EVALUATION OF NUTRACEUTICAL POTENTIAL OF *Morchella esculenta*

BY:-

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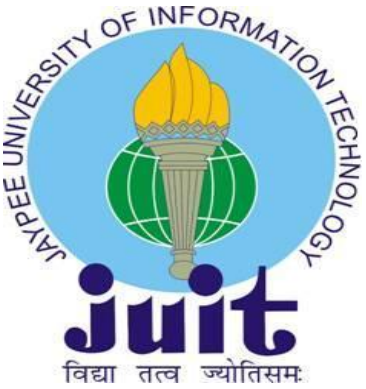
UNDER THE SUPERVISION OF DR. GARGI DEY



DEPARTMENT OF BIOTECHNOLOGY AND BIOINFORMATICS

JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY - WAKNAGHAT

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CERTIFICATE

This is to certify that the work titled **"EVALUATION OF NUTRACEUTICAL POTENTIAL OF*Morchella esculenta*"** submitted by **"Ms. Sadaf Ali and Ms. SachiVerma"** in the partial fulfillment for the award of degree of Bachelor of Technology (Biotech) of Jaypee University of Information Technology, Waknaghat has been carried out under my supervision. This work has not been submitted partially or wholly to any other university or institution for the award of this or any other degree or diploma.

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Abstract: The nutraceutical potential of *Morchella esculenta* gives them special status for the design of future strategies to develop novel dietary supplement. In the present study conducted, *M. esculenta* showed strong antioxidant activity with *Agaricus bisporus* taken as a control for different solvent extracts of petroleum ether, chloroform, methanol and aqueous. *A.bisporus* showed highest TPC and Flavanoid concentration in Methanolic Extract and *M. esculenta* showed in Aqueous Extract. Synergistic combination of both the mushrooms samples showed higher TPC and Flavanoid concentration than the individual mushroom extracts. Free radical scavenging activity of mushroom samples which showed that methanol extract of *A. bisporus* has highest % inhibition and methanol extract of *M. esculenta* has highest % inhibition. Synergistic combination of both the mushrooms samples for scavenging capacity showed a noticeable increase. *M. esculenta* showed more LPO inhibition than *A. bisporus*. Concluding from the results discussed above, *M. esculenta* showed higher free radical scavenging activity than *A. bisporus*. The synergy and additive effect of pair mushrooms proved to be beneficial as it increased the antioxidant activity many folds. Through *in silico* study we planned to screen out the best antioxidant components from *Ganoderma lucidum, Lentinus edodes, Cordyceps sinensis and Morchella esculenta* based on the basis of binding energy of the bioactive component to specific binding sites of proinflammatory markers such as LBP, LOX-2, COX-2, PLA-2, TLR-4, TNF-, and IL-1B. Higher Libdock scores indicate stronger receptor-ligand binding. LBP the best binding is seen with Ganoderal A (146.843) from *G. lucidum*, for COX-2 Galactomannan (142.256) from *M. esculenta*, for LOX-2 Galactomannan (142.988) from *M. esculenta,* for TLR-4 Galactomannan (146.156) from *M. esculenta,* for PLA-2 Galactomannan (128.877) from *M. esculenta,* for TNF-α Eritadenine (93.4152) from *L. edodes*, IL-1β (Interleukin-1β) was unable to dock any of the ligands. It was concluded that Galactomannan from *M. esculenta* showed highest Libdock scores for all the protein receptor and hence through this study predicted to be the best antioxidant bioactive component.

Keywords: *Morchella esculenta,* nutraceutical, antioxidant activity, synergistic, *in silico*

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LIST OF ABBREVIATIONS

ABBREVIATIONS	EXPANDED FORMS
ml	Milli Litres
mg	Milli grams
nm	nanometer
API	Active Pharmaceutical Ingredients
ROS	Reactive Oxygen Species
RNS	Reactive Nitrogen Species
AIDS	Acquired Immunodeficiency Syndrome
HIV	Human immunodeficiency virus
MEEP	*Morchella esculenta* extracellular polysaccharides
EP	Extracellular Polysaccharide
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
BHA	Butylated hydroxyanisole

LMW	Low Molecular Weight
FASSAI	Food Safety and Standards Authority of India
TNS	Total Nutraceutical Solutions, Inc.
PDB	Protein Database
MOE	Molecular Operating Environment
GAE	Gallic Acid Equivalent
BHT	Butylated Hydroxytoulene
TPC	Total Phenolic Content
ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
DPPH	2,2-diphenyl-1-picrylhydrazyl
LPO	Lipid Peroxidation
TBARS	Thiobarbituric acid reactive substances
TCA	Trichloroacetic Acid
TBA	Thiobarbituric acid
MDA	Malondialdehyde
NMR	Nuclear Magnetic Resonance
NCBI	National Center for Biotechnology Information

NIH	United States National Institues of Health
LBP	Lipolysaccharide binding protein
PLA-2	Phospholipase-A2
COX-2	Cyclo-oxygenase -2
LOX-2	Lipoxygenase-2
TNF-α	Tumour Necrosis Factor- α
TLR-4	Toll like receptor-4
IL-1β	Interleukin-1β

1. INTRODUCTION

A **mushroom** is the fleshy, spore-bearing fruiting body of a fungus, which is produced above ground on soil or on its food source. They are considered as having high nutritive value and low calorie food with good quality proteins, some vitamins and minerals. Mushrooms are an important and attractive natural source of food and medicine (Selima Khatun *et al*., 2011).

Mushrooms have gained importance over the last few decades and have become attractive as a functional food and as a source for the development of drugs and nutraceuticals (Lakhanpal and Rana, 2005) responsible with their antioxidant, antitumor (Jones and Janadhanan, 2000) and antimicrobial properties. Mushrooms have important present status for nutraceuticals which possess a potential to design future strategies for improving human health.

"A **nutraceutical** can be defined as a substance that maybe considered a food or a part of a food that provides medical or health benefits like the prevention and treatment of disease by **Dr.Stephen DeFelice**". There are many species of mushrooms which are cultivated and wild, edible and non-edible that have been studied for nutritional and nutraceutical components (Lakhanpal and Rana, 2005). The active components found in mushrooms include polysaccharides, dietary fibres, oligosaccharides, triterpenoids, peptides and proteins, alcohols and phenols, and mineral elements such as zinc, copper, iodine, selenium and iron, vitamins, amino acids etc. (Pardeshi and Pardeshi, 2009).

Morchella esculenta is one of the most expensive and wild edible mushrooms with a strong flavour and aroma.

Morchella esculenta possess anti-inflammatory, antitumor, antioxidant and antimicrobial activities (Mau, Chang, Huang, & Chen, 2004; Nitha, Meera, & Janardhanan, 2007; Nitha and Janardhanan, 2008; Nitha, Fijesh, & Janardhanan, 2011; Alves et al., 2012). *Morchella esculenta* species are reported to minimize oxidative damage in organisms that occurs in several chronic diseases (Ferreira et al., 2009). Phenolic compounds, tocopherols and organic acids are considered to be the most responsible for antioxidant activity of mushrooms (Ferreira et al., 2009; Reis et al., 2012; Leal et al., 2013). Steroids (mainly ergosterol derivatives) and polysaccharides isolated from *Morchella esculenta* was reported to possess both in vitro and in vivo antioxidant and NF-kappa B inhibiting properties (Meng et al., 2010; Kim, Lau, Tay, & Blanco, 2011). Furthermore, galactomannan was also isolated from it showed immunostimulatory properties (Duncan et al., 2002).

The particular characteristics of growth and development of *Morchella esculenta* in nature result in the accumulation of a variety of secondary metabolites such as phenolic compounds, steroids, polysaccharides, β-glucans, vitamins, proteins, several of them with biological activities (Lakhanpal and Rana, 2005) which are chemically diverse and explored in traditional medicines.

2. AIMS OF THE STUDY

The aims of our research project are:

1. To evaluate the nutraceutical potential of ***Morchella esculenta*** .

OBJECTIVES:

1. Extraction of bioactive components from ***Morchella esculenta.***

2. Estimation of Nutraceutical potential

   - Total Phenolic Content
   - Flavanoid Content
   - ABTS Assay
   - DPPH Assay
   - Lipid Peroxidation

3. Virtual Screening of mushroom nutraceuticals.

3. REVIEW OF LITERATURE

3.1 Mushrooms

Different mushroom species estimated on earth are about 1,40,000 of which only about 10% are known. Of approximately 14,000 species, 50% are edible, more than 200 are safe and about 700 species are known to possess pharmacological properties (Reshetnikov *et al*.2001).Mushrooms contain many substances and several of them can be used as active pharmaceutical ingredients (API's) which have potential to be developed as nutraceuticals. The long list includes polysaccharides, phenolics, proteins (fungal immunomodulating proteins; lectins, glycoproteins and non-glycosylated proteins and peptides), polysaccharide–protein complexes, lipid components (ergosterol), and terpenoids, alkaloids, small peptides and amino acids, nucleotides and nucleosides. This represents a great assortment of biological properties which include antioxidant, antitumor, anticancer, antimicrobial, immunomodulatory, anti-inflammatory, antiatherogenic and hypoglycemic actions. (Chang *et al*.2003).

A comparative study was done among the following *Ganoderma lucidum , Lentinus edodes , Morchella esculenta , Cordeceps sinensis , Cantherallus cibarius , Agaricus bisporus* to know their present research status. Table 3.1.1, Table 3.1.2, Table 3.1.3 represents the bioactive components present and their medicinal properties of the mushrooms specified above, phenolic and tocopherols presents in different mushroom species.

Table 3.1.1: Contains geographical location, species, bioactive component and functions of different mushroom species.

SPECIES OF MUSHROOMS	GEOGRAPHICAL LOCATION	SPECIES	MAIN BIOACTIVE PRINCIPLE	EXCLUSIVE FUNCTION
GANODERMA (REISHI)	**INDIA: Garhwal himalaya, uttarakhand (khirsuforests),Himachal Pradesh, Jammu and Kahmir**	**80 species *G. lucidum, G. tsugae, G.resinaceum, G. boninense, G. colossum, G.formosanum, G.neojaponicum, G. applantum,***	**Triterpenes (ganodermin acids), Polysaccharide (B- glucan), Peptidoglycan, Ergosterol, Coumarin, Mannitol, Alkaloids**	**Anti cancer, Anti-inflammatory, Liver problems, insomnia, gastric ulcers, Lowers blood pressure, Anti-allergic**

LENTINUS (Shiitake)	**INDIA: Western ghats (shimoga dist.), parts of North Western Himalaya**	**40 species *L. edodes,* *L. Sajar caju,* *L. torulosus,* *L. cladopus,***	**Eritadenine, Polysaccharide Lignin (EP3, EPS4), Centinamycin A & B, Ergosterol**	**Anti cancer, Immune stimulation, Treatment of HIV, Cholestrol reduction,**
AGARICUS *(*Button mushroom*)*	**INDIA: Garhwal himalaya (pauri garhwal & khirsu forest), Shimoga dist, Karnataka**	**200 species *A. bisporus,* *A. blazei,* *A. campestris,* *A. augustus,* *A. moelleri,***	**Lectins, B-glucan, Carbohydrate, Selenium**	**Antitumor, Immunological enhancement, Treatment for AIDS, Diabetes, Hypotension, Hepatitis**
CORDYCEPS (Caterpillar fungus) CHINA- DongChongXiaCho JAPAN- TocKuKaso	**INDIA: Uttarakhand, Shimoga dist., Karnataka**	**400 species *C. sinesis,* *C. militaris,* *C. friessi,* *C. cinerius,* *C. cibarius***	**Cordycepins, Adenosine, Ergosterol, Crude protein & other amino acids**	**cholestrol, Immune system stimulation, Asthma, arrhythmia, Prevent blood platelet aggregation, impotence**
MORCHELLA (Morels)	**INDIA: Garhwal Himalaya pauri gharwal, nagdev-Jandidhar forest), HP (shimla district)**	**≈60 species *M. esculenta,* *M. conica,* *M. elata***	**Ergosterol, Polysaccharide (MEP I, MEP II), Galactomannan , Beta- glucan**	**Immunostimulator activity, Anti-oxidant activity, Anti-inflammatory activity, Cardiovascular health, Reduces risk of breast and prostate cancer, Weight loss, Diabetes treatment**
CANTERALLUS (Chanterelle)	**INDIA: Khajjiya forest, HP, Garhwal dist., Shimoga dist, Karnataka,**	**≈100 species *C. cibarius,* *C. friessi,* *C subcibarius,* *C. cinerius***	**B-carotene, Vitamin D,C, Potassium**	**Antimicrobial Antibacterial Antifungal properties**

Table 3.1.2: Phenolic compounds present in different mushrooms

Phenolic compounds	Mushroom species	References
p-Hydroxybenzoic acid	*Agaricus bisporus, Lentinus edodes*	[19,20]
Protocatechuic acid	*Agaricus bisporus, Lentinus edodes, Morchella esculenta, Ganoderma lucidum*	[18,20]
Gallic acid	*Agaricus bisporus, Lentinus edodes, Morchella esculenta, Ganoderma lucidum, Cantherallus cibarius*	[18,21]
5-Sulfosalicylic acid	*Ganoderma lucidum*	[21]
Cinnamic acid	*Agaricus bisporus, Lentinus edodes, Cantherallus cibarius*	[19,20]
p-Coumaric acid	*Cantherallus cibarius*	[18,20]
Caffeic acid	*Cantherallus cibarius, Morchella esculenta*	[18,22]
Ferulic acid	*Cantherallus cibarius, Morchella esculenta*	[18]
3-*O*-Caffeoylquinic acid	*Cantherallus cibarius*	[22]
4-*O*-Caffeoylquinic acid	*Cantherallus cibarius*	[22]
5-*O*-Caffeoylquinic acid	*Cantherallus cibarius*	[22]
Quercetin	*Ganoderma lucidum*	[21]
Rutin	*Cantherallus cibarius*	[22]
Kaempferol	*Ganoderma lucidum*	[21]
Myricetin	*Ganoderma lucidum, Agaricus bisporus*	[21]
Catechin	*Ganoderma lucidum, Lentinus edodes*	[21]
Hesperetin	*Ganoderma lucidum*	[21]
Naringin	*Ganoderma lucidum, Agaricus bisporus*	[21]
Formometin	*Ganoderma lucidum*	[21]
Biochanin	*Ganoderma lucidum*	[21]
Pyrogallol	*Ganoderma lucidum, Agaricus bisporus*	[21]
Tannic acid	*Cantherallus cibarius*	[18]

Table 3.1.3: Tocopherols present in different mushrooms

Tocopherol	Mushroom species	References
α-tocopherol	*Agaricus bisporus, Cantherallus cibarius, Morchella esculenta*	[16,19,22]
β-tocopherol	*Agaricus bisporus, Cantherallus cibarius*	[19,22]
γ-tocopherol	*Ganoderma lucidum, Lentinus edodes, Cantherallus cibarius*	[19,22]
δ-tocopherol	*Morchella esculenta*	[16]

3.2 *Morchella esculenta*:

Morchella esculenta is an economically important wild species. The fruiting bodies are edible and are used as flavors in soups and gravies which enhances its taste and aroma. *Morchella esculenta* is an expensive product because of its rich nutritional value coupled with a unique flavour and its inability to be cultivated . The local people cook ascocarps (the fruiting body) which is mixed with rice and vegetables. It is considered as nutritious as meat or fish (Pankaj *et al.*2002).

Fig 3.2 Fruiting bodies of *Morchella esculenta*
(collected from upper region of Shimla, Kotkhai)

3.2.1 Taxonomy

Phylum: Ascomycota

Class: Pezizomycetes

Order: Pezizales

Family: Morchellaceae

Local name : Gutchichyau, Morelchyau, Chyau, Guche, Pote Chyau.

English name - Morel Mushroom

3.2.2 Habitat

The most therapeutic and highly priced mushroom found in Himalayan ranges of north western extremity of India. The species grow on soil rich in organic matter, in loamy soil, in pure and mixed coniferous forests, broad leaved forests, in open areas or grasslands, in apple orchard or under various types of shrubs in the open under direct and indirect shade.

Morchella esculenta mainly appear during the spring season from March to May when snow begins to melt and is accompanied by some precipitation (Lakhanpal and Rana, 2005).

3.2.3 Nutritive value

Morchella esculenta has an excellent flavour, reputed to be superior to that of other mushrooms. Not only morels but their mycelia have been reported to have the same nutritive value (Cochrane *et al*.1958). The vitamin content was also recorded with the presence of thiamine, niacin, riboflavin, pantothenic acid, pyridoxine and $B_{12.}$ Some minerals such as calcium, magnesium and iron are present (Samajhpati *et al*. 1978)

Table 3.2.3: Nutritive Value of *Morchella esculenta*

Content	% content	References
Moisture	**83.06**	**[2]**
Ash	**0.96**	**[2]**
Fat	**2.97**	**[2]**
Fibre content	**1.96**	**[2]**
Protein	**22-51%**	**[1,2]**

3.2.4 Traditional knowledge :

The studies were conducted to gather information on collection, myths associated with their cultivation, propagation in nature, local names and to see the impact of *Morchella esculenta* collection on social-economic aspects of the inhabitants.

The most common belief widespread among local people which they have heard from their ancestors is that *Morchella esculenta* emerge from ground after lightening strikes and that they would come up in greater abundance in the burnt up areas. As in this context they are considered wild and uncultivable. Only the local inhabitants know about them and they can easily identify them.

They are mostly collected by low income group people. The middlemen buy these collections at site at the rate of rupees 1000-2000 , whole sellers at the rate of 3000-4000 and these ultimately reach the consumers at the rate 10,000-12,000 /Kg.

Morchella esculenta collection adds a substantial amount to the income of the poor people. Thus it is a source of additional income for the marginal farmers in high hill zones of the Himachal Pradesh. (Lakhanpal and Rana, 2005)

3.2.5 Life cycle of *Morchella esculenta*

Morels' life cycle begins with plasmogamy between strain variant which create a heterokaryotic mycelium (secondary mycelium) which on later stages form heterokaryotic sclerotia. This ascocarp is covered with minute elongated sacs called ascus. Ascus contains microscopic spores, approximately eight spores per ascus lined up like small eggs. The beginning of the life cycle of the morels occurs when these spores escape in the air and land on appropriate food source such a moist dead, rotting or decaying plant life.

Hatching of the spores produces small mess like structures called hyphae. Hyphae spreads on the entire food source producing an interwoven mat or feeding mesh called mycelium. Under unfavourable conditions, the mycelium stays in a dormant condition forming hardened protective bodies called sclerotia. Sclerotia develops into ascocarps as favourable conditions occur.

(Volk and Leonard *et al*.1989)

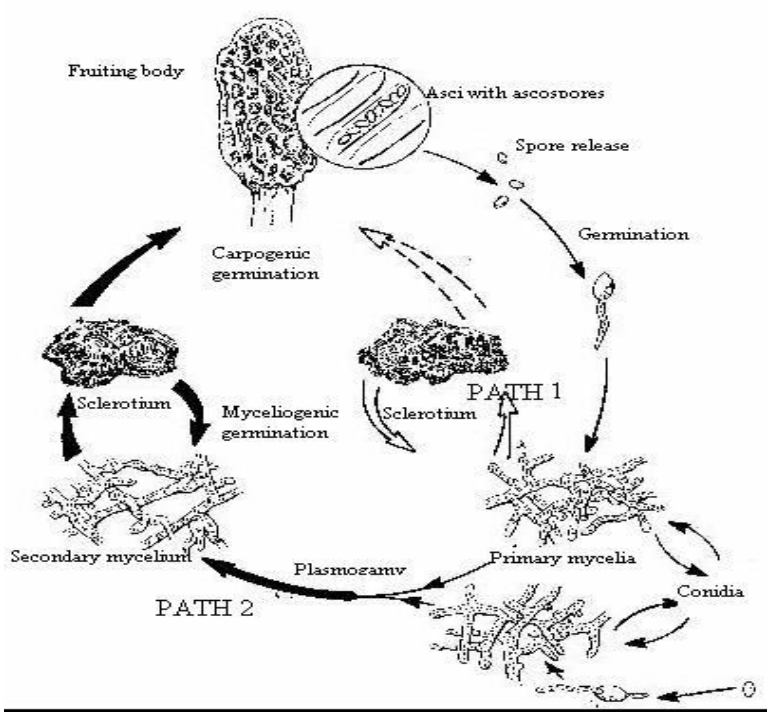


Fig 3.2.5 Life Cycle of Mushrooms

3.2.6 Cultivation of morchella esculenta through scientific intervention

A process for cultivating Morchella ascocarps using mycelium and a tree seedling, the tree seedling having a root system and shoot system. The process includes the steps of inoculating the root system with the mycelium to produce an inoculated tree seedling; stimulating the mycelium to form sclerotia by severing the shoot system from the root system; and inducing the sclerotia to produce ascocarps by providing conditions conducive to the formation of ascocarps. Also, the growth of trees may be accelerated by inoculating the root system with the mycelium. (PATENT NO. : US 6,907,691 B2)

3.3 Nutraceutical Potential of *Morchella esculenta*

The term *nutraceutical* is used to describe the substances or combination of substances that have considerable potential to be developed as dietary supplements and used in the prevention and treatment of various human diseases without having any side-effects.

A *mushroom nutraceutical* is a refined/partially refined extract from either the mycelium or the basidioma and ascoma, which is consumed in the form of capsule or tablet as a dietary supplement (not as a regular food) with potential therapeutic applications (Chang *et al.*2003).

When natural species are considered as a sources with potential medicinal properties, (Baker *et al 1995*) one should take into account the following:

i) evidence regarding the traditional usage of the substance by indigenous populations,
ii) abundance of the species in nature and
iii) sustainable utilisation of the species.

Another extremely important criterion when searching for novel bioactive compounds is the uniqueness of the organism and its potential to produce secondary metabolites (Donadio *et al*. 2002).

Nutraceutical potential of morels has been investigated (Lakhanpal *et al*.2010). Nutraceutical attributes of morels compare favourably with the other mushroom species. They are even a better source of Polysaccharides, crude fibre, nucleic acids, minerals especially Se, Zn, K, Cu, Na, and Ca, Vitamin (B1, B2, C,A, D and K; proteins and all the essential amino acids. They are free from cholesterol. Hence they are good of nutraceutical use.

Polyunsaturated fatty acids predominate over monounsaturated and saturated fatty acids. Linoleic, oleic and palmitic acids were abundant with considerable amounts of α- linolenic acid. Concerning the tocopherols, the α-, γ- and δ-tocopherols were also quantified.Oxalic acid, fumaric acids, malic acid , quinic acid and citric acid were also present. Protocatechuic and phydroxybenzoic acids were found and p-coumaric acid was quantified (Heleno *et al*.2013).

They represents a great assortment of biological and medicinal properties which include antioxidant, antitumor, anticancer, antimicrobial, immunomodulatory, anti-inflammatory.

3.3.1 Medicinal Properties of *Morchella esculenta*

3.3.1.1 Anti-Oxidant Activity

Natural products with antioxidant activity help the endogenous defense system. In this view the antioxidants present in the diet are supposed to be of major importance as protector agents reducing oxidative damage. An essential condition for normal organism functioning is maintenance of equilibrium between free radical production and antioxidant defenses. Free radicals are mainly in the form of Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS). Overproduction of free radicals disturbs the equilibrium and is known as oxidative stress. In this condition, excess free radicals damages DNA and proteins, cellular lipids which affects normal functioning and leads to various diseases. As a result they have emerged to be an attractive functional food and as a source for the development of potential nutraceutical, taking benefit of the additive and synergistic effects of the bioactive compounds present. The antioxidants present in them include phenolic compounds (phenolic acids and flavonoids), carotenoids, tocopherol and ascorbic acid that are important protective agents for human health.
Phenolic compounds, tocopherols, ascorbic acid, and carotenoids are antioxidants present in mushrooms which could be extracted for being used as active pharmaceutical ingredient (API) against chronic diseases related to oxidative stress. Also, mushrooms might be used directly in diet and promote health, taking advantage of the additive and synergistic effects of all the bioactive compounds present.

Morchella esculenta species are reported to minimize oxidative damage in organisms that occurs in several chronic diseases (Ferreira *et al*.2009). Phenolic compounds, tocopherols and organic acids are considered to be the most responsible for antioxidant activity of mushrooms (Ferreira *et al*.2009; Reis *et al*.2012; Leal *et al*. 2013).

Morchella esculenta extracellular polysaccharides (MEEP) showed anti-oxidative activity. An in vitro antioxidant assay showed that MEEP exhibited strong hydroxyl radical scavenging activity and moderate 1,1-diphenyl-2-picryldydrazyl radical scavenging activity and reductive power. For antioxidant testing *in vivo*, MEEP were orally administered over a period of 60 days in a D-galactose induced aged mice model. Administration of the polysaccharides inhibited significantly the formation of malondialdehyde livers and serums, and raised the activities of antioxidant enzymes and the total antioxidant capacity in a dose-dependent manner. Furthermore, we also observed that MEEPs markedly enhanced the body's immune system by measuring macrophage phagocytosis and splenocyte proliferation in D-galactose induced mice. These findings suggest that EPs from *Morchella esculenta* are a promising source of natural antioxidants and immunoenhancing drugs (LiHong Fu *et al*. 2013).

Methanolic extracts prepared from the mycelia of *Morchella esculenta* showed high antioxidant activity (85.4%) at 25 mg/ml; for comparison, the activities of the common antioxidants ascorbic acid, α- tocopherol and BHA were 36.9%, 80.5% and 98.1% (at 0.5 mg/ml), respectively (Mau *et al*. 2004).

High concentrations of ROS and RNS react with each other to form more potent reactive species such as peroxynitrite. ROS and RNS damges both nuclear and mitochondrial DNA, RNA, lipids and proteins by various reactions reactions such as nitration, oxidation and halogenations leading to mutations (Sawa *et al*.2006).

Morchella esculenta also showed higher lipid peroxidation inhibition (Sandrina A. Heleno *et al*.2013). The best biomarker of lipid peroxidation is set of arachidonic acid oxidation products termed as isoprostanes (Tang *et al*.2002). Short lived lipid hydroperoxides are initial products of unsaturated fatty acid oxidation which when reacted with metals produce a number of reactive products (e.g. aldehydes and epoxides). The major aldehyde product of lipid peroxidation is Malondialdehyde (MDA) which is mutagenic in mammalian cells and carcinogenic in rats (Yau *et al*.1979). MDA can react with DNA bases dG, dA and dC to form adducts.

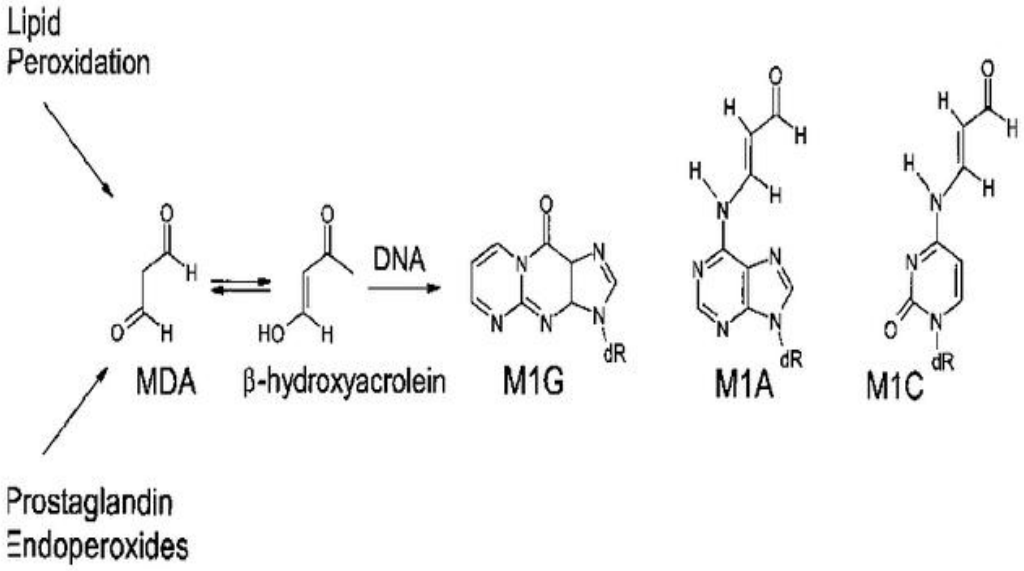


Fig3.3.1.1: Synthesis of MDA and its reaction with DNA bases

3.3.1.2 Immuno-Stimulatory Effect

The main nutritional components of *Morchella esculenta* were described as follows: protein 32.7%, fat 2.0%, fibre 17.6%, ash 9.7%, and carbohydrates 38.0%: polysaccharides were the main components of the extract from *Morchella esculenta.* Polysaccharides from natural sources are a class of macromolecules that could profoundly affect the immune system; they have potential as immunomodulators for a wide range of clinical applications. A water-soluble polysaccharide, MEP, was obtained from the fermentation broth of *Morchella esculenta*. Two fractions of this polysaccharide (MEP-I and MEP-II), MEP had typical immunostimulatory activity. The immunomodulatory activity of MEP was investigated to

provide some evidence for the development and application of functional food or polysaccharide drug. (Cui and Chel *et al*.2011)

Isolation of Galactomannan enhances Macrophage Activation from *Morchella esculenta*.
Galactomannan exhibits immunostimulatory activity. Galactomannam comprises about 2.0% of dry fungal material weight, and its glycosyl components include mannose (62.9%) and galactose (20.0%)

Additional polysaccharides have been isolated from the nutrient liquor resulting from fermentation of *Morchella esculenta*. These include two polysaccharide fractions, MEP-SP2 and
MEP-SP3, that have molecular weights of 23,000 Da and 44,000 Da, respectively. The glycosyl composition of MEP-SP2 is mannose, glucose, arabinose, and galactose (mole ratio of 1.75:4.13:0.71:0.68). The glycosyl composition of MEP-SP3 is xylose, glucose, mannose, fructose, arabinose, and galactose (mole ratio of 3.58:14.9:3.85:1.77:51.3:0.53). Another polysaccharide isolated from this material has a molecular weight of 11,500 Da and is composed of xylose, glucose, arabinose, and galactose (mole ratio of 0.29:0.24:0.61:0.39). (Christine *et al*.2002).

3.3.1.3 Anti-inflammatory activity

Anti-inflammatory activity of a 50% ethanolic extract of *Morchella esculenta* mycelium grown in submerged culture has been determined by carrageenan induced acute and formalin induced chronic inflammatory models. Oral administration of 500 mg/kg body weight of extract showed 66.6% and 64.2% inhibition of acute and chronic inflammation, respectively (Nitha and Janardhanan, 2005).

Later work showed further elaborated on the dose-dependent inhibition of both acute and chronic inflammation, and suggested that the activity is comparable to that of the standard reference drug, Diclofenac (Nitha et al., 2007).

3.3.1.4 Antitumor activity

The antitumor activity of a 50% ethanolic extract of *Morchella esculenta* mycelium grown in submerged culture was determined by the mouse solid tumor model induced by Daltons Lymphoma Ascites cells. Oral administration of 1 g/kg body weight of the morel extract resulted in a 74.1% inhibition in tumor volume and 79.1% decrease in tumor weight 30 days after tumor cell implantation (Nitha and Janardhanan, 2005).

Later research further confirmed the antitumor activity of the extract against both ascites and solid tumours (Nitha *et al*., 2007).

3.4 Synergistic Effects

Combinations of two or more components that would work together synergistically in order to have an increases medicinal effectiveness. For instance it would be useful to produce a potent combination of antioxidants that function synergistically to inhibit the generation of free radicals.
The antioxidant activity of different mushrooms is different, the level of single antioxidant in food do not necessarily reflect their total antioxidant capacity but because of the possible synergistic interactions among the antioxidant components in a food matrix.

In search of synergistic effects in antioxidant capacity of combined edible mushrooms (Bruno Queiros *et al.*2009). In this paper it showed that synergistic effects are better then individual antioxidant components.

3.5 Virtual screening of mushroom nutraceuticals

In this post genomic era, research increasingly focuses on omics approach for validation and successful optimization. Experimental and computational efforts are dedicated to large scale generation and analysis of information derived from 3D structures and dynamics of proteins.

Computational generation of protein structure via modeling and docking of protein with potential ligand are important steps in computational proteomics.

Docking is used for computational schemes that attempt to find the best matching between two molecules- a receptor and ligand. Top ranked may be tested for binding affinity which is the starting point for validation and optimization.

Key ingredients in docking:

- Representation of the system
- Accurate structural modeling
- Correct prediction of activity
- Ranking of potential solution.

In silico molecular docking plays a role in identifying novel ligands for receptors and identifying the interaction of nutraceuticals with different markers and to screen out the novel candidate for anti inflammatory response which screens molecules by orienting and scoring them into the binding side of the protein.

The process starts with the application of docking algorithms that POSE small molecules in the active site which involves prediction of ligand confirmation and orientation (posing) within targeted binding site and their interaction energies were identified using scoring functions.

The pharmacophore approach is applied which takes in consideration that molecules are active at the receptor binding site because they possess features that favor the target interaction site and are geometrically complementary to it (Daisy.P *et al*.2012).

There have been some reports of molecular docking done in some species of mushrooms.

Using molecular docking to investigate the anti-breast cancer activity of low molecular weight compounds present on wild mushrooms (Hugo J.C.Froufe *et al*.2011). Mushrooms represent an unlimited source of compounds with antitumor and immunostimulating properties and mushroom intake as been shown to reduce the risk of breast cancer. A large number of LMW (low molecular weight) compounds present in mushrooms have been identified which include phenolic acids, flavonoids, tocopherols, carotenoids, sugars and fatty acids. In order to evaluate which wild mushroom LMW compounds may be involved in anti-breast cancer activity they selected a representative dataset of 43 LMW compounds and performed molecular docking against 3 known protein targets involved in breast cancer (Aromatase, Estrone Sulfatase and 17β-HSD-1) using AutoDock4 as docking software. The estimated inhibition constants for all LMW
compounds were determined and the potential structure-activity relationships for the compounds with the best estimated inhibition constants was established.

Pharmacophore-based discovery of FXR-agonists, Part II: Identification of bioactive triterpenes from *Ganoderma lucidum* (Ulrike Grienke *et al*.2011). The farnesoid X receptor (FXR) belonging to the metabolic subfamily of nuclear receptors is a ligand induced transcriptional activator. Its central function is the physiological maintenance of bile acid homeostasis including the regulation of glucose and lipid metabolism. Accessible structural information about its ligand-binding domain renders FXR an attractive target for in silico approaches. Integrated to
natural product research these computational tools assist to find novel bioactive compounds showing beneficial effects in prevention and treatment of the metabolic syndrome, dyslipidemia, atherosclerosis, and type 2 diabetes. Virtual screening experiments with structure-based pharmacophore models, previously generated and validated, revealed mainly lanostane-type triterpenes of the *Ganoderma lucidum* as putative FXR ligands. To verify the prediction of the in silico approach, two Ganoderma fruit body extracts and compounds isolated were pharmacologically investigated. Pronounced FXR-inducing effects were observed for the extracts at a concentration of 100 lg/mL. Intriguingly, five lanostanes out of 25 secondary
metabolites from G. lucidum, that is, ergosterol peroxide, lucidumol A, ganoderic acid TR, ganodermanontriol , and ganoderiol F, dose-dependently induced FXR in the low micromolar range in a reporter gene assay. To rationalize the binding interactions, additional pharmacophore profiling and molecular docking studies were performed, which allowed establishing a first structure–activity relationship of the investigated triterpenes.

3.6 Regulatory perspective of nutraceuticals in India

Commercialization of mushroom based products is rapidly expanding with current market value of dietary supplement products worldwide estimated to be in excess of $ 12 million per year. However mushroom based dietary supplements are highly diverse and major problem faced is the lack of standard production protocols fir guaranteeing product quality. In 1990, US diet supplement sale were evaluated at $3 billion which increased $10 million 14 million. In 1994 worldwide sales of medicinal mushroom derived products was estimated around $3.8 billion which has risen to $6 billion in 2000 and $11 billion in 2002. Of this, US and Europe formed the largest markets accounting to 36 per cent and 25 per cent respectively.

In 2010, the Indian nutraceutical industry was estimated at $2 billion, roughly 1.5 per cent of the global nutraceutical industry. Broad segments of Indian nutraceutical industry include dietary supplement (40 per cent) and functional food and beverage market (60 per cent). The total Indian nutraceuticals market is expected to be approximately $5 billion in 2015.

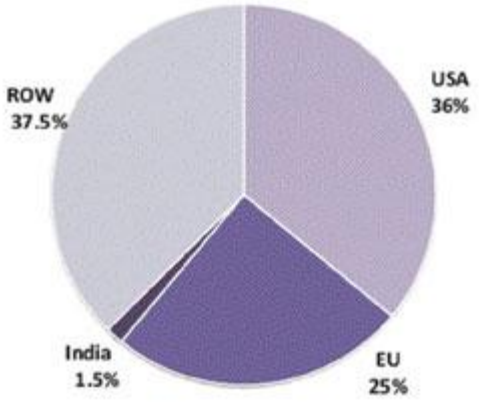


Fig 3.6: Nutraceutical industry

In 1995 the market value of *Ganoderma* based dietary supplements alone was estimated to be around $1,624 million and the value of equivalent products derived from *Lentinus edodes* was of same order.

3.6.1 Mushroom based Nutraceutical products

Immulink MBG® (Mushroom Beta Glucan) is a carbohydrate polymer isolated from the mycelium of *Ganoderma lucidum*, built from chains of D-glucose units linked by beta-glycosydic linkages.

Total Nutraceutical Solutions, Inc. (TNS) launched mushroom-based Vitamin D supplement from *Agaricus Blazei Murill*

***Ganoderma lucidum*:** Organo Gold's pure form of *Ganoderma*, certified organic in China, the United States and the European Union.

***Ganoderma lucidum* Mycelium:** Gathered from an 18-day-old *Ganoderma lucidum* mushroom, this supplement is known for its antioxidant properties, as well as the polysaccharide Beta-glucan, which can help stimulate immune function.

***Ganoderma* Spore Powder:** Harvested at the pinnacle of its potency, due to OG's patented shell-breaking technology, these spores are richer in naturally-beneficial compounds such as polysaccharides, triterpenes, and selenium than regular *Ganoderma lucidum*.

***Cordyceps sinensis* Mycelium Powder Capsule** richly cantains active components, *Cordyceps sinensis* polysaccharide,*Cordyceps sinensis* acid,etc. which will improve your immune system and long-term usage will benefit your health.

***Shiitake* mushroom extract powder with polysaccharide** and ***Shittake* mushroom mycelium extract powder** is found to be particularly valuable for treating hepatitis, viral infections and can also lower blood levels of cholesterol and lipids.

It has been proposed that mushroom nutraceuticals should be regulated as a special class of intermediate class of compounds between pharmaceuticals and healthy foods. To maintain good quality product need to improve quality control strategies with intervention of regulatory authorities.

3.6.2 Regulatory guidelines

Since nutraceuticals are not a part of pharma and drugs formulation, rules and regulations also tend to be different for this segment. Indian government has recently implemented the new law FSSAI (Food Safety and Standards Authority of India).

The Food Safety and Standards Act, 2006 consolidates eight laws governing the food sector and establishes the Food Safety and Standards Authority of India (FSSAI) and its other allied committees to regulate the sector.

FSSAI is aided by several scientific panels and a Central Advisory Committee to lay down standards for food safety. These standards include specifications for ingredients, contaminants, pesticide, biological hazards, labels and others.

The Act empowers the FSSAI and state food safety authorities to monitor and regulate the food business operators.

3.6.3 Current scenario

The Food Safety and Standard Rules, 2011 has been issued, effective from 5th May, 2011. The Food Safety and Standard Authority has also issued regulations about licensing and registration of food business, packing and labelling, food products standard and additive etc.

Thus, now there is one single legislation and specified authorities to regulate manufacture, sale and distribution of nutraceuticals, functional food and dietary supplement in India.

However, due to lack of clarity of specific regulations for registration of nutraceuticals and permitted additives, entrepreneurs intending to launch nutraceuticals in India is still facing the some challenges.

3.6.4 Regulatory requirements for entry in Indian market

In order to enter the Indian nutraceutical market, some of the very important areas of focus include **product evaluation, actual product analysis, procuring licenses and developing India specific health and label claims**.

Mushrooms are regarded as Super Foods due to the nutritional content. They are considered as an untapped reservoir of development of nutraceuticals. Mushrooms are often integrated in nutraceutical products targeting immune health because consumers nowadays are well-aware of the health benefits. In nutraceutical market only some of the mushrooms such as *Ganoderma Lucidum, Lentinus edodes, Cordyceps sinensis* have been successful in coming up with a nutraceutical product which creates a gap between others mushrooms and their potential to be developed as source of nutraceuticals.

Morchella esculenta found in Himalayan region which is considered a delicacy possess a potential to be developed as a nutraceutical. Uncloaking the mysteries in development of mushrooms as nutraceuticals there is a need for exploring natural resources through sustainable development. They represent an unlimited source of compounds with antitumor and immunostimulating properties .The antioxidants present in them include phenolic compounds (phenolic acids and flavonoids), carotenoids, tocopherol and ascorbic acid that are important protective agents for human health which could be extracted for being used as active pharmaceutical ingredient (API) and through fortification and enrichment forming a nutraceutical based product against chronic diseases related to oxidative stress which can be used directly in diet and promote health.

Intermolecular interactions between proteins and small ligands play essential roles in several life processes and understanding these interactions is critical for pharmaceutical and functional food industries. Molecular docking which is an *in silico* tool that predicts how a ligand interacts with a receptor usually by predicting the ligand free energy of binding and the three-dimensional structure of the ligand-receptor complex. Understanding these interactions of ligand and receptors the most suitable ligand that can be used in targeting of a particular disease can be identified and analyzed.

The development of potential nutraceutical, taking benefit of the additive and synergistic effects of the bioactive compounds present in different mushrooms can enhance their medicinal health effects as compared to the individuals.

4. Materials And Methods

An overview of the material and methods used in the study are described as follows:

4.1 MATERIALS

1. Study Subjects

- *Agaricus bisporus*
- *Morchella esculenta*

2. Apparatus and Instruments

- Hot Air Oven
- Soxhelet apparatus
- Rotary evaporator
- Spectrophotometer

3. Chemicals

Soxhelet Apparatus

- Petroleum Ether
- Methanol
- Chloroform
- Ethyl Acetate

Total Phenolic Test

- Gallic Acid (as standard)
- Sodium Carbonate
- Folin- Ciocalteu

Flavanoid content

- Quercetin (as standard)
- Sodium nitrite
- Aluminium chloride
- Sodium hydroxide

ABTS

- 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) or ABTS
- 80% ethanol
- Potassium Per Sulphate
- BHT(as standard)

DPPH

- 1,1-diphenyl-2-picrylhydrazyl
- 80% ethanol
- BHT(as standard)

Lipid peroxidation

- Tris–HCl buffer (pH 7.4, 20 mM)
- FeSO4 (10 µM; 0.1 ml)
- Ascorbic acid (0.1 mM; 0.1 ml)
- trichloroacetic acid (28% w/v, 0.5 ml)
- thiobarbituric acid (TBA, 2%, 0.38 ml)

4. Databases

- Pubchem
- PDB

5. Softwares

- MOE
- Discovery Studio Version 3.5

4.2 METHODS

4.2.1 Preparation Of Mushroom Extract

Morchella esculenta fruiting bodies were collected from upper region of Shimla, Himachal Pradesh. The fruiting bodies were dried in a hot air oven at 48°C for 48 hours. The dried fruiting bodies were crushed to powder by using electronic grinder.

Powered samples were taken in petroleum ether, chloroform, methanol, water in soxhlet extraction unit for extraction for about 24-30 hours .The residue was then obtained by rotary evaporator and was stored at 4°C for further use.(Chi *et al*. 2013)

The residue was redissolved in respective solvents to make different gradient of concentration ranging from 10 mg/ml to 80 mg/ml.

4.2.2 Synergistic preparation of Mushroom Pair Sample

In order to research the synergistic effect of the mushroom pair samples of *M. esculenta* and *A. bisporus*, they were mixed in equal proportion to make up to the final concentration. The screening out of the solvent extract chosen to test for synergy was done on the basis of best antioxidant activity with various assays in different solvents.

4.2.3 Estimation of Nutraceutical potential

4.2.3.1 Total Phenolic Activity

Total soluble phenolics in the individual mushroom extract and in the mushroom pair was determined with Folin-Ciocalteu reagent according to the method of Slinkard and Singleton (1977) by using gallic acid as a standard.

1 ml of extract of different concentration gradient ranging from 10 mg/ml to 80 mg/ml was mixed with 1 ml Folin-Ciocalteu reagent respectively with vigorous shaking and add 8 ml of distilled water to make up the volume in different test tubes. Mixed well and incubated for 90 min in dark. Absorbance was measured in 1cm cuvette at 765 nm by using spectrophotometer. The tests were performed in duplicates.

Gallic acid was used as a standard, and the total phenols were expressed as mg/g gallic acid equivalent using the standard standard curve equation: $y = 0.0014x$, $R^2 = 0.9801$ where y is absorbance at 765 nm and x is total phenolic content in the extracts.

4.2.3.2 Flavanoid Content

Flavanoid content in the individual mushroom extract and in the mushroom pair was determined by the method of Barros *et al.* 2008.Mushroom extract (250 µl) with different concentration gradient ranging from 10 mg/ml to 80 mg/ml was mixed with distilled water (1.25 ml) and $NaNO_2$ solution (5%, 75 µl) . After 5 min, $AlCl_3.H_2O$ solution (10%, 150 µl) was added. After 6 min, NaOH (1 M, 500 µl) and distilled water (275 µl) were added to the mixture . The solution was mixed well and the intensity of colour was measured at 510 nm. The tests were performed in duplicates.

The total flavanoid were expressed as mg/g Quercetin equivalent using the standard curve equation: $y = 0.110x$, $R^2 = 0.998$, where y is absorbance at 510 nm and x is total flavonoid content in the extracts.

4.2.3.3 ABTS ASSAY

ABTS assay in the individual mushroom extract and in the mushroom pair was determined by modified method of Paixao *et al*., (2007).

$ABTS^+$ was dissolved in water (7mM) to get the stock solution. ABTS radical cation was produced by reacting the stock solution with 2.45 mM (final concentration) potassium persulfate solution. Solution was kept in dark at room temperature for 12 hours prior to use. 2.9 ml ABTS and 100 µl of sample with different concentration gradient ranging from 10 mg/ml to 80 mg/ml was mixed and incubated for 30 minutes at room temperature. Absorbance of 80% ethanol was considered as blank while control (ABTS solution) was also run simultaneously. Absorbance was measured at 734 nm. **BHT** is taken as standard in this assay. The tests were performed in duplicates.

$$\%age\ inhibition = (Ac-As/Ac) \times 100$$

where
Ac = Absorbance of control
As = Absorbance of sample

4.2.3.4 DPPH ASSAY

DPPH assay in the individual mushroom extract and in the mushroom pair was determined by Barros *et al*.2008

DPPH solution was prepared by dissolving 32 mg in 1L 80% methanol. . 2.5 ml DPPH and 0.5 ml of sample with different concentration gradient ranging from 10 mg/ml to 80 mg/ml was mixed . Mixture was shaken well and kept in dark at room temperature for one hour. Absorbance was measured at 517 nm by using spectrophotometer . Absorbance of 80% ethanol was considered as blank while control (DPPH solution) was also run simultaneously. **BHT** is taken as standard in this assay.

The tests were performed in duplicates.

$$\%age\ inhibition = (Ac\text{-}As/Ac) \times 100$$

where
Ac = Absorbance of control
As = Absorbance of sample

4.2.3.5 Inhibition of lipid peroxidation using thiobarbituric acid reactive substances (TBARS)

TBARS assay in the individual mushroom extract and in the mushroom pair was determined by Barros and Ferreira *et al*.2007.

Add 0.2 ml mushroom extract with different concentration gradient ranging from 10 mg/ml to 80 mg/ml and Tris–HCl buffer (pH 7.4, 20 mM) in the presence of FeSO4 (10 μM; 0.1 ml) and ascorbic acid (0.1 mM; 0.1 ml) at 37°C for 1 h. The reaction was stopped by the addition of trichloroacetic acid (28% w/v, 0.5 ml), followed by thiobarbituric acid (TBA, 2%, 0.38 ml), and the mixture was then heated at 80°C for 20 min. After centrifugation at 3000g for 10 min to remove the precipitated protein, the colour intensity of the malondialdehyde (MDA)–TBA complex in the supernatant was measured by its absorbance at 532 nm.

The tests were performed in duplicates.

4.2.4 Virtual Screening of mushroom nutraceuticals

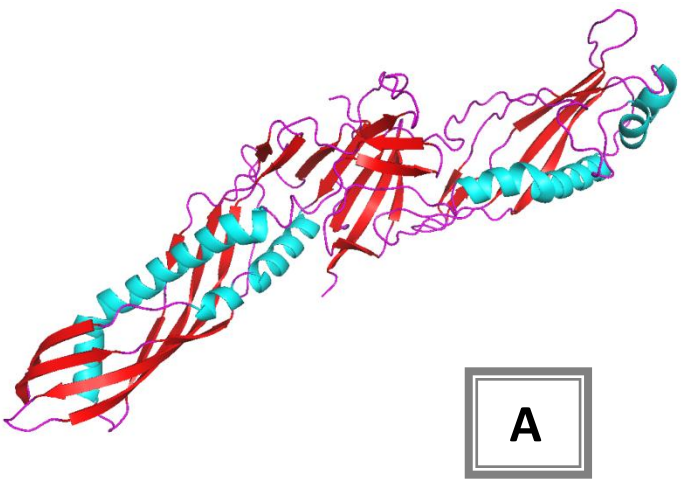
4.2.4.1 PDB (Protein Data Bank)

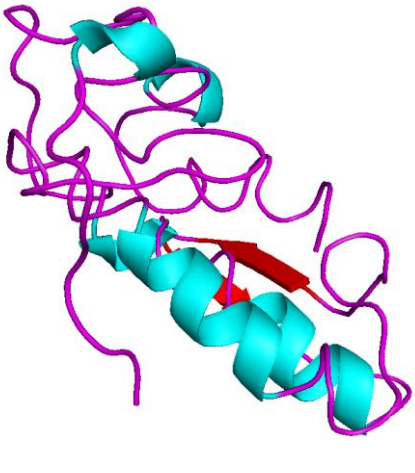
PDB (Protein Data Bank) is the worldwide library of structural data of biological macro molecules which established in Brookhaven National Laboratories. It contains structural information of the macromolecules based on X-ray crystallographic and NMR methods. 3D structure of inflammatory markers were taken from PDB.

Inflammatory markers include:

Table 4.2.4.1: Receptors (Inflammation markers)

BIOMARKER	SOURCE	PDB ID
LBP (Lipolysaccharide binding protein)	*Mus musculus*	4M4D
PLA-2(Phospholipase-A2)	*Homo sapiens*	ILE6
COX-2(Cyclo-oxygenase -2)	*Mus musculus*	4COX
LOX-2(Lipoxygenase-2)	*Homo sapiens*	4NRE
TNF-α(Tumour Necrosis Factor- α)	*Homo sapiens*	1TNF
TLR-4(Toll like receptor-4)	*Homo sapiens*	3FXI
IL-1β(Interleukin-1β)	*Homo sapiens*	2I1B





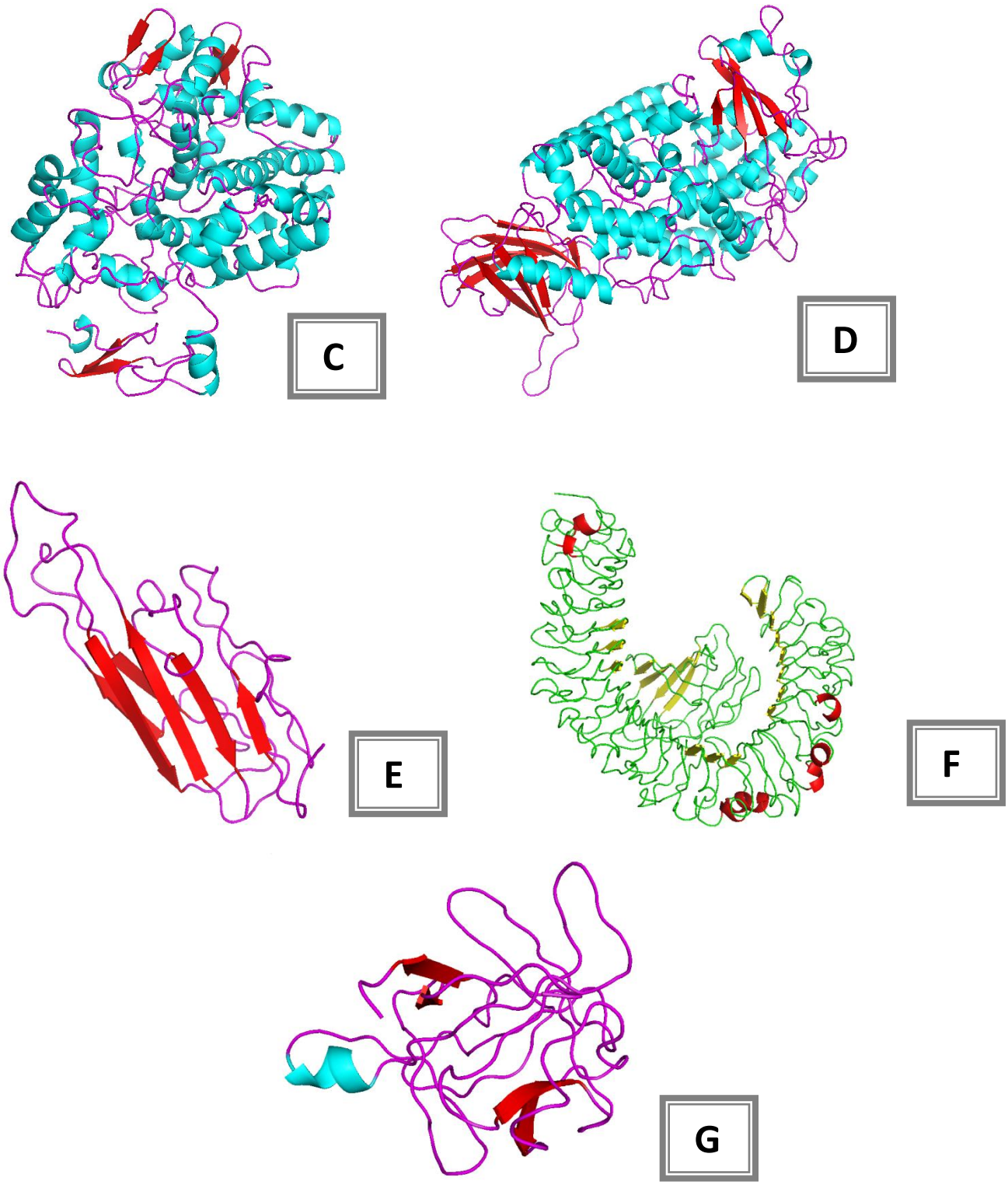


Figure 4.2.4.2: PYMOL images of Inflammation Markers

(A) LBP, (B) PLA-2, (C) COX-2, (D) LOX-2, (E) TNF-α, (F) TLR-4, (G) IL-1B

4.2.4.2 MOE 2008.10

3D structures obtained from PDB for COX-2 and LBP were from mus musculus species, so homology modeling was done on MOE using available structure as template and sequence of human as query. The query human sequence obtained was blast with PDB Database and highest similarity sequence was taken.

The modeled structures LBP and COX-2 have an rmsd value of 0.819 A° and 0.892 A°.

4.2.4.3 Pubchem

PubChem is a database of chemical molecules and their activities against biological assays. The system is maintained by the National Center for Biotechnology Information (NCBI), a component of the National Library of Medicine, which is part of the United States National Institutes of Health (NIH). Structure of mushroom bioactive components was obtained from Pubchem with the following ID's.

Table 4.2.4.3: Ligands (Bioactive Components of mushroom)

MUSHROOM	***BIOACTIVE COMPONENT***	***ID***
Ganoderma lucidum	***GANODERAL A***	***13934282***
	GANODERMIC ACID S	***6449828***
	GANOLUCIDENIC ACID A	***475412***
	GANOLUCIDIC ACID B	***20055994***
	GANODERIC ALDEHYDE A	***14484704***
	GANODERIOL F	***471008***
	GANODER-MANONDIOL	***46888221***

	GANODER-MANONTRIOL	***73177***
	GANODERIC ACID A	***471002***
	LUCIDENIC ACID A	***14109375***
	GANOLUCIDIC ACID A	***475412***
Cordyceps sinensis	***CORDYCEPINS***	***ZINC04514131***
	ADENOSINE	***ZINC02169830***
Lentinus edodes	***ERITADENINE***	***ZINC03872191***
	CENTANAMYCIN	***9825161***
	LENTINAN	***37723***
Morchella esculenta	***GALACTO-MANNAN***	***439336***

4.2.4.4 Discovery Studio 3.5 and Libdock

Libdock docking was performed using Discovery Studio 3.5. proteins were the inflammation markers and ligand were the mushroom bioactive components mentioned in the above table. The protein and ligands were first prepared. Hydrogen bonds were added and energy was minimized using CHARMm force field. The active site of the protein was then identified and defined. Then the ligands were docked into each active site using Libdock procedure. Libdock score, absolute energy were obtained from the study.

5. RESULTS

5.1 Solvent extraction

For ***Agaricus bisporus***, the dry weight of the mushroom was 25 gm and for ***Morchella esculenta***, 9.5 gm which further through soxhelet extraction unit and different solvents such as petroleum ether, chloroform,methanol and water. Residues were obtained and stored at 4◦C.

Table 5.1: Amount of residue Obtained

SOLVENT (300 ml)	*AMOUNT OF RESIDUE OBTAINED FOR Agaricus bisporus (per gm)*	*AMOUNT OF RESIDUE OBTAINED FOR Morchella esculenta (per gm)*
Petroleum Ether Extract	***0.0424±0.00212***	***0.055±0.00275***
Chloroform Extract	***0.048±0.0024***	***0.088±0.0044***
Methanol Extract	***0.152±0.0076***	***0.204±0.0102***
Aqueous Extract	***1.9±0.095***	***2.4±0.12***

5.2 Total Phenolic Activity

Total soluble phenolics in the individual mushroom extract was determined with Folin-Ciocalteu reagent using Gallic Acid as standard at765nm. **Table 5.2.1** represents the gallic acid standard

Table 5.2.1: Absorbance of Standard Compound (Gallic Acid)

Gallic concentration (mg/ml)	*Absorbance (λmax=765nm)*
100	***0.1925***
200	***0.304***
300	***0.41***
400	***0.662***
500	***0.704***
600	***0.89***
700	***1.05***
800	***1.137***
900	***1.292***
1000	***1.308***

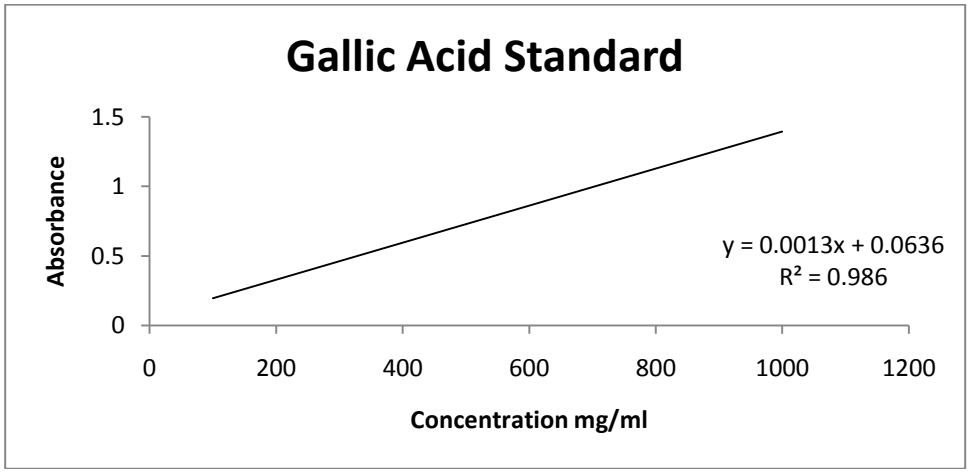


Figure 5.2.1: Standard curve of Gallic acid

It was observed from **Table 5.2.2** that methanol extract gave high concentration of phenols in *A. bisporus* (***496±24.8)*** whereas, aqueous extract gave higher phenol concentration in *M. esculenta(**769.57±38.4785)***. Comparing the two mushrooms, it was observed that *M. esculenta* have higher concentration of Total phenols than *A. bisporus*.

Table 5.2.2: Total Phenolic content in different Mushroom Extracts

Samples	***TPC for Agaricus bisporus (mg/g GAE)***	***TPC for Morchella esculenta (mg/g GAE)***
Methanol Extract	***496±24.8***	***113.403±5.67***
Chloroform Extract	***15.39±0.7695***	***414.57±20.7285***
Aqueous Extract	***344.065±17.203***	***769.57±38.4785***

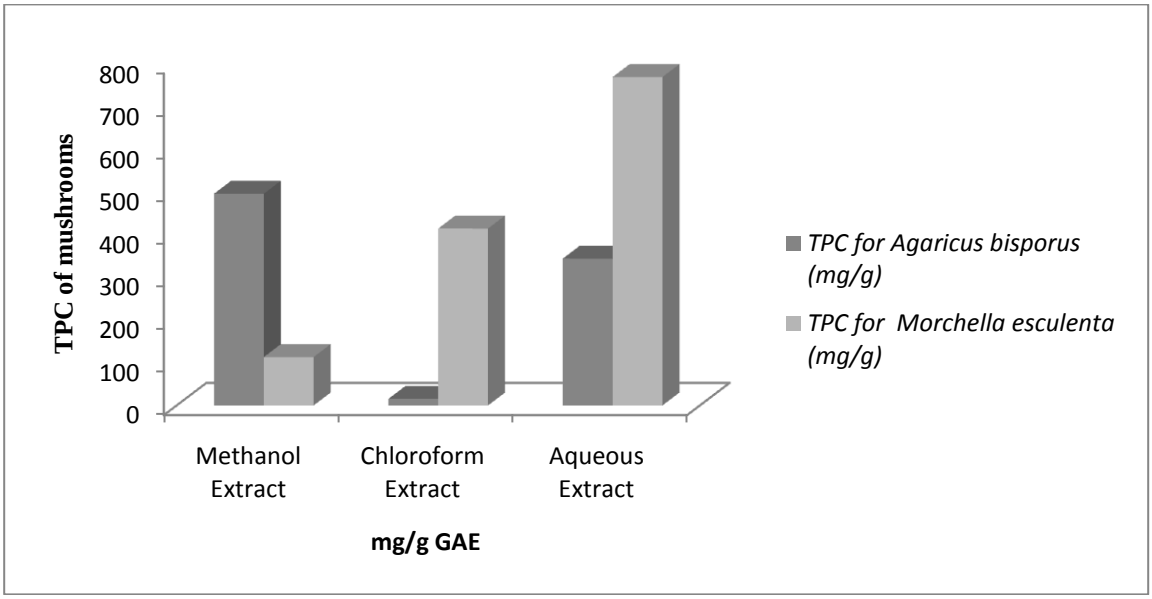


Figure 5.2.2: TPC of different mushrooms

Analyzing the results of TPC for Paired-Mushroom extract (***946.82±47.341)***, it was observed that the concentration increased folds than individual mushroom extract as shown in **Table 5.2.3**. Paired-Mushroom extract was a mixture of methanol extract of *A. bisporus* (***496±24.8***) and aqueous extract of *M. esculenta (**769.57±38.4785)*** which gave highest TPC.

Table 5.2.3: The measurement of TPC for Paired-Mushroom samples

TPC of Agaricus bisporus methanol extract (mg/g GAE)	***TPC of Morchella esculenta aqueous extract (mg/g GAE)***	***TPCof Mushroom pair extract (mg/g GAE)***
496±24.8	***769.57±38.4785***	***946.82±47.341***

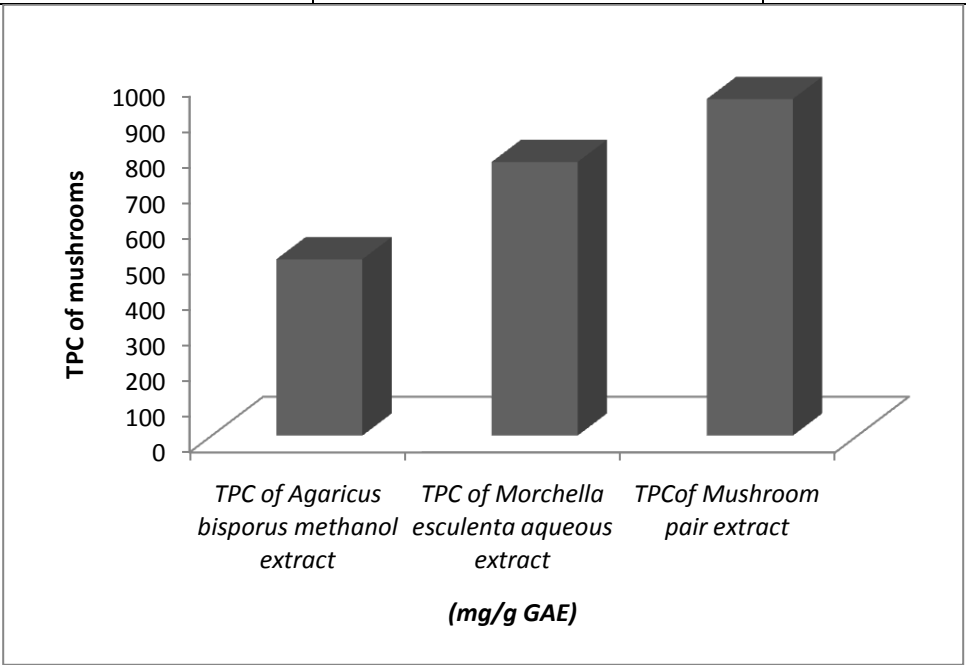


Figure 5.2.3: TPC for Paired-Mushroom samples

5.3 Flavanoid content

Mushroom extract (250 µl) with different concentration gradient ranging from 10 mg/ml to 80 mg/ml was taken. The absorbance was measured at 510 nm. The total flavanoid were expressed as mg/g Quercetin equivalent using the standard curve.

Table 5.3.1: Absorbance of Standard Compound (Quercetin Acid)

Quercetin concentration (µg/ml)	*Absorbance(λmax=510nm)*
2	***0.234***
4	***0.448***
6	***0.658***
8	***0.869***

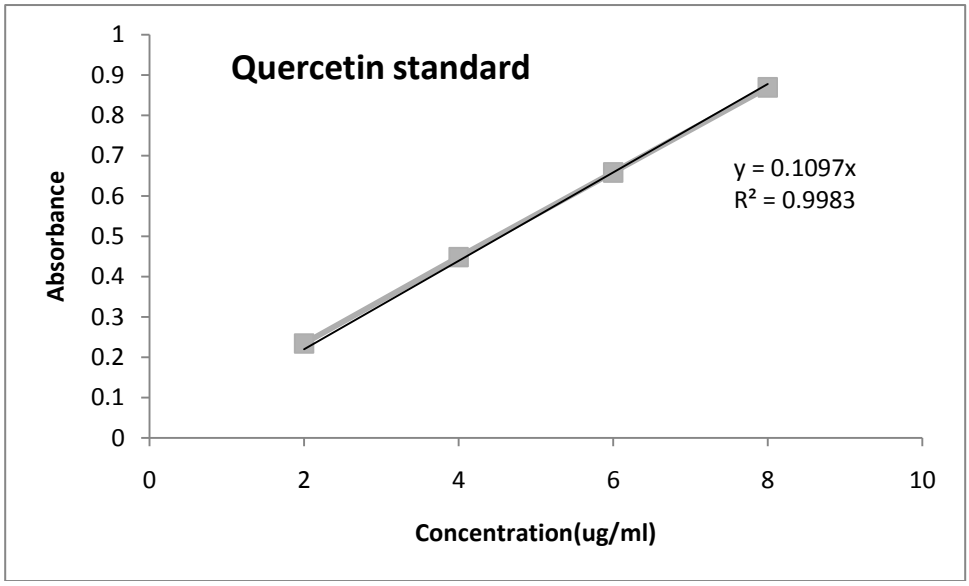


Figure 5.3.1: Standard curve of Quercetin acid

It was observed from **Table 5.3.2** that methanol extract showed highest concentration of flavonoids in *A. bisporus* ***(24.23±1.2165)*** and aqueous extract showed highest in *M. esculenta* ***(24.23±1.2165)***. In comparison to both the mushroom samples, *M. esculenta* is observed to have more flavonoid content.

Table 5.3.2: Flavanoid content in different Mushroom Extracts

Samples	***Flavanoid concentration for Agaricus bisporus (mg/g quercetin)***	***Flavanoid concentration for Morchella esculenta (mg/g quercetin)***
Methanol Extract	***6.52±0.326***	***2.39±0.1195***
Chloroform Extract	***1.71±0.0855***	***14.79±0.7395***
Aqueous Extract	***2.89±0.1445***	***24.23±1.2165***

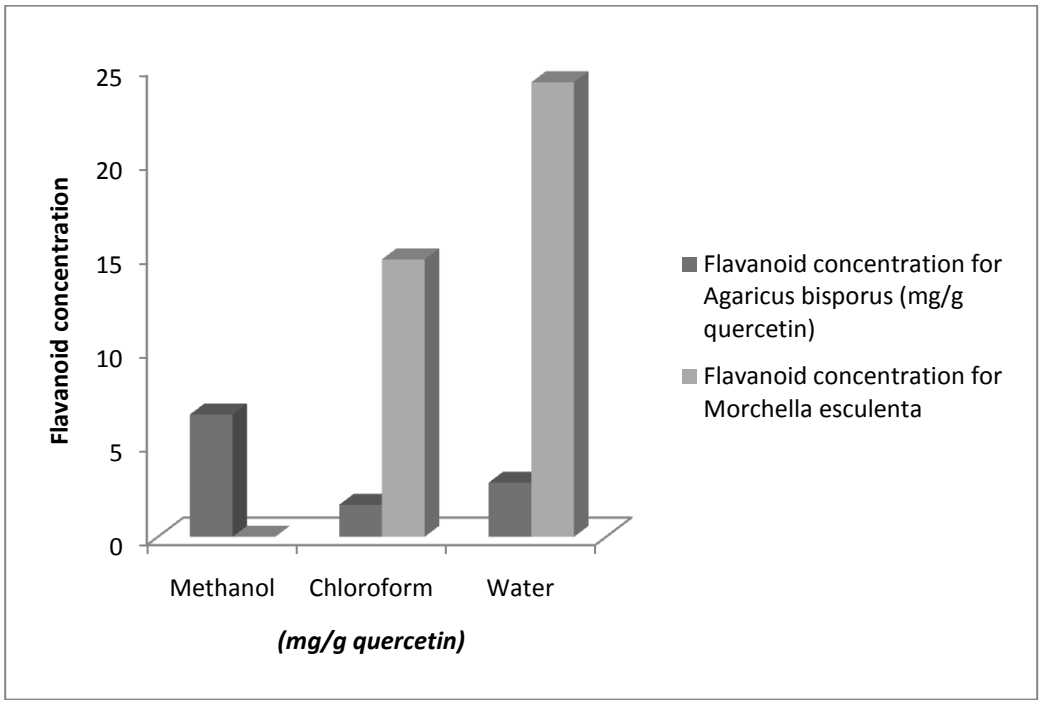


Figure 5.3.2: Flavanoid content of different mushrooms

Analyzing the results of Flavanoid content for Paired-Mushroom extract (***84.67±4.2)***, it was observed that the concentration of Flavanoids increased than individual mushroom extract as shown in **Table 5.3.3.** Paired-Mushroom extract was a mixture of methanol extract of *A. bisporus* (***6.52±0.326***) and aqueous extract of *M. esculenta* ***(24.23±1.2165)*** which gave highest Flavanoid content.

Table 5.3.3: The measurement of Flavanoid content for Paired-Mushroom samples

Flavanoid concentration of Agaricus bisporus methanol extract (mg/g quercetin)	***Flavanoid concentration of Morchella esculenta aqueous extract (mg/g quercetin)***	***Flavanoid concentration of Mushroom pair extract (mg/g quercetin)***
6.52±0.326	***24.23±1.2165***	***84.67±4.2***

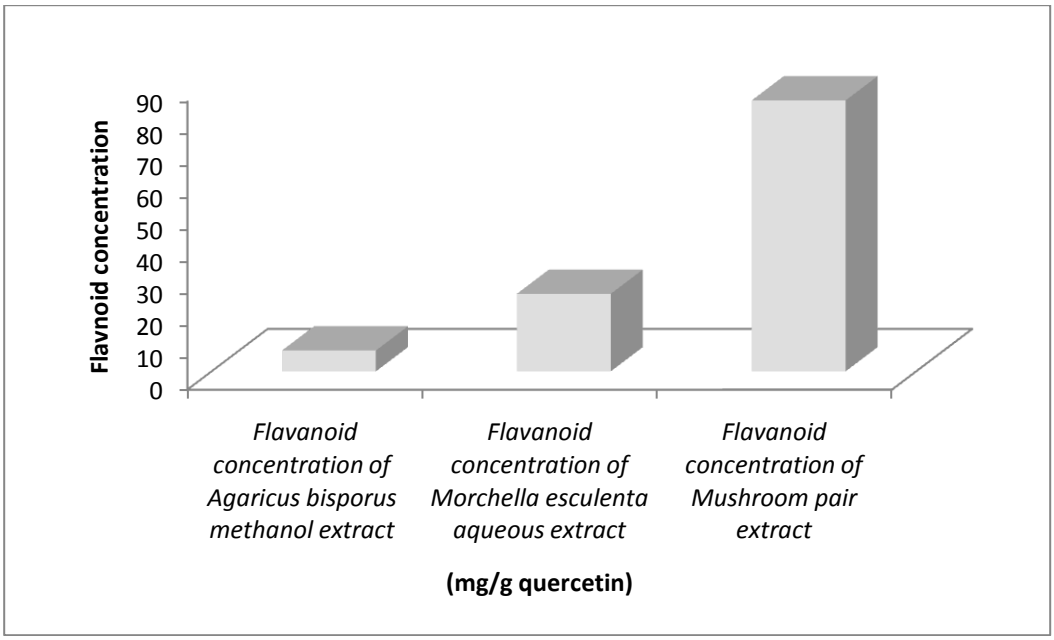


Figure 5.3.3: Flavonoid content for Paired-Mushroom samples

5.4 ABTS Assay

ABTS Assay with different concentration gradient ranging from 10 mg/ml to 80 mg/ml was performed. Absorbance was measured at 734 nm and BHT was taken as a standard in this assay. The **Table 5.4.1** below shows that the **methanol extract** at **50 mg/mL** of *A. bisporus* shows highest % inhibition = ***34.14±1.707*** which infers that the methanol extract has the highest free radical scavenging capacity for ABTS.

Table 5.4.1: ABTS Free radical scavenging activity of different extracts of *A. bisporus*

Concentration (mg/ml)	***% inhibition Agaricus bisporus methanol extract***	***% inhibition Agaricus bisporus chloroform extract***	***% inhibition Agaricus bisporus aqueous extract***
10	***7.2±0.36***	***2.32±0.116***	***4.242±0.212***
20	***10.154±0.508***	***5.67±0.28***	***8.34±0.417***
30	***15.24±0.762***	***7.89±0.39***	***12.667±0.64***
40	***23.43±1.171***	***11.74±0.59***	***20.34±1.017***
50	***34.14±1.707***	***15.7±0.79***	***19.05±0.95***
60	***33.124±1.656***	***19.88±0.99***	***18.73±0.94***
70	***32.16±1.608***	***18.23±0.911***	***18.22±0.911***
80	***32.229±1.611***	***18.02±0.901***	***18.09±0.91***

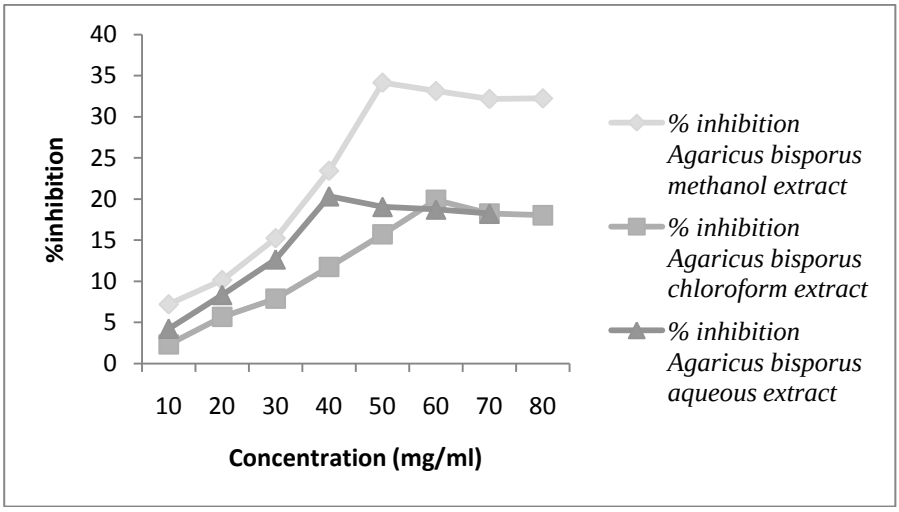


Figure 5.4.1: ABTS scavenging capacity of A. bisporus obtained from (♦) methanol extract, (■) chloroform extract, (▲) aqueous extract

The **Table 5.4.2** below shows that the **methanol extract** at **50 mg/mL** of *M. esculenta* shows highest % inhibition = ***47.51±2.37*** which infers that the methanol extract has the highest free radical scavenging capacity for ABTS.

Table 5.4.2: ABTS Free radical scavenging activity of different extracts of *M. esculenta*

Concentration (mg/ml)	***% inhibition Morchella esculenta methanol extract***	***% inhibition Morchella esculenta chloroform extract***	***% inhibition Morchella esculenta aqueous extract***
10	***10.81±0.54***	***7.21±0.36***	***9.11±0.45***
20	***13.43±0.67***	***12.01±0.60***	***12.81±0.64***
30	***19.322±0.97***	***22.32±1.12***	***26.98±1.35***
40	***29.83±1.49***	***44.56±2.23***	***34.87±1.74***
50	***47.51±2.37***	***42.98±2.15***	***42.32±2.12***
60	***46.91±2.34***	***40.77±2.03***	***46.69±2.33***
70	***46.56±2.33***	***40.051±2.00***	***43.44±2.17***
80	***45.824±2.29***	***39.72±1.98***	***42.61±2.13***

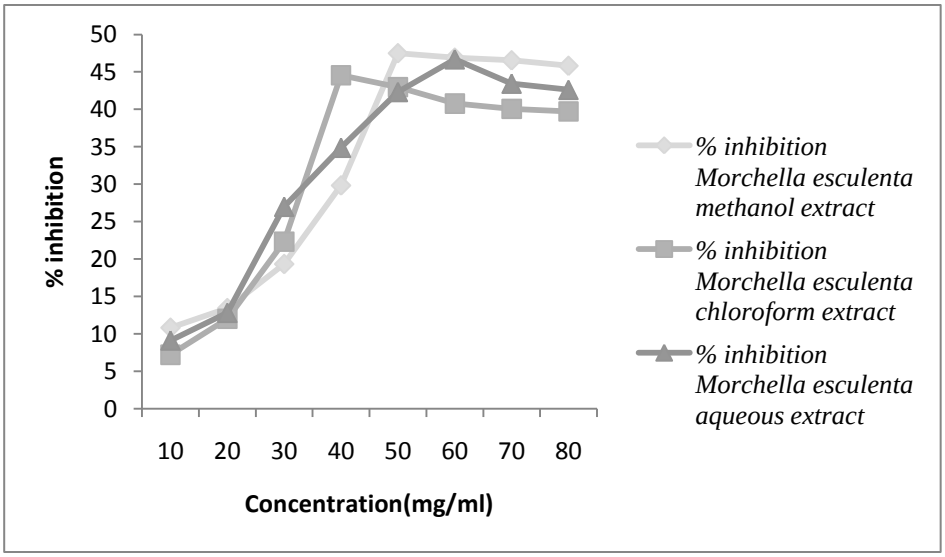


Figure 5.4.2: ABTS scavenging capacity of M. esculenta obtained from (♦) methanol extract, (■) chloroform extract, (▲) aqueous extract

Comparing the methanol extract of *A. bisporus* and *M. esculenta*, as shown in **Figure 5.4.3** it was observed that at **50 mg/mL** concentration of sample, *M. esculenta* showed higher free radical scavenging activity or % inhibition, i.e, ***47.51±2.37.***

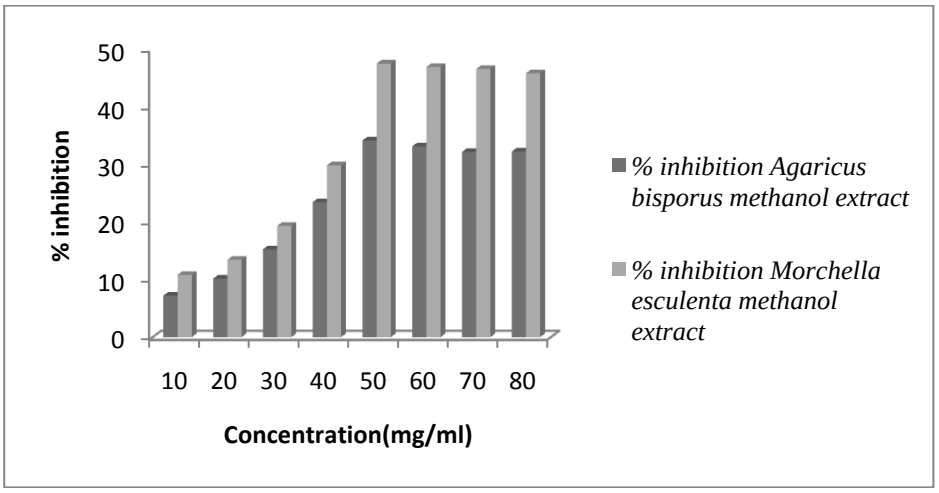


Figure 5.4.3: Comparison between ABTS scavenging capacity of A. bisporus and M. esculenta for methanol extract

Comparing the chloroform extract of *A. bisporus* and *M. esculenta*, as shown in **Figure 5.4.4** it was observed that in *A. bisporus* highest % inhibition was showed at **60 mg/mL**, i.e., ***19.88±0.99***, whereas in *M. esculenta* it was observed at **40 mg/mL**,i.e., ***44.56±2.23***

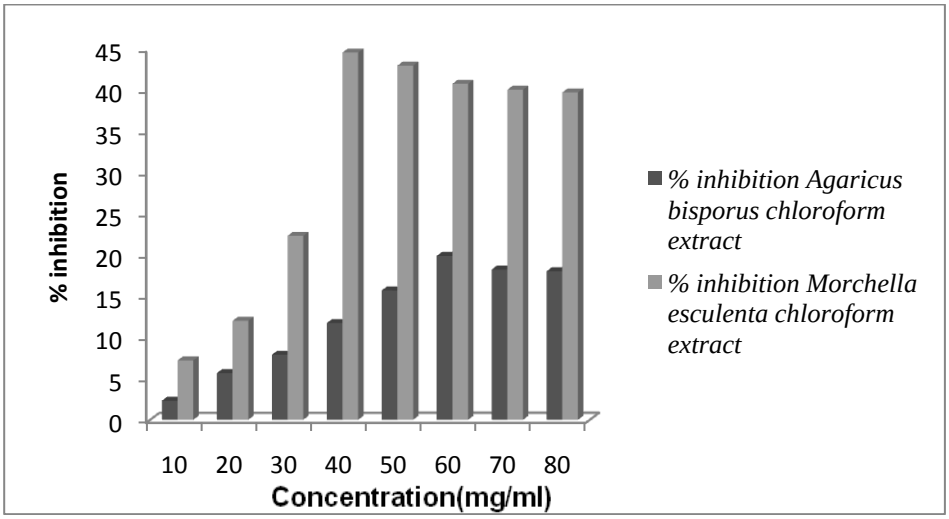


Figure 5.4.4: Comparison between ABTS scavenging capacity of A. bisporus and M. esculenta for chloroform extract

Comparing the aqueous extract of *A. bisporus* and *M. esculenta*, as shown in **Figure 5.4.5** it was observed that in *A. bisporus* highest % inhibition was showed at **40 mg/mL**, i.e., ***20.34±1.017***, whereas in *M. esculenta* it was observed at **60 mg/mL**,i.e., ***46.69±2.33***

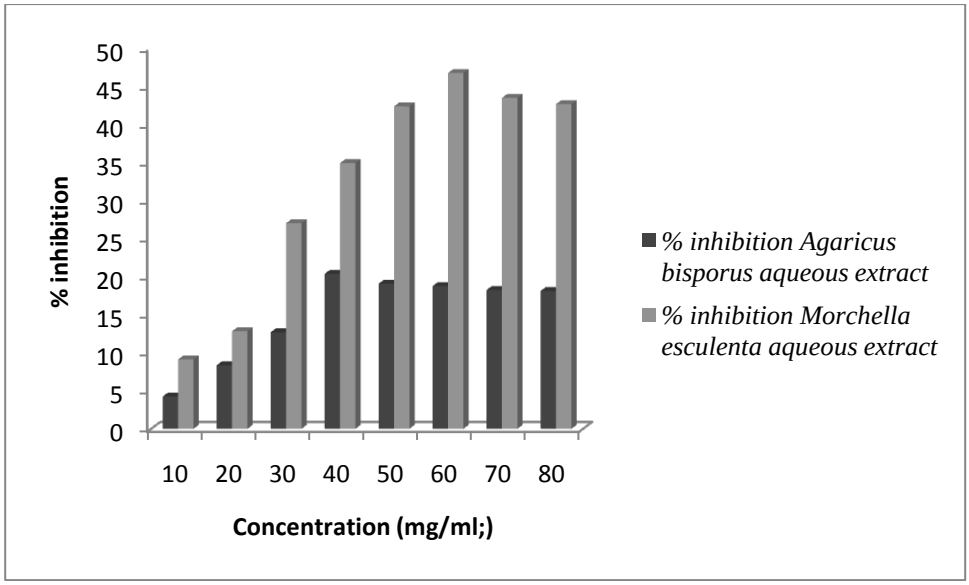


Figure 5.4.5: Comparison between ABTS scavenging capacity of A. bisporus and M. esculenta for aqueous extract

Analyzing the results of free radical scavenging activity by ABTS for Paired-Mushroom extract (***70.5±3.53)***, as shown in **Table 5.4.3** it was observed that the scavenging capacity was increased many folds in Paired-Mushroom sample than individual mushroom extract. Paired-Mushroom extract was a mixture of methanol extract of *A. bisporus* (***34.14±1.707***) and methanol extract of *M. esculenta* ***(47.51±2.37)*** which gave highest % inhibition.

Table 5.4.3: ABTS Free radical scavenging activity of Paired-Mushroom samples

% inhibition of Agaricus bisporus methanol extract	***% inhibition of Morchella esculenta methanol extract***	***% inhibition of Mushroom pair extract***
34.14±1.707	***47.51±2.37***	***70.5±3.53***

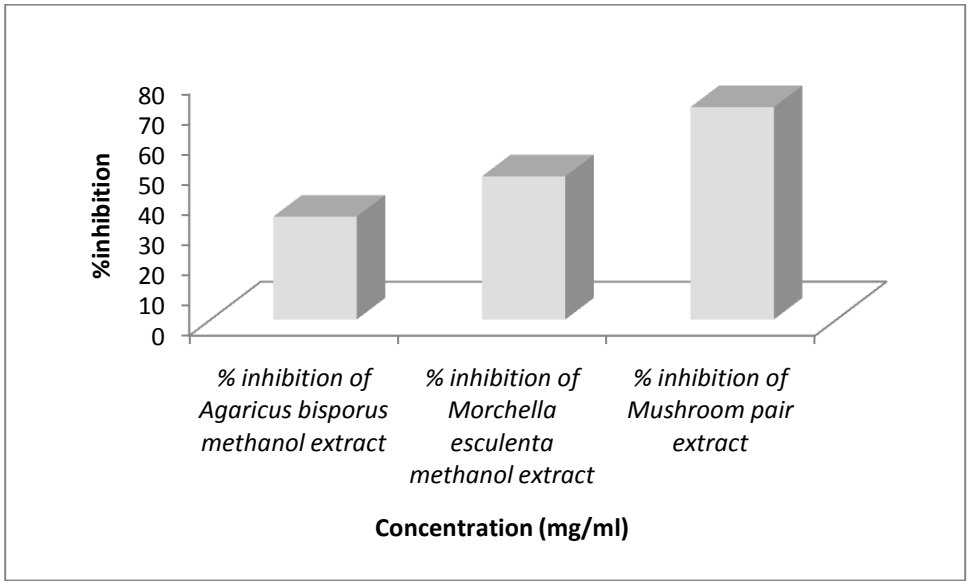


Figure 5.4.6: ABTS scavenging capacity of A. bisporus, M. esculenta and Paired-Mushroom samples

5.5 DPPH Assay

DPPH Assay with different concentration gradient ranging from 10 mg/ml to 80 mg/ml was performed. Absorbance was measured using spectrophotometer at 517 nm. The **Table 5.5.1** below shows that the **methanol extract** at **50 mg/mL** of *A. bisporus* shows highest % inhibition = ***45.99±2.29*** which infers that the methanol extract has the highest free radical scavenging capacity for DPPH. It was also observed that beyond 50mg/mL, the % inhibiton becomes gradually constant for methanol extract.

Table 5.5.1: DPPH Free radical scavenging activity of different extracts of *A. bisporus*

Concentration (mg/ml)	*% inhibition Agaricus bisporus methanol extract*	*% inhibition Agaricus bisporus chloroform extract*	*% inhibition Agaricus bisporus aqueous extract*
10	***12.65±0.63***	***5.667±0.28***	***8.78±0.44***
20	***19.89±0.99***	***12.45±0.62***	***15.677±0.78***
30	***26.75±1.33***	***16.97±0.84***	***22.32±1.12***
40	***35.32±1.77***	***20.81±1.04***	***32.92±1.65***
50	***45.99±2.29***	***23.12±1.156***	***31.23±1.56***
60	***44.71±2.23***	***26.69±1.33***	***30.98±1.54***
70	***44.2±2.21***	***24.85±1.24***	***30.25±1.51***
80	***43.81±2.19***	***24.69±1.23***	***30.11±1.50***

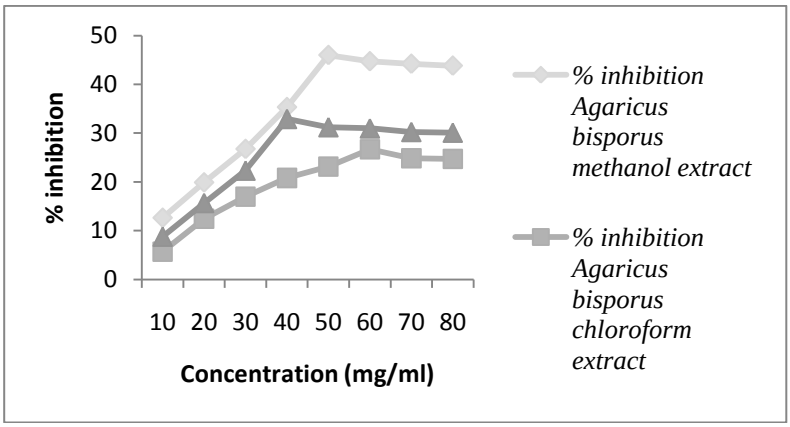


Figure 5.5.1: DPPH scavenging capacity of A. bisporus obtained from (♦) methanol extract, (■) chloroform extract, (▲) aqueous extract

The **Table 5.5.2** below shows that the **methanol extract** at **50 mg/mL** of *M. esculenta* shows highest % inhibition = ***54.28±2.71***which infers that the methanol extract has the highest free radical scavenging capacity for DPPH. It was also observed that beyond 50mg/mL, the % inhibiton becomes gradually constant for methanol extract.

Table 5.5.2: Free radical scavenging activity by DPPH for different extracts of *M. esculenta*

Concentration (mg/ml)	***% inhibition Morchella esculenta methanol extract***	***% inhibition Morchella esculenta chloroform extract***	***% inhibition Morchella esculenta aqueous extract***
10	***13.23±0.66***	***4.87±0.24***	***6.87±0.34***
20	***22.37±1.12***	***12.31±0.62***	***14.72±0.74***
30	***28.78±1.43***	***20.64±1.03***	***22.45±1.122***
40	***38.9±1.94***	***31.92±1.59***	***32.94±1.65***
50	***54.28±2.71***	***30.85±1.54***	***40.96±2.05***
60	***52.99±2.65***	***29.87±1.49***	***41.175±2.06***
70	***52.64±2.63***	***28.73±1.44***	***39.31±1.96***
80	***52.14±2.61***	***27.12±1.36***	***38.78±1.94***

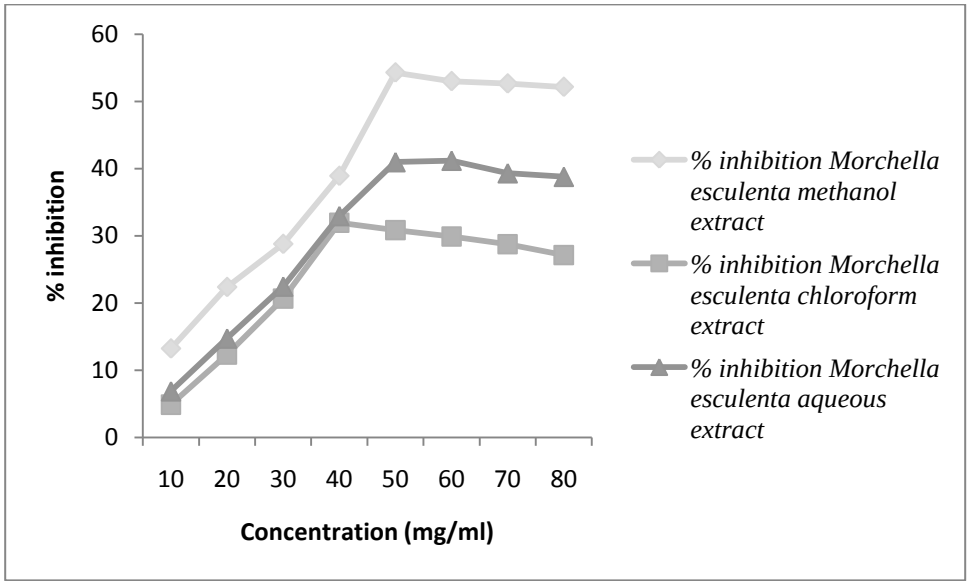


Figure 5.5.2: DPPH scavenging capacity of M. esculenta obtained from (♦) methanol extract, (■) chloroform extract, (▲) aqueous extract

Comparing the methanol extract of *A. bisporus* and *M. esculenta*,as shown in **Figure 5.5.3** it was observed that at **50 mg/mL** concentration of sample, *M. esculenta* showed higher free radical scavenging activity or % inhibition, i.e, **54.28±2.71.** Both the mushroom samples showed highest % inhibition at 50mg/mL.

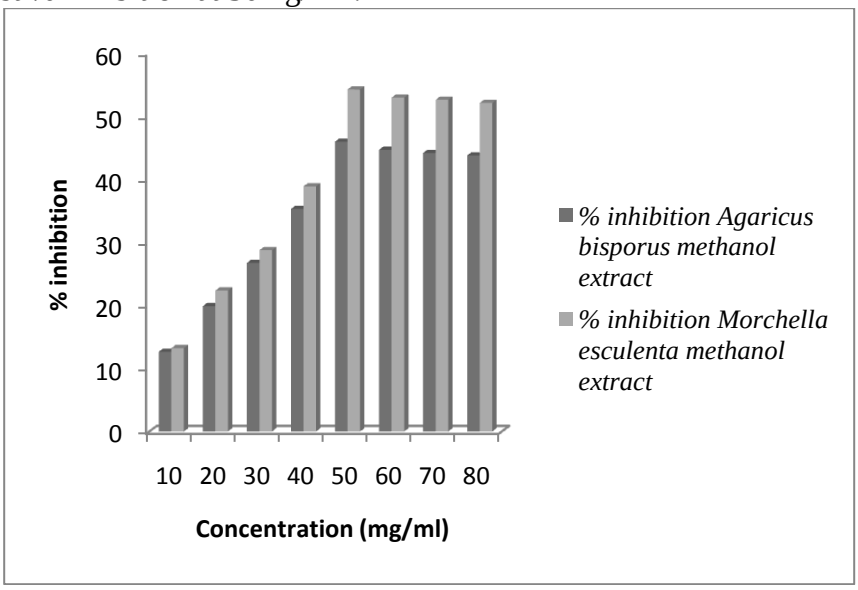


Figure 5.5.3: Comparison between DPPH scavenging capacity of A. bisporus and M. esculenta for methanol extract

Comparing the chloroform extract of *A. bisporus* and *M. esculenta*, as shown in **Figure 5.5.4** it was observed that at **50 mg/mL** concentration of sample, *M. esculenta* showed higher free radical scavenging activity or % inhibition, i.e, ***30.85±1.54.***

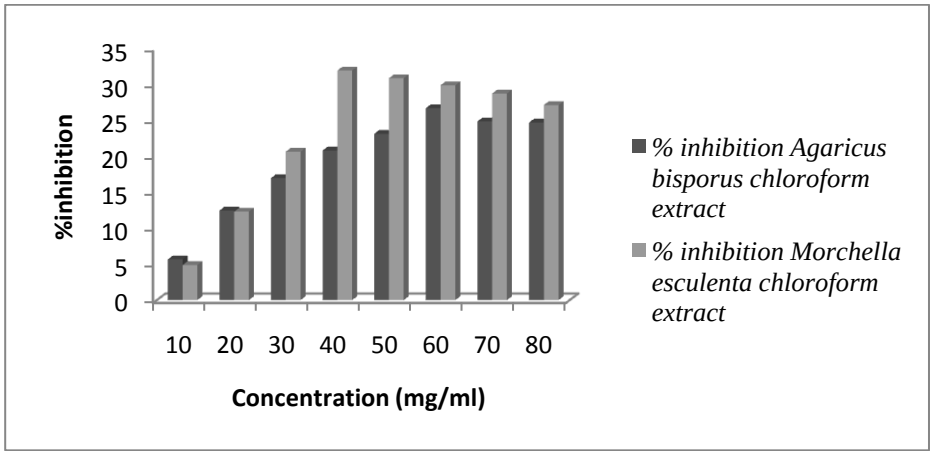


Figure 5.5.4: Comparison between DPPH scavenging capacity of A. bisporus and M. esculenta for chloroform extract

Comparing the aqueous extract of *A. bisporus* and *M. esculenta*, as shown in **Figure 5.5.5** it was observed that at **60 mg/mL** concentration of sample, *M. esculenta* showed higher free radical scavenging activity or % inhibition, i.e, ***41.175±2.06.***

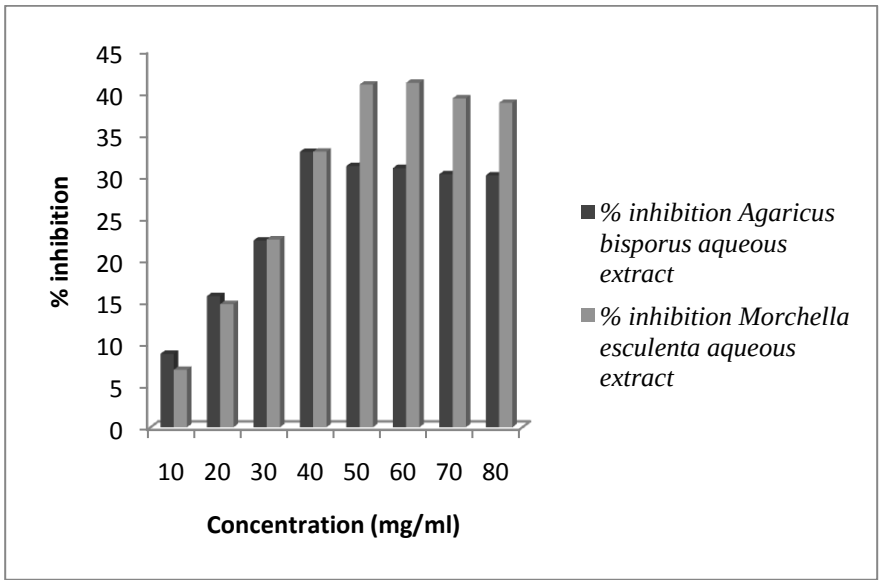


Figure 5.5.5: Comparison between DPPH scavenging capacity of A. bisporus and M. esculenta for aqueous extract

Analyzing the results of free radical scavenging activity by DPPH for Paired-Mushroom extract (***90±4.5)***, as shown in **Table 5.5.3** it was observed that the scavenging capacity was increased many folds in Paired-Mushroom sample than individual mushroom extract. Paired-Mushroom extract was a mixture of methanol extract of *A. bisporus* (***45.99±2.29***) and methanol extract of *M. esculenta* ***(54.28±2.71)*** which gave highest % inhibition.

Table 5.5.3: DPPH Free radical scavenging activity for Paired-Mushroom samples

% inhibition of Agaricus bisporus methanol extract	***% inhibition of Morchella esculenta methanol extract***	***% inhibition of Mushroom pair extract***
45.99±2.29	***54.28±2.71***	***90±4.5***

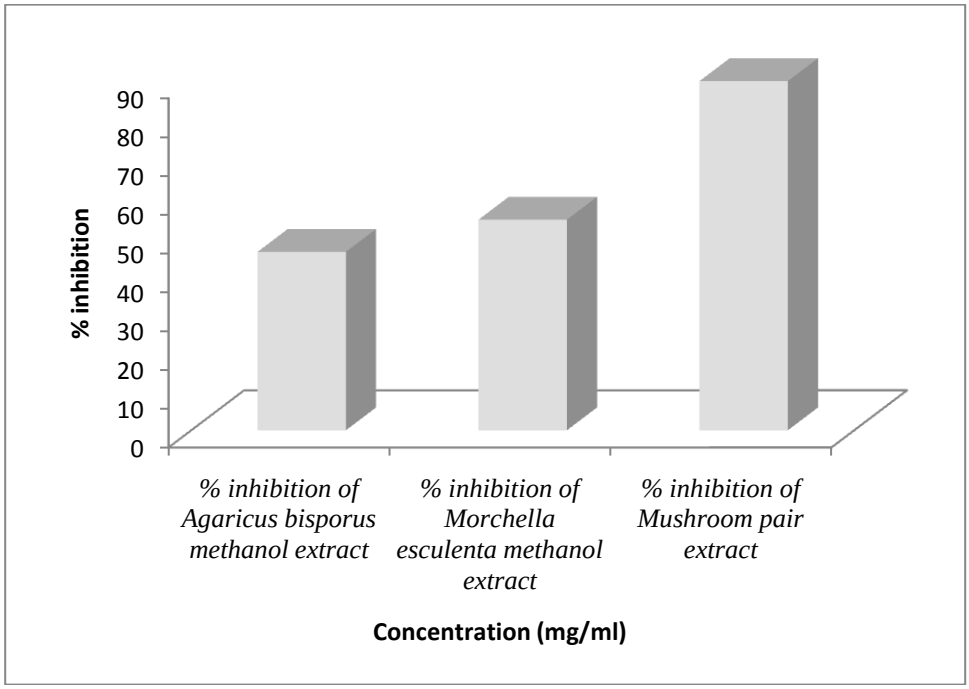


Figure 5.5.6: DPPH scavenging capacity of A. bisporus, M. esculenta and Paired-Mushroom sample

5.6 Lipid peroxidation

LPO was determined by using using thiobarbituric acid reactive substances (TBARS) with petroleum ether extract of different concentration gradient ranging from 10 mg/ml to 80 mg/ml was prepared. The colour intensity of the malondialdehyde (MDA)–TBA complex in the supernatant was measured by its absorbance at 532 nm.As shown in **Table 5.6.1** it was observed that the highest % inhibition for *A. bisporus* was shown at **50 mg/mL**, i.e., ***51.62±2.58.***

Table 5.6.1: Lipid peroxidation (LPO) inhibition (%) for Petroleum Ether extract of *A. bisporus*

Concentration (mg/ml)	***% inhibition Agaricus bisporus petroleum ether extract***
10	***8.9±0.445***
20	***18.42±0.921***
30	***26.65±1.33***
40	***38.91±1.94***
50	***51.62±2.58***
60	***50.39±2.51***
70	***49.23±2.46***
80	***48.34±2.41***

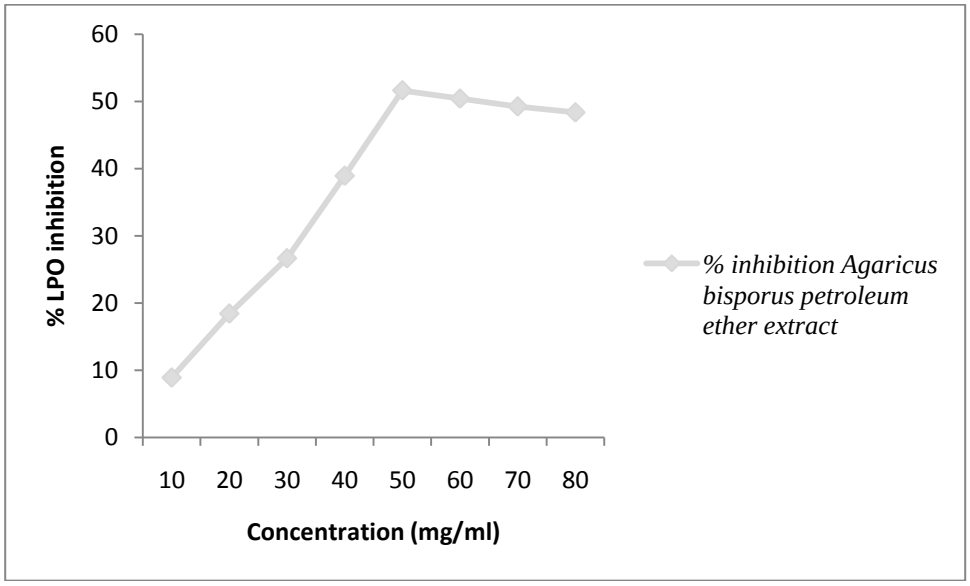


Figure 5.6.1: Lipid peroxidation (LPO) inhibition (%) for Petroleum Ether extract of A. bisporus

In *Mochella esculenta* , as shown in **Table 5.6.2** it was observed that the highest % inhibition for lipid peroxidation was shown at **50 mg/mL**, i.e., ***64.63±3.23.***

Table 5.6.2: Lipid peroxidation (LPO) inhibition (%) for Petroleum Ether extract of *M. esculenta*

Concentration (mg/ml)	***% inhibition Morchella esculenta petroleum ether extract***
10	***12.22±0.611***
20	***21.34±1.07***
30	***34.56±1.73***
40	***45.87±2.29***
50	***64.63±3.23***
60	***62.32±3.11***
70	***61.01±3.05***
80	***59.68±2.98***

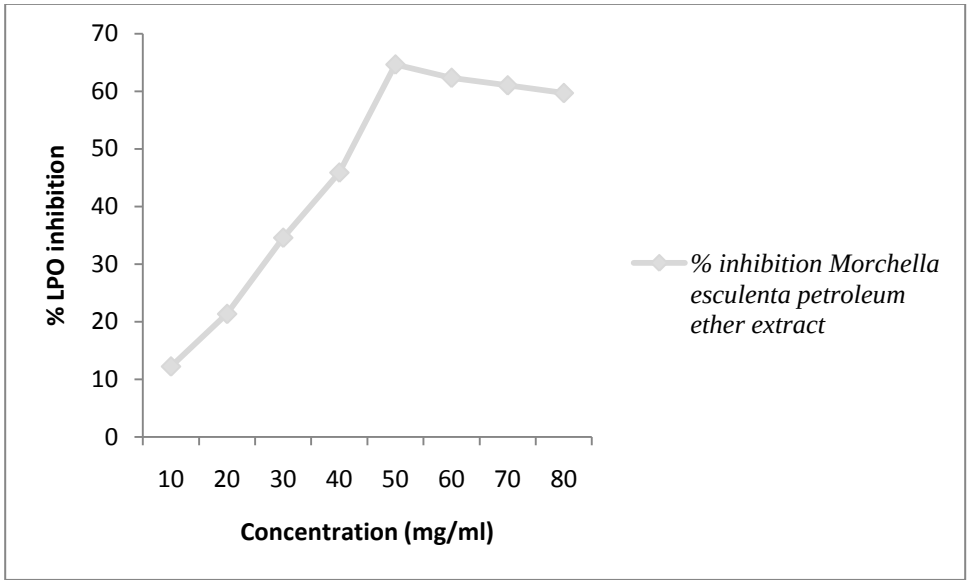


Figure 5.6.2: Lipid peroxidation (LPO) inhibition (%) for Petroleum Ether extract of M. esculenta

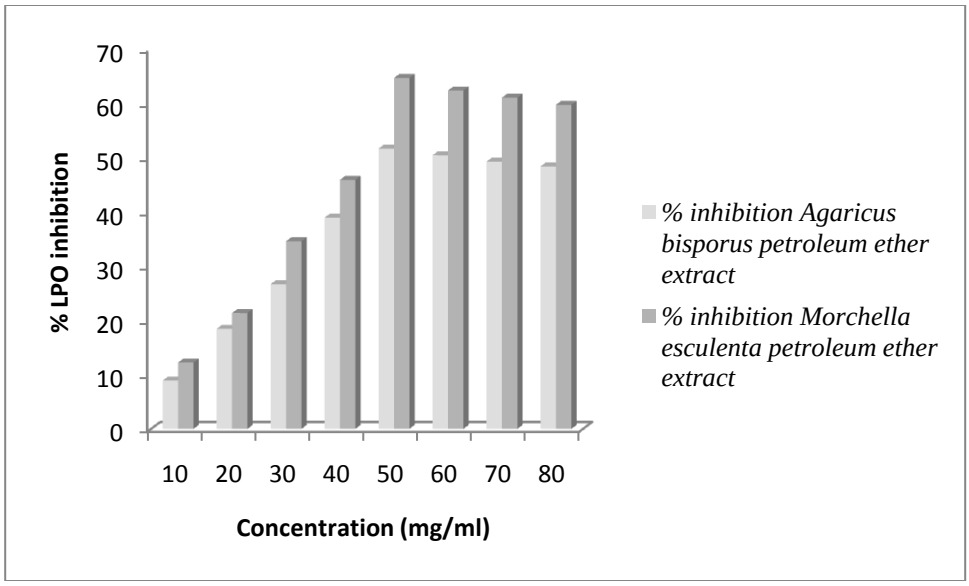


Figure 5.6.3: Comparison between the LPO inhibition (%) of A. bisporus and M. esculenta for Petroleum Ether extract

Analyzing the results of Lipid peroxidation inhibition for Paired-Mushroom extract (***82.41±4.12)***, as shown in **Table 5.6.3** it was observed that the scavenging capacity was increased folds in Paired-Mushroom sample than individual mushroom extract. Paired-Mushroom extract was a mixture of methanol extract of *A. bisporus* (***51.62±2.58***) and methanol extract of *M. esculenta* ***(64.63±3.23)*** which gave highest % inhibition.

Table 5.6.3: Lipid peroxidation (LPO) inhibition (%) for Petroleum Ether extract of *A. bisporus*, *M. esculenta* and Paired-Mushroom sample

% inhibition of Agaricus bisporus petroleum ether extract	***% inhibition of Morchella esculenta petroleum ether extract***	***% inhibition of Mushroom pair extract***
51.62±2.58	***64.63±3.23***	***82.41±4.12***

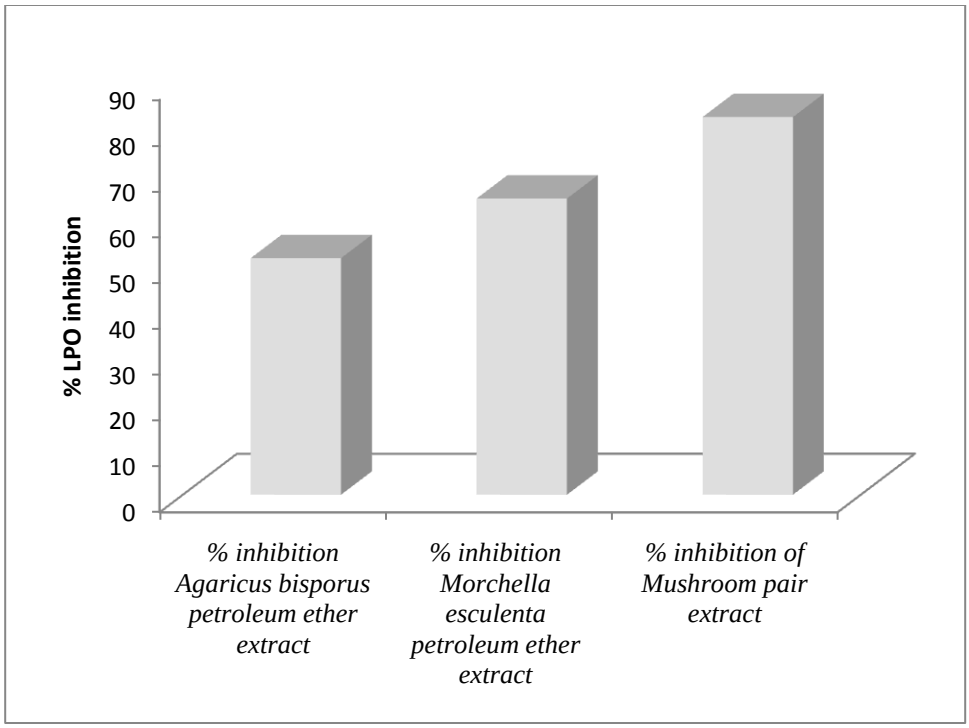


Figure 5.6.4: Lipid peroxidation (LPO) inhibition (%) for Petroleum Ether extract of A. bisporus, M. esculenta and Paired-Mushroom sample

5.7 Virtual Screening of mushroom nutraceuticals

Libdock docking was performed using Discovery Studio 3.5 with different proteins as inflammation markers and mushroom bioactive components as ligands. The ligands were docked into each active site using Libdock procedure. Through this we screen out the best antioxidant components from *Ganoderma lucidum, Lentinus edodes, Cordyceps sinensis and Morchella esculenta* based on the binding energy of the ligand (bioactive component) to specific binding sites of the protein, here, LBP, LOX-2, COX-2, PLA-2, TLR-4, TNF-, and IL-1B. Higher Libdock scores indicate stronger receptor-ligand binding. **Table 24** represents the libdock score.

Table 5.7: LiBDOCK SCORE FROM DISCOVERY STUDIO 3.5

PROTEIN (INFLAMMATORY MARKERS) ⇨ / **LIGANDS** (BIOACTIVE COMPONENTS OF MUSHROOM) ⇩	**LBP**	**COX-2**	**LOX-2**	**TLR-4**	**PLA-2**	**IL-1B**	**TNF-ALPHA**
(Ganoderma lucidum) **GANODERAL A**	146.843	115.549	114.158	104.619	88.2962	0	0
GANODERIC ALDEHYDE A	130.9	118.785	112.607	109.677	83.4543	0	0
GANODERIC ACID A	118.666	127.036	112.143	111.907	0	0	0
GANODERMIC ACID S	92.9878	127.765	99.5874	115.564	0	0	0
GANOLUCIDIC ACID A	114.179	124.522	113.082	101.853	115.186	0	0
GANOLUCIDIC ACID B	109.171	126.192	109.371	119.325	60.3763	0	0
LUCIDENIC ACID A	122.802	109.562	117.495	97.9677	91.7208	0	0

GANODERIOL F	141.742	122.448	120.58	107.494	70.1437	0	0
GANODER-MANONDIOL	141.714	114.604	116.104	110.566	0	0	0
GANODER-MANONTRIOL	145.188	115.406	117.719	109.37	0	0	0
METHLY GANODERATE A	120.027	112.609	108.936	113.51	0	0	0
(Lentinus edodes) **ERITADENINE**	118.437	111.476	111.206	121.719	97.6062	0	93.4152
CENTANAMYCIN	125.944	103.213	132.504	119.843	57.8931	0	0
LENTINAN	0	0	0	0	0	0	0
(Cordyceps sinensis) **CORDYCEPINS**	118.907	114.472	106.251	115.325	95.8137	0	88.3173
ADENOSINE	129.246	122.808	106.155	122.013	102.889	0	93.3171
(Morchella esculenta) **GALACTO-MANNAN**	144.395	142.256	142.988	146.156	128.877	0	0

6. DISCUSSION

According to the data shown in Table 5.1, the amount of residue obtained was more from *Morchella esculenta* as compared to that obtained from *Agaricus bisporus* .

Morchella esculenta is a popular edible mushroom. As reported in the previous studies, we showed its nutritional and nutraceutical properties, as well as its strong antioxidant activity.
The total phenolic content in the mushroom extracts was determined with the Folin-Ciocalteu. Gallic acid standard was used determine the concentration of phenols in the extract. Total phenolics was expressed in mg/g gallic equivalents (GAE). Table 5.2.2 showed that TPC varies from the range 15.39±0.7695 to 496±24.8 mg/g for various extracts of *Agaricus bisporus* and 113.403±5.67 to 769.57±38.4785 for various extracts of *Morchella esculenta*. *M. esculenta* (769.57±38.4785) showed highest concentration of TPC in Aqueous Extract. Whereas, looking at the results of *A. bisporus* (496±24.8), highest TPC was observed in Methanolic Extract. Table 5.2.3 shows the Synergistic combination of both the mushrooms which was tested for TPC and it was observed that the Paired-Mushroom extract of both the mushroom samples showed higher TPC than individual mushroom extracts.

The amount of total flavonoid was determined using Quercetin as the standard and the total flavonoid were expressed as mg/g Quercetin equivalent using the standard curve. Table 5.3.2 shows the Flavonoid content varying from the range 1.71±0.0855 to 6.52±0.326 mg/g for various extracts of *Agaricus bisporus* and 2.39±0.1195 to 24.23±1.2165 for various extracts of *Morchella esculenta.* It was observed that *M. esculenta* (24.23±1.2165*)* showed highest flavonoid concentration in Aqueous extract, whereas, *A. bisporus* (6.52±0.326 *)* showed highest flavonoid content in Methanol extract. Flavonoid content was measured for Paired-Mushroom extract shown in Table 5.3.3. It was observed that it showed many folds increase in concentration of Flavanoid content (84.67±4.2). The mixture was made of methanol extract of *A. bisporus* (6.52±0.326) and aqueous extract of *M. esculenta* (24.23±1.2165).

The scavenging effects of ABTS and DPPH radicals were determined measuring the decay in absorbance at 734 nm and 517 nm respectively. Table 5.4.1 and Table 5.4.2 represents ABTS Free radical scavenging activity of mushroom samples which showed that methanol extract of *M. esculenta* has highest % inhibition = 47.51±2.37 at 50mg/ml whereas, methanol extract of *A. bisporus* has highest % inhibition =34.14±1.707 at 50mg/ml. Analyzing the DPPH scavenging capacity from Table 5.5.1 and Table 5.5.2 of the two mushroom samples, it was observed that for both the mushroom samples the highest DPPH scavenging activity was seen in methanol extract at 50 mg/ml with *M. esculenta* 54.28±2.71 % inhibition and *A. bisporus* 45.99±2.29 % inhibition. It was also observed from the graphs (*Figure 4,5,9,10*) that beyond the concentration at 50 mg/ml, the curve gradually became constant showing reduced scavenging activity. ABTS and DPPH scavenging capacity was also tested for Paired-Mushroom sample. It was observed that for ABTS and DPPH a noticeable increase was seen, 70.5±3.53 and 90±4.5 respectively.

Petroleum Ether extract was used to evaluate the Lipid peroxidation inhibition by the two mushroom samples. *A. bisporus* showed 51.62±2.58 % LPO inhibition and *M. esculenta* showed 64.63±3.23 % inhibition. Both the mushroom samples showed highest % inhibition at 50 mg/ml concentration.

Concluding from the results discussed above, *Morchella esculenta* contains higher free radical scavenging activity than *Agaricus bisporus* as the Total phenolic content and Flavonoid content are more in *M. esculenta*. Observing the extract results of methanol, chloroform and aqueous, it was noticed that methanol and aqueous extract shows high anti-oxidant activity in *M. esculenta* and methanol extract shows in *A. bisporus*.

The synergy and additive effect of pair mushrooms proved to be beneficial as it increased the antioxidant activity many folds.

Discovery of bioactive compounds from natural products have gained importance in recent years. Demand for natural antioxidant has been increasing due to concerns about the safety of synthetic antioxidants. Through *in silico* study we planned to screen out the best antioxidant components from *Ganoderma lucidum, Lentinus edodes, Cordyceps sinensis and Morchella esculenta* based on the binding energy of the ligand (bioactive component) to specific binding sites of the protein, here, LBP, LOX-2, COX-2, PLA-2, TLR-4, TNF-, and IL-1B. Higher Libdock scores indicate stronger receptor-ligand binding. The docking results further more explained the ligands fitting with a specific pose into the receptor cavities. Analyzing the results in Table 5.7, we observed that for LBP (lipopolysaccharide binding protein) the best binding is seen with Ganoderal A (146.843) from *G. lucidum* , Centanamycin (125.944) from *L. edodes*, Adenosine (129.246) from *C. sinensis*, and Galactomannan (144.395) from *M. esculenta*. For COX-2 (cyclooxygenase-2) Ganoderic acid A (127.765) from *G. lucidum* , Eritadenine (111.476) from *L. edodes*, Adenosine (122.808) from *C. sinensis*, and Galactomannan (142.256) from *M. esculenta.* For LOX-2 (lipoxygenase-2), Ganoderiol F (120.58) from *G. lucidum,* Centanamycin (132.504) from *L. edodes*, Cordycepins (106.251) from *C. sinensis*, and Galactomannan (142.988) from *M. esculenta.* For TLR-4 (toll like receptor-4) , Ganolucidic Acid B (119.325) from *G. lucidum,* Eritadenine (121.719) from *L. edodes*, Adenosine (122.013) from *C. sinensis*, and Galactomannan (146.156) from *M. esculenta.* For PLA-2 (phospholipase A2), Ganolucidic Acid A (115.186) from *G. lucidum*, Eritadenine (97.6062) from *L. edodes*, Adenosine (102.889) from *C. sinensis*, and Galactomannan (128.877) from *M. esculenta.* For TNF-α (Tumour Necrosis Factor- α), no ligands docked from *G. lucidum*, Eritadenine (93.4152) docked from *L. edodes,* Adenosine (93.3171) showed higher Libdock score from *C. sinensis*, and Galactomannan also didn't show any binding affinity towards TNF-α . IL-1β (Interleukin-1β) was unable to dock any of the ligands from the four mushrooms. Further analyzing the results, it can be concluded that Galactomannan from *M. esculenta* showed highest Libdock scores for all the protein receptor and hence through this study predicted to be the best antioxidant bioactive component.

7. FUTURE WORK

Now with *in vitro* and *in silico* studies done we planned to make a dietary supplement using the synergistic effect of different mushroom components and their response to inflammation. *In vivo* studies the anti-inflammatory activity of the extract would be studied through using acute and chronic inflammation models using swiss albino mice. In acute models, inflammation will be induced by carrageenan and dextran and chronic inflammation by formalin.

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