EVALUATION OF ANTIDIABETIC POTENTIAL, TOXICITY TESTING AND PHYTOCHEMICAL SCREENING OF *Ganoderma applanatum* AND *Stereum ostrea*

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RECOMMENDATION

This thesis entitled, "**EVALUATION OF ANTIDIABETIC POTENTIAL, TOXICITY TESTING AND PHYTOCHEMICAL SCREENING OF *Ganoderma applanatum* AND *Stereum ostrea***", carried out at Nepal Academy of Science and Technology has been performed by **Mr. Brishav Dhoj Rajbahak** and worked under our guidance for the partial fulfillment of the requirement for the degree of Master of Science in Biotechnology.

We, hereby, approve this report under the course code (BT 621) for the final evaluation and approval.

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CERTIFICATE OF APPROVAL

This thesis entitled "**EVALUATION OF ANTIDIABETIC POTENTIAL, TOXICITY TESTING AND PHYTOCHEMICAL SCREENING OF *Ganoderma applanatum* AND *Stereum ostrea***", submitted by **Mr. Brishav Dhoj Rajbahak** has been examined and approved by each member of the report evaluation committee and found to be satisfactory regarding content, English usage, formats, citations, referencing style and consistency, and has been submitted to Tribhuvan University as a partial fulfillment of the requirement for the degree of Master of Science in Biotechnology.

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Brishav Dhoj Rajbahak

DECLARATION

I hereby declare that the work reported in this thesis entitled "**EVALUATION OF ANTIDIABETIC POTENTIAL, TOXICITY TESTING AND PHYTOCHEMICAL SCREENING OF *Ganoderma applanatum* AND *Stereum ostrea***", submitted to Central Department of Biotechnology, Tribhuvan University, is my original work. It is done in the partial fulfillment of requirement for the Master of Science (MSc) in Biotechnology, under the supervision and guidance of Prof Dr. Krishna Das Manandhar, Head, Central Department of Biotechnology, Tribhuvan University, Kirtipur, Kathmandu and Dr. Deegendra Khadka, Senior Scientific Officer, Molecular Biotechnology Unit, Nepal Academy of Science and Technology, Khumaltar, Lalitpur.

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ABSTRACT

Ganoderma applanatum is a bracket mushroom growing on woody tree, found in the forests in many countries of the world including Nepal. It is a member of the Polyporaceae family and is mostly used in traditional Asian culture as medicine and by artists to create artwork. Research on *Ganoderma applanatum* has attracted increasing attention in Nepal as a result of its therapeutic properties (antitumor, antibacterial, and antifibrotic).

Stereum ostrea, also referred to as the false turkey tail mushroom, is renowned for its therapeutic properties. It is a member of the Stereaceae family. The anti-diabetic potential of ethanol extracts of *Ganoderma applanatum* and *Stereum ostrea* was examined. The phytochemical analysis of these mushrooms revealed the presence of alkaloids, flavonoids, phenolic compounds, saponins, tanins and steroidal compounds.

Using alpha amylase inhibition assay, *Ganoderma applanatum* and *Stereum ostrea* extracts were examined in vitro for their potential as anti-diabetic agents. The IC_{50} values were determined to be 896.32 ± 20.35 µg/ml and 723.55 ± 15.80 µg/ml for *Ganoderma applanatum* and *Stereum ostrea* respectively. The latter of the two mushrooms had superior alpha amylase inhibitory activity. Testing for toxicity involved giving albino mice ethanol extracts of both mushrooms. At 2000 mg/kg, extract from both mushrooms was confirmed to be non-toxic.

Keywords: *Ganoderma applanatum*, *Stereum ostrea*, antidiabetic potential, *in-vitro* antidiabetic test, toxicity test

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

Abs	Absorbance
Avg	Average
EGAE	Ethanolic *Ganoderma applanatum* Extract
ESOE	Ethanolic *Stereum ostrea* Extract
ELISA	Enzyme Linked Immunosorbent Assay
GA	*Ganoderma applanatum*
Gm	Grams
L	Liter
Rpm	Revolution per minute
SD	Standard Deviation
SO	*Stereum ostrea*
IC_{50}	Half maximal Inhibitory concentration
LD_{50}	Median lethal dose
Mg/ml	Milligrams per Milliliter
Ml	Milliliters
Mg	Milligrams
T2DM	Type 2 Diabetes Mellitus
UV	Ultraviolet
NaCl	Sodium Chloride

CHAPTER 1. INTRODUCTION

1.1 Background

Nature has given us resources which make our life livable and easy. It has provided us food, water, air, land and medicinal plants which helps to meet our needs. To enjoy these resources and live life to the fullest an individual must be healthy. For being healthy one must be equipped with the required defenses that can neutralize the attacks from havoc-wreaking pathogens. This is where medicine comes in handy. Plants and fungi are incredible sources of biologically active metabolites. Fungi, particularly mushrooms, have huge potential for medicinal use (Badalyan et al., 2019). However, only a fraction of these resources has been studied. We are still unknown about majority of these resources and their potential use in the medical field. Our ancestors used some of these mushrooms for medicinal purpose. Such practices are still prevalent today in many rural settings where modern medicine is not easily accessible. The medicines were determined by trial and error, and further investigation into those mushrooms is required for scientific validation.

It is a known fact that mushrooms have been used as a food and medicine since ancient times (S. T. Chang & Buswell, 1996; Chang & Miles, 2004). They are considered highly nutritional food as they contain 19 to 35% protein on a dry weight basis and low total fat content with good amount of fibre and vitamins (S. T. Chang & Buswell, 1993). In Asian countries, mushrooms have been used for treating various diseases. Various species of mushrooms like *Auricrlariu* spp., *Tremellu fisiformis, Hericim erinucew, V. volvacea, L. edodes* is traditionally used for treating ailments like hemorrhoids and various stomach ailments; for maintaining healthy lung tissue, gastric ulcers, blood pressure and accelerating the healing of wounds, and prevention of rickets and the relief of gastric acidity respectively. Traditionally used medicinal mushrooms can be tested scientifically in a laboratory to know about their actual medicinal value.

Mushrooms are still an enigma for us. Many species of mushrooms have not been under scientific investigation. Perhaps, it is due to the fact that mushrooms are seasonal, they are not easily accessible. Fortunately with new scientific methods of tissue culture and mass propagation we are now able to produce the mushrooms all year round. With the help of different extraction techniques, the biologically active metabolites can be isolated from the mushrooms. The crude extract can be obtained by using different types of solvent. These

extracts can be examined for antioxidant, antidiabetic, antibacterial, anticancer activities, etc. They can be screened for biologically active compounds useful for the human beings.

1.2 Fungi

The kingdom fungi consist of eukaryotic microorganisms such as yeast, molds, and mushrooms. The presence of chitin-containing cell walls is the defining feature of fungal cells. Fungi are responsible for contributions that are crucial to the biosphere, industry, medicine, and research (Jason et al., 2009). In comparison to other eukaryotes, the typical fungal genome size of 30–40 Mb is relatively small (Rogers & Bendich, 1994). According to phylogenetic studies, animals and fungi have a closer relationship than plants (Baldauf & Palmer, 1993). They are found in different shape and size ranging from unicellular to multicellular and are composed of filaments or hyphae, which together are known as the mycelium. The hyphae can be septate or aseptate and coenocytic. Both sexual and asexual mode of reproduction occurs in fungi (Evans et al., 2009).

Fungi get the required nutrients from their surroundings. The hypha grows through the substrate like wood, soil, allowing it to receive the nutrient from them. Secretion of digestive enzymes which break down the substrate helps to absorb the nutrients. The hyphae help cover a large surface area. Fungi produce bioactive compounds capable of exhibiting different therapeutic effects such as pain relieving, protection from bacteria, fungi and viruses, inhibiting inflammation, antioxidant, cytotoxic, liver protective, cholesterol lowering, sugar lowering, blood pressure lowering, immunostimulating, immunosuppressive, etc. (Mizuno et al., 1995; Wasser & Weis, 1999; Hawksworth, 2001)

1.3 Mushrooms:

Mushrooms are macrofungus with a distinctive fruiting body, composed of mycelial, hyphal structures, which can be either hypogeous or epigeous, large enough to be seen with the naked eye and to be picked by hand (S. Chang & Miles, 1992). They play important role in human life and ecosystem. They have been around the earth for a very long time. They are nature's decomposers that play a vital role in keeping the earth clean and recycle the various nutrients (Stamets P, 2004). They are biological elements without chlorophyll which reproduce through spores. The mode of nutrition can be saprophytic, parasitic, or symbiotic.

Pileus, lamellae, stipe, annulus and volva together comprises a mushroom. The majority of woody fungus lacks a distinct stipe. Mushrooms can have spores of various colors like white, yellow, black, creamy, chocolaty, rosy, green, and rusty. Due to its diverse geomorphology, variety in altitude, and climatic circumstances, Nepal has a particularly rich mushroom diversity. There are 1291 different varieties of mushrooms reported, of which 159 are edible, 100 are poisonous, 74 are used medicinally, and 25 have other purposes (Devkota & Aryal, 2020). Mushrooms are superfoods that boost human health. They can be substituted in diets as low-calorie foods. They may aid in the early intervention of unhealthful states and maybe stop the effects of a number of fatal diseases (Raut & Adhikari, 2021).

1.4 Medicinal mushrooms

Mushrooms with medicinal properties have been a subject of interest in the scientific world. There is a growing interest in investigating the extracts from mushrooms, purifying and studying individual constituents to know their interactions and biological activity. Different mushrooms have been utilized to treat a variety of illnesses all around the world. Tribal societies have passed the information about the medicinal and other useful properties of the mushrooms they used from generation to generation. As every coin has two sides, there are both pros and cons of modern medicines. The major drawbacks are unwanted side effects and development of resistance. As a result, interest in the herbal and homoeopathic medical systems has greatly increased recently (Evans et al., 2009). Acting like miniature factories, mushrooms are capable of producing a variety of novel enzymes and other biologically important chemicals which can have pharmacological potential. (Lindequist et al., 2005)

Recognizing the value of mushrooms, their cultivation for exploring the medicinal potential is gaining a lot of traction in modern times. The secondary metabolites are a top priority in the scientific community. Exploring and investigating the mushrooms for their biologically active components has helped in identifying novel compounds with medicinal benefits (Stamets & Zwickey, 2014). These mushrooms can be used in conjunction with other treatments for various disorders. Desirable properties of medicinal mushrooms include protection against microbes, inhibition of inflammation, protection of heart, liver, control of diabetes, and anticancer properties. They are capable of slowing tumor growth, regulating tumor genes, decreasing new tumor formation, and increasing engulfment of malignant-cell. (Guggenheim et al., 2014)

1.4.1 Mushrooms as a source of antimicrobial

Antibiotics are a boon for humanity. Undoubtedly the discovery of antibiotics has saved a lot of lives. However, haphazard use of antibiotics has led to the rise of multi drug resistance. Some antibiotics have undesirable side effects. So to safeguard us from the resistant strains of microbe, new antibiotics should be introduced. Secondary metabolites having antimicrobial properties isolated from medicinal mushrooms have been reported from various parts of the world. Ganomycins produced by the mushroom species *Ganoderma pfeifferi Bres.* has been shown to suppress the growth of methicillin-resistant *S. aureus* (Hobbs, 2002) and other gram positive and gram negative bacteria (Mothana et al., 2000).

Oxalic acid found in *L. edodes* (Berk.) is reported to have the ability to inhibit *S. aureus* and other microbes (Bender, 2003). The extract from mycelia of *L. edodes* generated using ethanol has shown inhibition of growth of *P. caudatum* (Badalyan et al., 2004). The phenolic and polyphenolics are one of the largest groups of secondary metabolites that have exhibited antimicrobial activity (James et al., 2018). Bacterial proliferation is inhibited by polyphenols (Efenberger-Szmechtyk, 2020). Additionally, polyphenols have an impact on protein biosynthesis, alter cell metabolism, and prevent the formation of ATP and DNA.

1.4.2 Mushrooms as a source of antioxidant

Free radical species can have deleterious effect on the body. They can originate from sunlight, UV light, ionizing radiation, chemical reactions, and physiological processes. These free radical species have been linked to cellular aging, cancer, and DNA damage (Marx, 1987). They might lead to decline of the immune system (Pike and Chandra, 1995). Antioxidant compounds protect against the damage due to free radical species. Synthetic antioxidants are very good radical scavengers but they come with side effects (Grice, 1988). Such antioxidants can have adverse effects on health (Branen, 1975). Antioxidants from natural sources are considered to be safer and are more preferable. They can be found in fruits, vegetables, mushrooms etc. (Kanner et al., 1994; Madsen and Bertelsen, 1995; Cao et al., 1996).

Mushrooms derived antioxidants are the most promising natural source of antioxidant. Different species like *Agaricus bisporus, Boletus badius, Hericium erinaceus, Hypsizigus marmoreus, Lentinula edodes, Lepista nuda, Pleurotus sp., Polyporus squamosus, Russula delica, Termitomyces sp., Volvariella volvacea* and *Verpa conica*, show antioxidant potential

in vitro and may have application in vivo (Elmastas et al., 2007; Fu et al., 2002; Puttaraju et al., 2006). The mushroom shows the ability to neutralize the reactive oxygen species due to presence of secondary metabolites (González-Palma et al., 2016; Sevindik, 2018). The redox characteristics of phenolics, which enable them to function as reducing agents, hydrogen donors, singlet oxygen quenchers, and metal chelators, are primarily responsible for their anti-oxidant activity (Liang et al., 2010).

1.4.3 Mushrooms as a source of antidiabetic

Diabetes mellitus is a metabolic condition, which affects carbohydrate, protein and fat metabolism (Barceló & Rajpathak, 2001). Insulin helps in the metabolism of carbohydrate, fat and protein (Geser, 1976). About 537 million people around the world live with diabetes (International Diabetes Federation, 2021). This metabolic disorder can have adverse effect on the health of people. Close monitoring and control of the blood glucose level is of paramount importance for the well-being of the patients (DeFronzo, 1999). There are modern medicines which help to achieve the targeted blood glucose level in the patients. These medications are useful but they come with side effects as well as resistance, which in the long run make the condition miserable. So the best possible treatment options with more effectiveness and safety should be searched. Various studies have revealed the antidiabetic potential of different mushroom species.

Hot water and ethanol extracts of *G. lucidum* and *G. sinense* have been reported to exhibit antioxidant and antidiabetic activity. In terms of the phenolic content and antioxidant potential, hot water extracts surpassed the ethanol extracts. Ethanol extract of *G. lucidum* had the strongest α-glycosidase inhibitory capacity and also had the largest amount of total ganoderic acids. It was found that the ethanol extracts from both mushroom species had better antidiabetic effects compared to the hot water extracts. Ganoderic acids may contribute to the antidiabetic effects of both the Ganoderma species (Tang et al., 2016). A polysaccharide component of *G. frondosa* was found to have hypoglycemic effects in T2DM individuals (Wu et al., 2021). Coriolan, a b-glucan-protein complex isolated from submerged culture of *Tremella versicolor* (Bashir et al., 2017), and an acidic glucuronoxylomannan from the fruiting bodies of *Tremella aurantia Schwein* showed hypoglycemic effects (Hsu T, 2014) in several test systems and reduced the symptoms of diabetes (Hikino H et al., 1985).

In another study, by Su C, *Grifola frondosa*'s n-hexane extract had the strongest anti-glucosidase activity, while *Coriolus versicolor* had the strongest anti-alpha amylase activity. When compared to acarbose, all mushroom extracts had weaker anti-alpha-amylase activity, but greater anti-alpha-glucosidase activity. Oleic acid and linoleic acid concentrations in the extracts showed distinct effects on the extracts' capacity to inhibit alpha-glucosidase activity, according to GC-MS analyses (Su C, 2013). D. Stojkovic et al. investigated the in vitro antidiabetic properties of some edible as well as medicinal mushrooms. Methanol extracts were used for performing in vitro assays on α-amylase and α-glucosidase enzyme inhibition. *I. obliquus* extract showed the most promising potential, while no inhibition of α-amylase was recorded with *M. conica* and *C. militaris* methanol extract at the tested concentration (D. Stojkovic, 2019).

In a study, when patients with T2DM were treated with polysaccharide fractions from *G. lucidum*, mean post-prandial glucose values were found to be lower in the test group (Gao et al., 2004). It was found that the Cordyceps extract help to improve diabetes in streptozotocin-induced diabetic rats (Hsu T, 2002). The anti-diabetic potential is attributed to phytochemicals like flavonoids, terpenoids, saponins, carotenoids, alkaloids, and glycosides (Afrisham et al., 2015; Kooti et al., 2015). No side effects could be seen due to which they could be used for the treatment of diabetes around the world (Gupta & De, 2012). The antidiabetic activity is manifested through several mode of action.

1.4.4 Mushrooms as antitumor agents

The number of tumor-related deaths is skyrocketing every year. Mushrooms could be used to curb these numbers by preventing as well as treating the disease. Several extracts of *I. obliquus* have shown antitumor effects in tumor cell systems and animal experiments (Kahlos, 1987; Burczyk et al., 1996). In addition to affecting the cytokine production, mushrooms can regulate immune response cells, such as hematopoietic stem cells, lymphocytes, macrophages, T cells, dendritic cells (DCs), and natural killer (NK) cells (Moradali et al., 2007). Mushrooms can destroy tumors by modulating the immune system and thereby aid in cancer treatment. In a study, done by Ohwada et al., polysaccharide K (PSK) derived from turkey tail mushroom resulted in better survival rates versus the control group in stage II/III colorectal patient. Immunomodulatory action could be seen at higher doses which resulted in increase of CD8+ T cells and CD19+ B cells (Ohwada et al., 2005).

The use of *G. lucidum* polysaccharide (Ganopoly) in advanced lung cancer patients was found to be beneficial in the study done by Gao et al. The study checked patient's condition based on their quality of life and disease severity (Karnofsky score) as well as hematological, immunological, and biochemical markers. Mushrooms ability to change the immune response of the major players of the immune system like the lymphocytes helps to safeguard against the bone marrow suppression due to chemotherapy. Reducing the bone marrow suppression provides better overall response for treatment. The medicinal mushrooms help to raise the effectiveness of tumor treatment (Gao et al., 2003). It was found that PSK extracted from turkey tail increased the efficacy of the drug docetaxel in the treatment of human gastric carcinoma. In both in vitro and in vivo conditions, researchers found that PSK helped in apoptosis by inhibiting antiapoptotic molecules (Kinoshita et al., 2010). Thus, to achieve comparable levels of apoptosis, the researchers were able to employ a lower dose of the medication.

1.4.5 Immunosuppressive and antiallergic mushrooms

The immunological response can be both stimulated and suppressed by mushroom extracts. Because they can assist to decrease allergic reactions, this intriguing characteristic of mushrooms is beneficial for the treatment of allergic illnesses. In numerous studies, it was discovered that the mushroom extract obtained using ethanol were capable of inhibition of allergic reactions. The extracts were found to minimize the allergic reactions even with application on skin. Mushrooms like *H. marmoreus, F. velutipes, Pholiota nameko* (*T. Ito*), and *Pleurotus eryngii* significantly reduced allergy in mice (Sano et al., 2002). Ganoderic acids C (8d) and D (8e), obtained from *G. lucidum* antagonizes the generation of histamine from mast cells in rat (Tasaka et al., 1988; Kohda et al., 1985).

1.5 Diabetes Burden and Risk

Diabetes is a rapidly growing global epidemic. It is a chronic, metabolic disease characterized by a high level of blood glucose. If not treated accordingly, it gives raise to various health problems. It can deteriorate health by causing damage to various parts of the body like the heart, blood vessels, eyes, kidneys and nerves (Rubin et al., 2012). Type 2 diabetes is more prevalent worldwide. It manifests itself when the body develops insulin resistance or doesn't make enough insulin. Diabetic patients need timely and affordable treatment in order to live

a life without complications. People in low-and middle-income countries are more prone to diabetes and its complications (International Diabetes Federation, 2011). It is perhaps due to the rapid urbanization and shift in routine like increased consumption of fast food, and sedentary lifestyle (Hu, 2011).

Asia is a becoming a hub of this global epidemic. Diabetes has started to be seen in younger adults as well. The demographic to be more affected by diabetes are obese, have a family history of T2DM, and come from less affluent socioeconomic groups (Wilmot et al., 2010). Modern day inactive lifestyle, consumption of unhealthy food is leading cause of the current global epidemic. Lifestyle changes like exercising and consuming a healthy diet can help prevent the occurrence of diabetes. Diabetes brings several other complications. Cardiovascular complications are the most lethal for diabetic patients. Similarly, hypertension, nephropathy and hyperlipidemia are prevalent in patients with diabetes. Aggressive management is required for controlling and limiting the damage that can arise due to diabetes (Tomic et al., 2022).

1.6 Principle of toxicity test in lab animals

The concept of acute oral toxicity is based on the idea that a small number of test animals can be used to evaluate the toxicity of chemical or plant extracts and essential oils while still providing sufficient data on the test substance's acute toxicity to aid in classification. One of the predefined dosages of the medicine is administered orally to a group of research animals. The next phase is determined by whether compound-related death occurred in the animals dosed at the previous stage, in which case no further testing, the dosing of three additional animals with the same dose, or the dosing of three further animals at the subsequent higher or lower dose level, is necessary. The method will enable a choice to be made regarding the test sample's classification into toxicity classes based on previously established LD_{50} cut-off values (OECD guideline for testing of chemical, 2001).

1.7 STATEMENT OF PROBLEM

The number of people with Diabetes Mellitus is increasing year after year. If the appropriate treatment is not provided, it can wreak havoc in the human body. It is a metabolic disorder which requires immediate attention and strict medical control to make sure that the matters don't go out of hand and lead to disastrous results. Constant monitoring and tight control of the blood glucose levels is of paramount importance to ensure the optimal health status of the patients. Even though there are modern medicines available for controlling this disease, they are not effective for long term use (Davies, 1994; Harbottle et al., 2006). The drugs have their fair share of side effects which eventually wears down a patient. Safer alternatives to the present drugs are urgently needed to resolve these problems. Thus, search for compounds with high efficiency and less side effects is essential.

Since mushrooms are packed with different biologically active compounds, natural products with medicinal benefits can be derived from them. Different parts of the mushroom like fruiting bodies, mycelium, can be used for this purpose. In rural parts of Nepal traditional medicine is still in practice to treat the diseases. People still don't have access to modern medicines so they have no other option except natural therapy. They use available natural resources and their extracts to cure the diseases. People have been using different species of mushrooms as a medicine to cure different ailments. Ethnobotanical use of these mushrooms found in the wild has not been studied scientifically. These extracts may have the potential to be used as alternative or complementary medicines. The *in-vitro* antidiabetic potential of the *G. applanatum* and *S. ostrea* mushroom has not been evaluated. Also, the comparison between antidiabetic activities of these species has not been performed.

1.8 RATIONALE AND JUSTIFICATION

Somewhere in a corner of a deep dark forest, a mushroom is waiting to be discovered. Perhaps it has desirable properties like anti-bacterial, antidiabetic, antioxidant, anti-cancer etc. Research in these areas is limited. Medicinal mushrooms often show potent antioxidant, antidiabetic, anticancer activity and can be used for the management of various ailments. This study is intended for investigating the antidiabetic potential of *G. applanatum* and *S. ostrea* and toxicity testing in albino mice. It is very unfortunate that still people in the rural parts of our country are deprived of proper healthcare and medicine facilities. They don't have the privilege of health services and modern medicines. Still they have to rely on the traditional

herbal remedies to treat their illness. Nonetheless we are rich in biodiversity which can be explored as source of medicine. Measurement of *in-vitro* antidiabetic property gives us a peek at how effective the mushrooms are in reducing the blood glucose levels. Comparison of the blood glucose lowering rates shows us which mushroom is better suited for using against diabetes. Toxicity testing in mice model helps to figure out whether or not the extracts from the mushroom is toxic and determines their usability as alternative and complementary medicine.

1.9 OBJECTIVES

1.9.1 GENERAL OBJECTIVES:

- To evaluate the antidiabetic potential of *Ganoderma applanatum* and *Stereum ostrea*, perform toxicity testing and phytochemical screening.

1.9.2 SPECIFIC OBJECTIVES:

- To obtain ethanol extract from fruiting bodies of *Ganoderma applanatum* and *Stereum ostrea*
- To perform in-vitro alpha amylase inhibition assay of the ethanol extracts of *Ganoderma applanatum* and *Stereum ostrea* using DNSA.
- To perform toxicity testing of the extracts in albino mice.
- To perform phytochemical screening of the extracts.

1.10 RESEARCH HYPOTHESIS

Null Hypothesis (H_0): The extracts have antidiabetic potential and are non-toxic.

Alternative Hypothesis (H_1): The extracts don't have antidiabetic potential and are toxic.

CHAPTER 2. LITERATURE REVIEW

In the recent decades articles on different species of *Ganoderma* and *Stereum* mushroom has been published. There is an increasing interest in the culture and exploitation of these mushrooms due to its medicinal importance. This has boosted the number of people getting into the race to explore these mushrooms for scientific investigation.

2.1 *Ganoderma applanatum*

Locally, the mushroom *Ganoderma applanatum* is referred to as "Kathey chyau." It belongs to the phylum Polyporaceae, class Agaricomycetes, and family Ganodermataceae. Sunsari, Kaski, Syangja, Makwanpur, Lalitpur, Gorkha, Bajhang, Jumla, Kathmandu, Solukhumbu, and Dolakha are just a few of the districts in Nepal where it has been found (Balfour & Browne, 1968; Pandey, 1976; Adhikari et al., 2019). The carpophore which ranges from 10-40 cm in length has a bracket like structure which is initially white and changes to reddish brown as it ages. The tubes are white, 8 to 25 mm in length, and have tiny pores that quickly turn brown when handled. Spore is cream colored and ranges from 7-9 x 5.5-6.5 µm, and is oval shaped (Pandey, 2007). It has a dull brown cap surface that is unvarnished, furrowed, and lumpy (Binion et al., 2008). The hue of the pore surface is white. The mode of nutrition is saprobic and sometimes parasitic. It can be found growing either alone or in clusters on most species of hardwoods or from the wounds of injured trees.

It produces white rot of sapwood and heartwood. It is perennial and is fairly common and widely distributed. The stem is usually absent. The flesh is thin brown to cinnamon brown and woody. It doesn't have distinctive odor and taste. The spore print is brown in color (Breitenbach & Kränzlin, 1986; Gilbertson & Ryvarden, 1986; States, 1990). Mushrooms of *Ganoderma* spp. are globally distributed and are well known for their medicinal property (Lin & Yang, 2019; Ma et al., 2011) . This genus has about 300 discovered species, the majority of which reside in tropical areas (Richter et al., 2015). They are useful for curing diabetes and insulin resistance (Wińska et al., 2019). It has found applications as functional food and nutraceuticals as well. Previous studies have reported various components with biological activity including primary and secondary metabolites (Grienke et al., 2015).

These mushrooms can be used as alternative to conventional therapy of diabetes. The most active ingredients of those mushrooms are polysaccharides and triterpenoids. It has been used to treat various chronic diseases, such as chronic hepatitis, immunological disorders,

neurasthenia, arthritis, and nephritis (Chen et al., 2021). The antidiabetic potential of five popular Ganoderma species has been studied by researchers: *G. lucidum, G. sinense, G. tsugae, G. applanatum,* and *G. leucocontextum* which revealed that triterpenes were superior in inhibiting α-glucosidase and α-amylase activity (Chen et al, 2019). When tested on mice, the residual polysaccharides of *G. applanatum* have been shown to have protective properties against carbon tetrachloride induced liver damage. It is a potential candidate for drug and functional food development against chemical hepatic injury (Gao et al., 2019).

In a study done by Mfopa, it was found that water soluble polysaccharides from *G. applanatum* had antidyslipidemic potential. High fat diet fed obese rats was given the polysaccharide orally for two months at different dose level (50, 100, and 150 mg/kg bodyweight) which was able to significantly decrease the lipid parameters (total cholesterol, triglyceride, low-density lipoprotein cholesterol levels). Also it was revealed that the extract improved the high-density lipoprotein cholesterol level in obese rats (Mfopa et al., 2021). It has been reported that the triterpenoids like lanostane from *G. applanatum* exhibit significant anti-adipogenesis effects (Peng et al., 2022). Some steroidal compounds recovered from it, were capable of inhibiting both gram-positive and gram-negative microbes (Smania et al., 2004). It has been reported that the polysaccharides of *G. applanatum* has antitumor effect and the polysaccharide were capable of inhibiting the sarcoma 180 implanted subcutaneously in mice (Gao et al., 1991).

In search of newer medications to combat the alarming threat posed by multi-resistant microbes, many researchers have found mushroom extracts with antibiotic potential. It has become vital to create new antibiotics which target multiple proteins and has lesser side effects. According to a study, the bacterial strains *P. aeruginosa, P. fluorescens, B. subtilis, S. epidermidis,* and *M. luteus* were strongly inhibited by water extracts of *G. applanatum* (Hassan et al., 2019). Kozarski found that the polysaccharide extracts of *G. applanatum* had antioxidant potential. It had high DPPH scavenging action. The reducing power and the overall quantity of phenols and alpha-glucans were found to be strongly correlated in the polysaccharide extracts. Despite the use of hot water, ethanol and dialysis, the extracts retained components like polysaccharides, proteins and polyphenols. The polysaccharides of *G. applanatum* had uronic acid (Kozarski et al., 2012).

2.2 *Stereum ostrea*

Stereum ostrea is a white rot mushroom belonging to Stereaceae family that infects a broad range of hardwood. Also known as the false turkey tail mushroom, it comes with colorful cap that displays zones of brown, red, orange colors. This widely distributed mushroom lacks a pore surface, and therefore has a smooth underside. It has relatively large size and can reach widths of 5-7 cm. It develops individual, sliced-funnel-shaped fruiting bodies. It is saprobic found on dead hardwoods, growing densely. It is found in spring, summer, fall, and winter (Blume & Nees, 1826; Fries, 1838; Welden, 1971). Concentric zones of different colors like red, orange can be seen. It doesn't have a stem. It is tough and woody. The white spore print is difficult to obtain and the spores range from 5.5-7.5 to 2-3 µ (Phillips, 1991; Lincoff, 1992; Metzler & Metzler, 1992) .One of the major metabolite groups of this mushroom are sesquiterpenoids, such as hirsutanes (Yun et al., 2002; Yoo et al., 2006; Liermann et al., 2010), stereumanes (Li et al., 2011), and drimanes (Kim et al., 2006), respectively.

The extraction process with different types of solvent like water, ethanol, methanol, acetone, ethyl acetate, etc. is usually done. Sesquiterpenes sterosterins A-E has been collected from *S. ostrea* cultures (Isaka et al., 2011) and 10 new illudalanes and norilludalanes, sterostreins F–O has been isolated (Isaka et al., 2011, 2012). It was found that the crude culture filtrate of *S. ostrea* is highly inhibitory versus the methanol extract and microbes like *B. subtilis* and *K. pneumonia* were inhibited by the extract (Praveen et al., 2012). The antibacterial activities was shown by acetone, ethanol and aqueous extracts of fruiting body of *S. ostrea* and the most effective inhibition was seen against *B. subtilis* (Prust et al., 2014). Antioxidant and total oxidant status of *S. ostrea* (Blume & T. Nees) Fr. has been assessed and as a result of the HPLC scans, Gallic acid, Benzoic acid, Sringic acid, Chlorogenic acid, and Catechin were determined. The element measurements using atomic absorption spectrometry revealed that Cr content is higher than expected (Akata, 2020).

The liquid culture filtrate, water and ethanol extract (solid culture) of *S. ostrea* has antibacterial and antifungal activities. Tests were conducted against bacteria and fungi separately using concentrations of 5–300 mg/ml. It was found that the liquid culture filtrate was more effective against Gram positive than Gram negative bacteria, and *S. aureus* was the most inhibited bacterium. By comparison, water extract was found to be superior to ethanol extract (Imtiaj et al., 2007). Similarly, in another study using ethanol and water extracts of *Stereum*, it was revealed that the water extract was superior to the ethanol extract in terms of

effectiveness against both Gram positive and Gram negative bacteria (Ferreira-Silva et al, 2017). *S. ostrea* shows a promising result in decoloration of dyes as well. It can be used for bioremediation purpose (Praveen et al., 2015). In all bioassays tested, *S. ostrea* methanol extracts showed strong antioxidant activity (Kim et al., 2012). Results showed that methanol extract had greater ABTS scavenging activity than α -tocopherol, leading researchers to speculate that *Stereum* species might be a source of antioxidants.

CHAPTER 3. METHODOLOGY

3.1 Research Methodology and Data Analysis

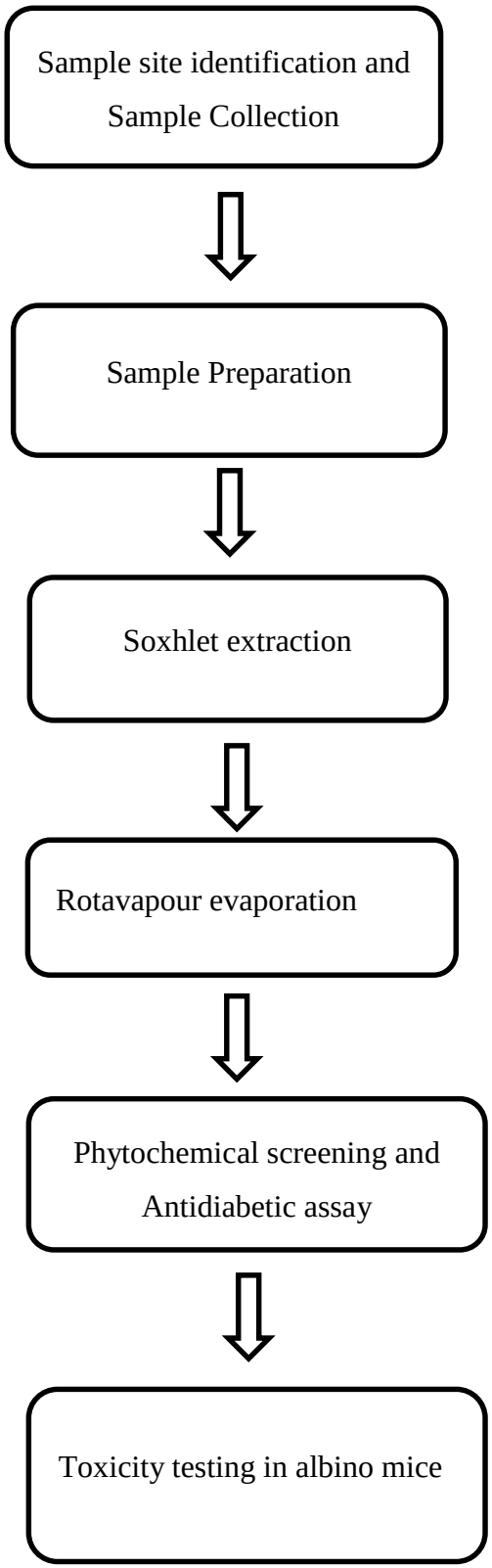

Figure 1: Overall study design in Flowchart

3.2 Sample and Material Collection

Two different species of Mushroom, namely, *Ganoderma applanatum* and *Stereum ostrea* were used in this experiment. The mushrooms were collected from Gaurishankhar Conservation Area, Dolakha, Nepal. Before collecting the samples, the samples were carefully examined. A voucher specimen was preserved. The samples were air dried and kept in the paper bags.

Figure 2: *Ganoderma applanatum*

Figure 3: *Stereum ostrea*

3.3 Morphological Identification and species description

The mushroom samples were macroscopically examined for the presence of cap, gills, ring, pileus, stipe, and volva. Taxonomic identification was made by expert, Dr. Jaya Kant Raut, Senior Scientific Officer, Biological Resource Unit, Nepal Academy of Science and Technology.

3.4 Alpha-Amylase Inhibitory Assay

The alpha--amylase inhibition assay was used to assess the anti-diabetic potential of the extracts of *G. applanatum* and *S. ostrea*. A little modification to the available standard protocol was made (Jamuna et al., 2012). A tube containing 125 μl of extract at various concentrations (20, 40, 80, and 160 μg/ml) was taken and 125 μl of 0.02 M phosphate buffer (pH 6.9) with 0.5 mg/ml of -amylase were added. Pre-incubation at 25 °C for 10 min was done. After that, 125 μl of a 1% starch solution was added, and incubated at 25 °C for 10 min. Dinitrosalicylic acid (DNS) reagent was added (125 μl) to stop the process. The tubes were

then cooled to room temperature after being incubated in boiling water for 5 min. 2.5 ml distilled water was added to the reaction mixture to dilute it. Then, a spectrophotometer was used to measure the absorbance at 540 nm. Using the same method but substituting distilled water for the extract, a control was created.

The α-amylase inhibitory activity was calculated as percentage inhibition:

$$\% \text{ Inhibition} = [\text{Abs}_{\text{control}} - \text{Abs}_{\text{extracts}} / \text{Abs}_{\text{control}}] \times 100$$

IC_{50} values were determined graphically.

3.4.1 Preparation of Phosphate buffer (0.02 M)

Disodium hydrogen phosphate (Na_2HPO_4) crystals (0.1174 gm) were weighed out, poured in 100 ml volumetric flask, dissolved and distilled water was added to make 100 ml of 0.02 M Na_2HPO_4 solution. Similarly, sodium dihydrogen phosphate (NaH_2PO_4) crystals (0.209 gm) were weighed out and 100 ml of 0.02 M NaH_2PO_4 solution was prepared in distilled water. Then 50 ml of 0.02 M Na_2HPO_4 solution and 50 ml of 0.02 M NaH_2PO_4 solution were mixed and 0.0196 gm (6.7 mM) NaCl was added. Finally, the pH of 0.02 M phosphate buffer was maintained at 6.9 by adding dilute HCl and NaOH.

3.4.2 Preparation of the starch solution (1% starch)

1 gm of soluble starch was weighed and dissolved in boiling water (80 ml). It was left for cooling and pH was adjusted to 7 and volume makeup was done by adding 20 ml distilled water.

3.4.3 Preparation of mushroom extracts

The mushroom samples were air-dried for a month under the shade at room temperature at the NAST plant specimen collection room. The dried samples were grinded using a grinder into a fine powder and used for extraction. 25 gm of the dried and powdered samples of *G. applanatum* and *S. ostrea* were kept in a thimble and extracted with 90 % ethanol in a Soxhlet extractor. The extraction process was continued until the color of the final drop of the extract became colorless. Then ethanol was removed from the extract using a Rotavapour at 45 ℃ under vacuum. The extract was kept in a refrigerator until further use.

3.4.4 Preparation of stock solution of sample extracts and Acarbose

The stock solution of concentration 1000 µg/ml of dry extracts was prepared by dissolving 10 mg of extracts in 10 ml of Phosphate Buffer. Serial dilution of thus obtained stock solution was done to prepare 4 different concentrations i.e. 20, 40, 80, 160 µg/ml for each extracts. Finally, this solution was kept in different Eppendorf tubes and stored for the future use. Following the same protocol above, acarbose solution of different concentration was also prepared and stored.

3.5 Experimental animals

Adult male albino mice bred in the Department of Plant Resources (DPR), Thapathali, Kathmandu, Nepal were used for the experiment. Eight weeks old mice with weights ranging from 40 to 60 gm were used. The animals were kept in cages at 23 ± 2 ℃. Throughout the experiment 12:12 light/dark cycle was maintained. They were given standard diet and water. The treatment and handling of the animals complied with internationally accepted ethical standards for the use of laboratory animals.

3.6 Acute toxicity test

Following a night of fasting with just water intake, the extracts were administered orally to the animals. Each mouse's weight was noted before the extract was given to them. Randomly the animals were divided into a control and treatment group (separately for both extracts), consisting of five mice.

The treatment group received extracts at 2000 mg/kg and the control group received distilled water. They were closely monitored for the first 4 hour for any toxicity along with behavioral changes like restlessness, tremor, diarrhea, sluggishness, weight loss and paralysis at regular intervals. Thereafter, they were monitored every day for 2 weeks to look for any changes in general behavior and/or other physical activities. After the extracts had been administered for 4 hours, the food was made accessible. Additionally, comprehensive care was provided for 7 days (Yankell & Loux, 1977).

3.7 Phytochemical screening

Using the established methodology, *G. applanatum* and *S. ostrea* extracts were screened for chemical content. The goal was to determine whether secondary metabolites such alkaloids, steroidal chemicals, phenolic compounds, flavonoids, saponins, and tannins were present or absent.

3.7.1 Tests for alkaloids

- Mayer's Test: Few drops of Mayer's reagent were added to a few ml of the extract (along the test tube's sides). Formation of creamy white or yellow colored precipitate indicates the presence of alkaloids (Evans et al., 2009).
- Wagner's Test: 1-2 drops of Wagner's reagent was added to a few ml of the extract (along the test tube's sides). Formation of brown or red color precipitate indicates the presence of alkaloids.

3.7.2 Test for Tannins

In the test for tannins, Extract was boiled in 20 ml of water in a test tube and filtered. Few drops of ferric chloride (0.1%) was added and observed for a brownish green or blue black coloration as an indication of tannins.

3.7.3 Test for Saponins

Distilled water (5 ml) was mixed with crude extract in a test tube. The frothing was mixed with few drops of oil and mixed vigorously and the foam appearance showed the presence of saponins.

3.7.4 Tests for Flavonoids

2 ml of 2% NaOH mixture was mixed with crude extract; concentrated yellow color was produced, which became colorless when 2 drops of diluted acid was added to mixture. This ensured the presence of flavonoids.

3.7.5 Test for Terpenoids

Chloroform (2 ml) was added with the aqueous extract (5 ml) and kept on the water bath and then boiled with H_2SO_4 concentrated (3 ml). Formation of grey color indicates the presence of terpenoids.

3.7.6 Test for Steroids

Chloroform (2 ml) and concentrated H_2SO_4 were mixed with the crude extract (5 ml). Formation of reddish coloration makes sure that the steroids are present in the sample.

3.8 Statistical analysis

The data are presented as mean ± standard deviation. Software used for the analysis of data was Graphpad prism along with MS Excel. Bar graphs and line charts were drawn using Excel software.

CHAPTER 4. RESULTS

4.1 Sample collection and preparation of extract:

The mushroom samples were collected from Gaurishankhar Conservation Area, Dolakha, Nepal. The fruiting body of the mushrooms was used for extraction purpose. The collected samples were dried and grinded into fine powder, and subjected to soxhlet extraction using ethanol as solvent. Powdered samples of *G. applanatum* and *S. ostrea* were used for the extraction process. Extraction was continued till the solvent became colorless. Then the residual ethanol was removed from the extract under vacuum using a Rotavapour at 45 ℃. Then it was left to dry in room temperature for a week. Then the resulting extract was scrapped and collected. The weight was recorded using a weighing balance. Then it was kept in Eppendorf tubes and stored in the refrigerator.

4.2 Extraction yields:

G. applanatum had higher yield than that of the *S. ostrea* as presented in table 1. The yield of ethanol extract of *G. applanatum* was found to be 9.4% whereas, the yield of ethanol extract of *S. ostrea* was found to be 7.6%.

Table 1: Percentage yield of the extracts

SN	Name of mushroom	Ethanol extract (%)
1	*Ganoderma applanatum*	9.4
2	*Stereum ostrea*	7.6

The yield comparison of *G. applanatum* and *S. ostrea* extracts can be visualized graphically as presented in the graph in figure 4.

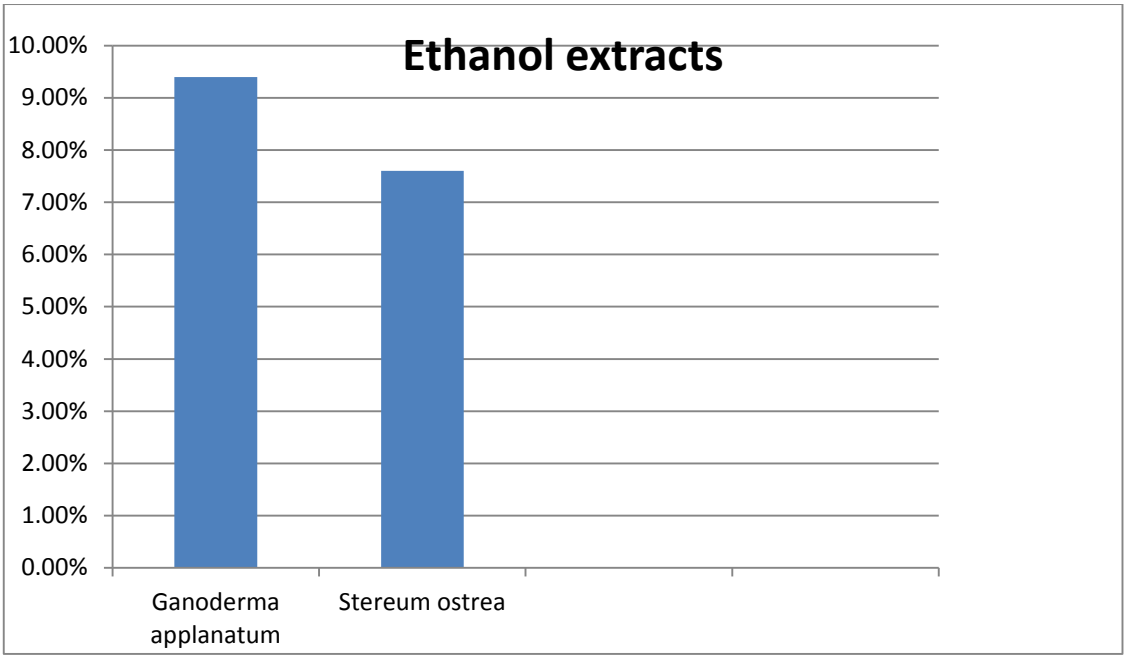

Figure 4: Bar graph comparing the percentage yield of the extracts

4.3 Alpha amylase inhibition activity:

Alpha amylase inhibition activity of the ethanol extracts of *G. applanatum* and *S. ostrea* was tested using DNSA reagent. IC_{50} values for extracts of both samples were calculated. The IC_{50} values of the extracts are shown in table 2.

Table 2: Comparison of α-amylase inhibitory activities (IC_{50})

SN	Sample	IC_{50}
1	*Ganoderma applanatum*	896.32
2	*Stereum ostrea*	723.55

The concentration of 20, 40, 80, 160 µg/ml was used to measure the percentage inhibition of alpha amylase for both the extracts as shown in table 3. *G. applanatum* had shown 24.86%, 39.40%, 47.73%, and 75.03% inhibition at 20, 40, 80, 160 µg/ml concentrations respectively. Similarly, *S. ostrea* had shown 48.61%, 72.80%, 73.58%, and 83.35% inhibition at 20, 40, 80, 160 µg/ml concentrations respectively. Among the extracts of the mushrooms, ethanol

extract of *S. ostrea* had highest % inhibition i.e. 83.35% with IC_{50} value of 723.55 µg/ml followed by ethanol extract of *G. applanatum* with 75.03% inhibition and IC_{50} 896.32 µg/ml

Table 3: Alpha (α)-amylase inhibition % by different concentration of the mushroom extract

Concentration (µg/ml)	Ethanol extract	
	Ganoderma applanatum	*Stereum ostrea*
20	24 .86	48.61
40	39.40	72.80
80	47.73	73.58
160	75.03	83.35

The comparison of IC_{50} of ethanol extracts of *G. applanatum* and *S. ostrea* can be visualized in bar graph as shown in figure 5.

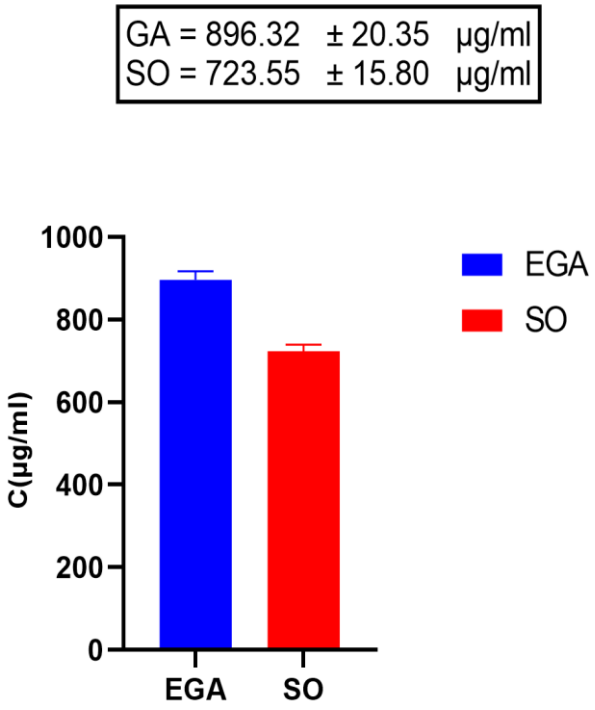

Figure 5: Bar graph comparison of IC_{50} of *Ganoderma applanatum* and *Stereum ostrea*

Acarbose, the standard drug had the inhibition percentage of 62%, 69%, 72% and 88% at the concentration of 20, 40, 80, and 160 µg/ml respectively. The IC_{50} value was found to be 86.79. It is shown in table 4.

Table 4: Percentage inhibition of Standard Acarbose

Sample	Concentration (µg/ml)	Absorbance	% inhibition	IC50 (µg/ml)
Acarbose	20	0.343	62	86.79
	40	0.280	69	
	80	0.278	72	
	160	0.154	88	

4.4 Phytochemical screening

To identify the mushrooms components in their extracts, primary phytochemical screening was done with the help of color-forming and precipitating chemical reagents. Table 5 summarizes the test results and lists the substances that were found. Alkaloids, flavonoids, phenolic compounds, steroidal compounds, tanins and saponins were found in the fractions of *G. applanatum* extracts after the screening was conducted. Alkaloids, flavonoids, phenolic chemicals, steroidal compounds, tanins and saponins were also found in *S. ostrea* extracts. This outcome agreed with earlier studies (Adhikari et al., 2019).

4.5 Acute toxicity test

The acute toxicity test revealed that while using a dose of up to 2000 mg/kg, there were no signs of toxicity following the administration of the ethanol extracts of either *G. applanatum* or *S. ostrea*. Extracts was given to five mice, all of whom survived. They did not display any signs of toxicity. By 24 hours they were moving and eating normally. The lack of any noticeable changes in alertness, motor activity, weight loss, sluggishness, paralysis, respiration, restlessness, diarrhea, convulsions, and coma served as the confirmation of non-toxic nature of the extracts. Additionally, they were physically active and no deaths were noticed for two weeks. The outcome shows that the tested doses of the mushroom extracts had no discernible negative effects; this suggests that the mice medium lethal dose is larger than 2000 mg/kg. A LD_{50} dose of more than 2000 mg/kg classified an oral toxicity category

as Category 5 under the Globally Harmonized System of Classification and Labeling of Chemicals (GHS). As a result, both *S. ostrea* and *G. applanatum* are found to be non-toxic.

Table 5: Phytochemical screening results for *Ganoderma applanatum* and *Stereum ostrea*

TEST	REAGENT	INFERENCES	
		Ganoderma applanatum	*Stereum ostrea*
Alkaloids	Dragendorff's	+	+
	Mayer's	+	+
Flavonoids	Sodium Hydroxide	+	+
Phenolic compounds	Ferric chloride and potassium ferrocyanide	+	+
Saponins	Froth test	+	+
Steroidal compounds	Chloroform and conc. Sulfuric acid	+	+
Tanins	Ferric chloride	+	+

CHAPTER 5. DISCUSSION

5.1 Extract yield

The extract of these mushrooms obtained using ethanol as the solvent showed variation in yield percentage (Table 1). The highest yield of extract was found to be *G. applanatum* (9.4%) followed by *S. ostrea* (7.6%). The extraction method, temperature, extraction time, phytochemical makeup, and solvent utilized all have a large impact on extraction efficiency (Ngo et al., 2017). These parameters determine the quantity of the extracts obtained. Variation in the yield can be due to the differences in these parameters between the two species.

This could be due to the difference in the composition in regards to the phytochemical makeup between *G. applanatum* and *S. ostrea.* The content of bioactive compounds like phenolics, alkaloids, flavonoids, and terpenoids is different between the extracts. *G. applanatum* had higher yield perhaps due to higher levels of primary and secondary metabolites as compared to *S. ostrea.* Over 600 distinct compounds have been isolated and identified from Ganoderma, including alkaloids, terpenoids, nucleobases, nucleosides, polysaccharides, proteins, steroids, and triterpenes (Galappaththi et al., 2022). Also above 200 various lanostane-type triterpenes have been reported from Ganoderma (Paterson, 2006).

5.2 Antidiabetic potential

Antidiabetic potential of the ethanol extracts of the mushrooms were calculated using acarbose as standard. The IC_{50} of ethanol extract of the *G. applanatum* was found to be 896.32 µg/ml and IC_{50} of ethanol extract of *S. ostrea* was found to be 723.55 µg/ml. The percentage inhibition of enzyme increases with respect to the concentration of acarbose as it ranges from 64% to 88%. There is concentration dependent increase in the percentage inhibition. Acarbose as the standard drug had highest percentage inhibition of 88% at 160 µg/ml. In the ethanol extract of *G. applanatum* the lowest inhibition rate was 24.86% at the concentration of 20 µg/ml and highest inhibition rate was 75.03% at the concentration 160 µg/ml. Similarly, ethanol extract of *S. ostrea* had the lowest inhibition rate of 48.61% and the highest inhibition of 83.35%. *S. ostrea* was found to be better in inhibiting the enzyme alpha amylase when compared to *G. applanatum*. The lower IC_{50} values indicate more potent anti-diabetic potential. Thus, ethanol extract of *S. ostrea* was found to be better anti-diabetic agent

than the ethanol extract of *G. applanatum*. Among the extracts of the mushrooms, extract of *S. ostrea* had highest % inhibition i.e. 83.35% with IC_{50} of 723.55 µg/ml followed by ethanol extract of *G. applanatum* with 75.03% and IC_{50} 896.32 µg/ml. The bioactive components in the extracts of *G. applanatum* and *S. ostrea* may be the cause of the antidiabetic effects. These elements might work in conjunction or individually to show antidiabetic activity.

Research indicates that tannins and terpenoids have ability to bind carbohydrates and proteins (Khan et al., 2014; Poongunran et al., 2015) due to which antidiabetic activity is shown. Beside these phenolic and flavonoids present in the mushrooms also effectively inhibit alpha-amylase activity based on the ability to form quinone with the 4-oxo-pyrane structure of the enzyme through the hydroxyl group at C-3 and C-4 of ring B (Sim et al., 2010). Inhibitory action of extracts increases with increased concentration. There is a dose dependent inhibitory activity.

5.3 Phytochemical screening

The phytochemical analysis was done with the help of reagents and reactions that produced different colors or precipitations which helped to indicate the presence or absence of a particular substance in the extract. *G. applantum* and *S. ostrea* are mushrooms which are packed with primary and secondary metabolites that can be useful for us. They contain biologically active mycochemicals. Primary screening of the fractions of *G. applanatum* extracts revealed the presence of alkaloids, flavonoids, phenolic compounds, tanins, steroid and saponins. Similarly, the *S. ostrea* extracts revealed the presence of alkaloids, flavonoids, phenolic compounds, steroids, tanins, saponins. Besides this *S. ostrea* has been found to have syringic acid, chrologenic acid, gallic acid, and catechin which exhibit anticancer, antitumor, anti-inflammatory, antioxidant, and antibacterial properties (Geetha et al., 2004; Lou et al., 2011; Taylor et al., 2005).

5.4 Acute toxicity test

Using the extracts of *G. applanatum* and *S. ostrea* to test for the toxicity in albino mice revealed that the extracts are non-toxic. No discernible behavioral changes were caused by administering the extracts orally in doses of 2000 mg/kg, and no signs of toxicity were found during the experiment. No issues with movement or sensitivity to touch were displayed. Water and food intake were both typical. These outcomes indicated that the extracts are not

hazardous to mice under the tested circumstances. Alkaloids, phenolic compounds, flavonoid, saponins, and steroids were found to be present in the extracts. Some of these substances are thought to be bioactive components that help with diabetes treatment. The extracts have the capacity to inhibit alpha amylase. Therefore, the chemical components of the extracts may aid in preventing diabetic problems or possibly act as a substitute for them.

CHAPTER 6. CONCLUSION

In this study two mushroom species *Ganoderma applanatum* and *Stereum ostrea* were used for evaluation of antidiabetic potential, toxicity testing and phytochemical screening. The alpha amylase inhibition assay was carried out in this study to test the antidiabetic potential of the extracts of *G. applanatum* and *S. ostrea*. The ethanol extracts of *G. applanatum* and *S. ostrea* obtained with soxhlet extraction were used for test. In the comparison of *in-vitro* alpha amylase inhibition of *G. applanatum* and *S. ostrea*, the latter was found to have higher inhibition percentage. The phytochemical screening revealed the presence of alkaloids, flavonoids, phenolic compounds, tanins, steroid and saponins in both the mushroom extracts.

Similarly, toxicity testing was performed with the ethanol extracts of *G. applanatum* and *S. ostrea.* In the toxicity testing study, both *G. applanatum* and *S. ostrea* extracts were found to be non-toxic when given orally to mice at 2000 mg/kg. Comparison of antidiabetic potential between the two extracts showed that ethanol extract of *S. ostrea* has better alpha amylase inhibition activity. This finding indicates that the mushroom has potential to be used in controlling diabetes. With proper research, *S. ostrea* can be used to develop alternate antidiabetic drug in the near future.

RECOMMENDATION

Based on the study following recommendation are made for further investigation:

- Studies for molecular characterization of the collected mushroom species.
- Study and comparison of antidiabetic potential of the mushroom extracts in in vivo condition with diabetes induced mice model.
- Studies to find the active phytochemical responsible for antidiabetic activity
- Studies to evaluate the mode of action and pathway of the antidiabetic activity

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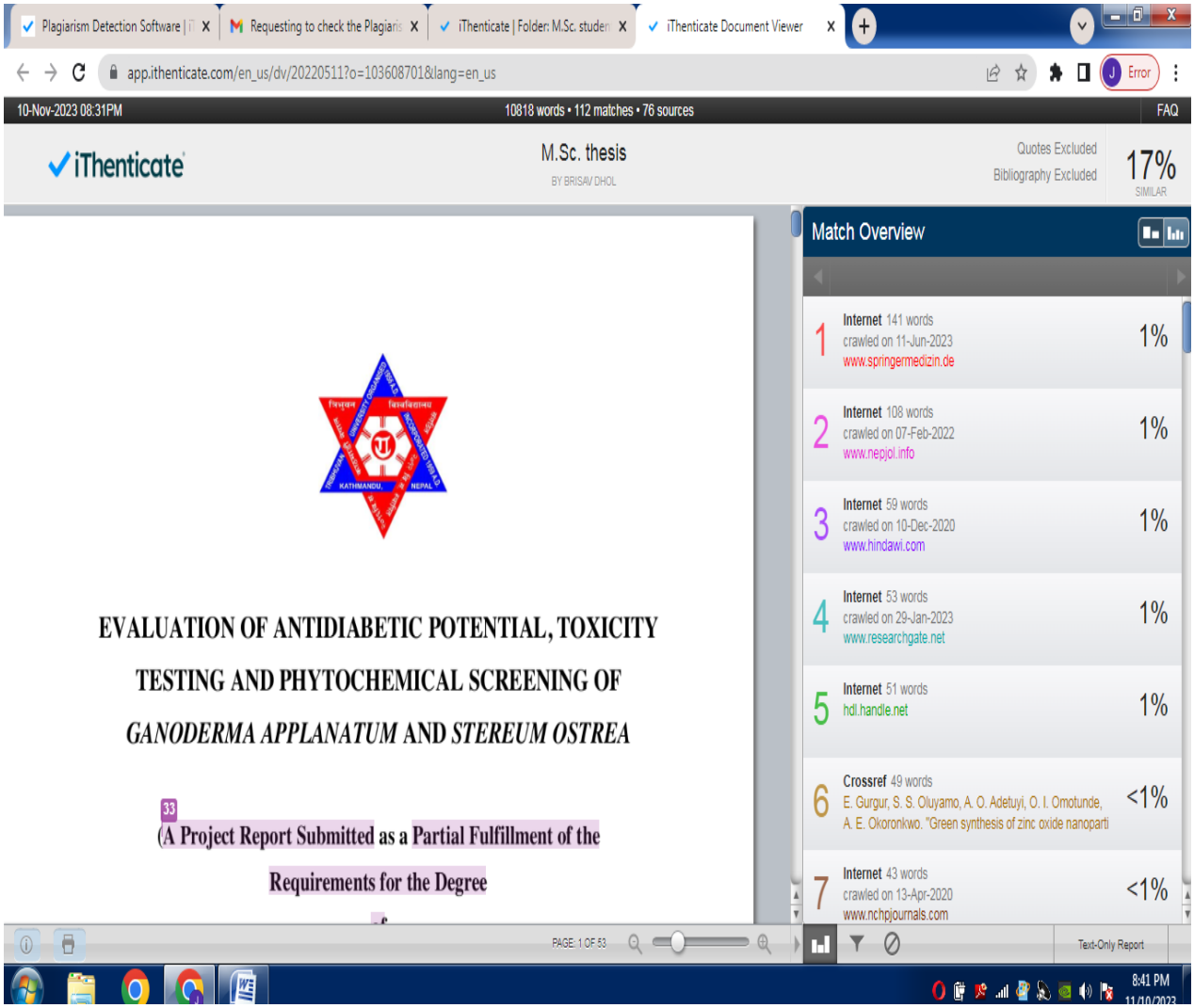
APPENDICES

Instruments required:

Sn	Name of the instruments	Company
1.	pH meter	Deluxe
2.	Petri plates	Borosil
3.	Beaker	Borosil
4.	Conical flask	Borosil
5.	Gloves	BSC care
6.	Autoclave	Indfos, Universal
7.	Mask	Face mask
8.	Cotton	Jajoo surgicals pvt. Ltd
9.	Separating funnel	Borosil
10.	Forceps	Surgicals forceps
11.	Culture jars	Borosil
12.	Laminar flow hood	Acco
13.	Measuring cylinder	Borosil
14.	Surgical blades	Ribbel
15.	Weighing balance	
16.	Heater	Vitco
17.	Scalpel	Ribbel
18.	Butter paper	Home foil
19.	Parafilm tape	Borosil
20.	Micropipette	Bionit
21.	Scissor	
22.	Aluminum Foil	

Chemicals Required

SN	Name of Chemicals	Company
1	Agarose	Rankem
2	KNO_3	CD4,sd Fine chemical
3	$(NH_4)SO_4$	Fisher
4	$MgSO_4.6H_2O$	Qualigens
5	$NaH_2PO_4.H_2O$	Sigma-Aldrich
6	$CaCl_2.2H_2O$	Qualigens
7	KH_2PO_4	Fisher scientific
8	Hcl	Qualigens
9	$MnSO_4.4H_2O$	Qualigens
10	$ZnSO_4.7H_2O$	Qualigens
11	$CuSO_4.5H_2O$	MERCR
12	Ethyl acetate	Qualigens
13	$Na_2MoO_4.2H_2O$	Qualigens
14	KI	Qualigens
15	Na_2EDTA	HIMEDIA
16	$FeSO_4$	Qualigens
17	Potato Dextrose Agar	HIMEDIA
18	Potato Dextrose Broth	HIMEDIA
19	NaCl	SIGMA
20	EDTA	LOPA
21	CTAB	HIMEDIA
22	Tris base	HIMEDIA
23	Ethanol	Bengal pharmaceuticals
24	Alpha amylase from malt	HIMEDIA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