex at acid pH.

The molybdate reagent was prepared by mixing equal volumes of 0.6M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate. One milliliter of the extracts (1mg/ml) was added to 3ml of Molybdate reagent solution. These tubes were kept incubated at 95°C for 90 minutes. After incubation, these tubes were normalized to room temperature for 20-30 minutes and the absorbance of the reaction mixture was measured at 695nm. Ascorbic acid was used as the standard.

Table 1: Concentration of total antioxidant capacity of different mushroom-herb combinations

Label	Ratio	Concentration (ug/ml)
MU/MO	5:45	32.11
MU/MO	10:40	31.86
MU/MO	20:30	38.50
MU/SL	5:45	40.47
MU/SL	10:40	37.42

MU/SL	20:30	33.72
MU/BL	5:45	34.14
MU/BL	10:40	42.19
MU/BL	20:30	34.41

Where,

MU/MO: Mushroom-moringa combination

MU/SL: Mushroom-scent leaf combination

MU/BL: Mushroom-bitter leaf combination

CHAPTER THREE

3.0 RESULTS

• **Sensory Evaluation**: Two stages of sensory evaluation were conducted: a taste test and an acceptability test. 50 participants took part in both phases, which were carried out on the nine (9) combinations of the mushroom-herb formulations. These participants were picked from various locations and the evaluation was done at different times. The participants varied in terms of gender, age, and educational statuses. The selection was based on each participant's desire and commitment to take part in the sensory evaluation. They received background information about the raw materials used to make the infusions.

Warm water was added to each combination in a plastic cup, and the mixture was thoroughly stirred to ensure a uniform infusion. No other ingredient, like sugar, was added. After every taste, the participants were asked to properly rinse their mouths with warm water and wait 60 seconds before tasting the next combination in order to reduce any potential carry-over effects. In order to fully savour the entire sensory nature of the tea, the participants were instructed to leave the infusions in their mouths for 5 seconds before swallowing.

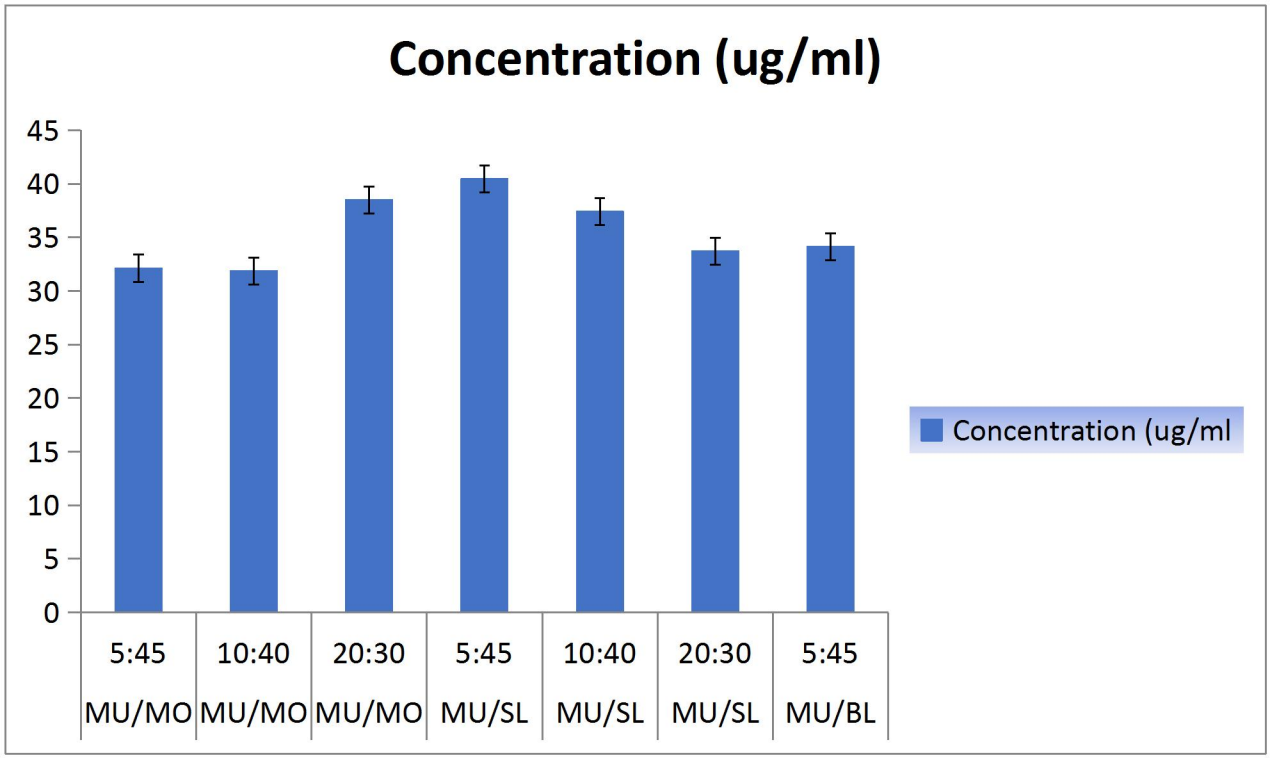

Figure 1: A chart showing concentrations of the total antioxidant capacity of different mushroom-herb tea combinations

Table 2: Data for the number of each gender

Gender	Number
Female	30
Male	20

Table 3: Data for the level of education of participants (using O' level as standard)

Level of educatio	Percentage (%
Educated	32
Non-educated	18

Table 4: Data for the age ranges of participants

Age ranges of particpar	Percentages (%
0-10 years	9
11-20 years	16
21-30 years	14
31-40 years	6
41-50 years	3
51 and above	2

Table 5: Data for taste test

No. of participants	Taste scale
3	1 (bitter)
6	2 (sour)
20	3 (insipid)
13	4 (bittersweet)
8	5 (sweet)

Table 6: Data for acceptability test

No. of participants	Acceptability scale
1	1 (dislike very much)
4	2 (dislike)
21	3 (okay)
15	4 (like)
9	5 (like very much)

CHAPTER FOUR

4.0 DISCUSSION

The result obtained from the total antioxidant capacity experiment showed that the antioxidant activity of this mushroom-herb tea formulation is quite high, as the average total antioxidant capacity (ATAC) of MU/MO is 34.16ug/ml, ATAC of MU/SL is 37.20ug/ml and ATAC of MU/BL is 36.91ug/ml. Meaning that this tea formulation seems to fit the description of an ideal component of a healthy dietary habit that can be used to promote health. The tea also appears to be safe and without significant side effects as no participant made any complaints of such like stomach upset, dizziness and allergies. Although, there are no clear-cut evidence for the effects of mushroom tea consumption, it is still believed that an overdose may cause slight and rare medical problems. The latest scientific insights into the beneficial elements provided mushroom tea, that was made from recent research, dispelled the misconceptions that only herbal tea, most especially green tea, provide health benefits (Trevisanato and Kim, 2000).

The results gotten in the total antioxidant capacity experiment agrees with the fact that mushroom tea also provides biologically active ingredients (e.g., flavonoids, vitamins, fluoride etc). It is important to note that flavonoids are antioxidants, a broad class of molecules in our body that neutralize free radicals, which are potent oxidizing compounds that damage the cells, while be minerals such as fluoride help fight dental cavities. The mechanism of action of flavonoids on the digestive system is poorly understood and maybe related to the absorption of flavonoids into the mucosal lining of the gastrointestinal tract (Trevisanato and Kim, 2000). The sclerotium produced by *Pleurotus tuber-regium* has been reported to constitute about 64.31% WW and 72.21% WW protein, 20.00% WW and 22.15% DW carbohydrate, 2.20% WW and 2.44

DW ash and 2.89% WW and 3.20 DW crude fibre (Ikewuchi and Ikewuchi, 2009). This here gives credence to the rich nutritional value of P. *tuber-regium.*

Of the fifty (50) participants, eight (8) said the formulation was sweet, thirteen (13) said it was bittersweet. So by observation, the feedback of the participants showed that the tea is easily ingested, tasty and palatable. This correspondents with the data shown in the acceptability test table, as nine (9) liked it very much while fifteen (15) liked it. Most of the persons in this set were 30 years and above. And this was so because of their previous knowledge of mushroom in their local communities during their childhood/juvenile days. The value for insipid, twenty (20), which is the highest in the table, could be addressed by adding little amount of sweetener in the process of making the formulation or by adding sugar while making the tea into an infusion.

The rest of the participants (those that said it was sour and bitter) gave their response because they were used to beverage drinks, so their judgment was because of their bias to beverage drinks. But this problem was solved when they were briefed on the health benefits of mushrooms. A category of this set contained persons that had little knowledge of mushrooms. Some were oblivious to the existence of these macrofungi, while others believed all mushrooms to be poisonous, which also formed basis for their bias against the tea formulation. Majority in this category were below 30 years and lived in urban areas, a few were uneducated (using O' level as a standard for measurement of education).

Generally, mushroom tea contains approximately zero calories (which would come from either added sugar or milk), has less caffeine than coffee, has no significant side effects, and provide several health benefits. Pleurotus species are known for producing polysaccharides like B-glucans with important medicinal properties as a constituent of the cellular wall of the fruiting body or of the mycelium Gern *et al.,* (2006). So, the larger the pileus the greater the polysaccharide content of the mushroom. However,

previously there is no documentation related to the bioactive metabolites composition and therapeutic properties of *Pleurotus tuber-regium* tea formulations (both infusion and decoction), as P. *tuber-regium* is the least grown of Pleurotus species. This could be as a result of its wild nature, difficulty in cultivation and inferior taste when compared to other Pleurotus species. So future research needs to be carried out on this Pleurotus species, to define the actual magnitude of its health benefits, establish the safe range of the mushroom tea consumption associated with the benefits, and elucidate potential mechanisms of action.

Youngabi *et al.,* (2004) and Osemwengie *et al*., (2006) stated that the mycogaphy is more commonly found in rural and suburban areas compared to the urban areas. Nigeria has been reported to have low level of mycogaphy among those living in urban areas because of poor mushroom domestication ideas, small numbers of commercial mushroom production industries, difficulties in obtaining adaptable mushroom cultivation technology, lack of innovative concepts in mushroom marketing, amongst others. Because of this, P. *tuber-regium* faces the risk of becoming an endangered species as it is underutilized, not to talk of the fact that it is least loved of the known Pleurotus species among locals dwelling in rural areas (Osemwengie *et al*., 2010). It is therefore necessary that several improvements should be made on artificial cultivation and/or domestication of edible mushrooms especially P. *tuber-regium*, to increase the awareness of its benefits as a nutraceutical food. This will inclusively enhance the consumption of P. *Tuber-regium* and make it more accessible to the public. Also, making food products from this mushroom will promote its local production and eliminate any likelihood of it being endangered.

With the diverse health benefits of *Pleurotus tuber-regium* and and with the fact that it may face the risk of gradually becoming an endangered mushroom species in Nigeria, further studies should be done in order to salvage the medicinal properties locked up in this edible macrofungi, so as to improve health living. Also more studies are needed to further elucidate the therapeutic effects of the tea.

4.1 RECOMMENDATION

It is recommended that

• tea formulations with other wild mushrooms is done

• a full microbiological analysis is carried out on these formulations

• a full mineral and vitamin analysis of the infusions is performed

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CONCLUSION

From this study, it can be concluded that *Pleurotus tuber-regium* like all other mushrooms, is a species with lots of nutritional and medicinal characteristics can help improve his health, essence beneficial to him. With the positive and encouraging responses from the participants, P. *tuber-regium* as a tea formulation should be encouraged, especially with its practically low calorie, extremely rich protein and/or little or no side effects. But it is believed that an overdose may cause slight and rare medical issues. However, an increase in the consumption of mushroom tea especially this Pleurotus species that has negligible calorie load, should be encouraged.

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