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Identification of Fungal Metabolites with Antiaging Effects-An Insilico Study

by

Javeria

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I dedicate this thesis to my loving and supportive family and friends who have fully helped me in achieving my life goals.

CERTIFICATE OF APPROVAL

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Abstract

Aging is an intricate biological process, characterized by a steady decline in cellular functions and an increased risk of chronic disorders. Among the behavioral, environmental, and genetic factors contributing to aging are inflammation and oxidative stress. As the world's population ages, research into safe and practical methods to reduce the consequences of aging is becoming more and more vital. Fungi are a crucial source of bioactive metabolites, providing diverse compounds with significant pharmaceutical and industrial applications due to their unique biochemical pathways. Their metabolites have led to the discovery of antibiotics, immunosuppressants, and anticancer agents, underscoring their importance in drug development. This study explores the potential anti-aging properties of many fungal metabolites using an in-silico approach. Ergothioneine, kojic acid, and ganoderic acid were selected as potential metabolites and examined for their interactions with key aging-related proteins such as PI3K, Akt, and IGF1R. The 3D (Dimensional) structure of the target proteins and the ligands served as the input for docking. The best ligand was selected based on physicochemical properties, ADMET properties, docking score, and lipinski rule. MD Simulation were performed to prove

disapprove the docking results. By considering all these parameters ganoderic acid was seen to obey all drug-like properties with a docking score of -8.8 against insulin-like growth factor 1 receptor. The ganoderic acid Combined with AKT protein has mean RMSD value of 1.24Å. The research also underscores the promising ADMET profile of ganoderic acid, indicating its suitability as a therapeutic agent due to its moderate water solubility, good intestinal absorption, and minimal toxicity. To check further effectiveness of ganoderic acid, it was compared with commercially available antiaging drug metformin. A comparison of all drug-like characteristics showed that ganoderic acid is much better in many aspects than metformin. Metformin showed a docking score of -4.8 while ganoderic acid has -8.8, other pharmacokinetic properties of ganoderic acid are also good than metformin. So, it is concluded here that ganoderic acid can prove itself as a potential anti-aging drug candidate in future therapeutics.

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Abbreviations

Admet	Absorption, Distribution, Metabolism, Excretion, Toxicity
Akt/Pkb	Protein Kinase B
Bbb	Blood Brain Barrier
Cadd	Computer Aided Drug Designing
Cb-Dock	Cavity Detection Guided Blind Docking
Cns	Central Nervous System
Cyp2d6	Cytochrome P450 2d6
Gravy	Grand Average of Hydropathicity
Hba	Hydrogen Bond Acceptor
Hbd	Hydrogen Bond Donor
Igf1r	Insulin-Like Growth Factor 1 Receptor
Kegg	Kyoto Encyclopedia Of Genes And Genomes
Mw	Molecular Weight
Nr	Negative Residues
Pdb	Protein Data Bank
Pi3k	Phosphoinositide 3-Kinases
Pr	Positive Residues
Vdss	Volume Of Distribution At Steady State

Chapter 1

Introduction

Fungi as microorganisms have been increasingly utilized for obtaining biologically active substances, due to their expected biosynthetic capacity. These metabolites produced by fungi boast broad industrial applications, and their production proves cost-effective in difference to plant-derived compounds [1]. The primary use of fungal metabolites includes pigment, antimicrobial immunosuppressant, immune enhancer, antioxidant, cytotoxic agent, enzyme blocker, and other substances active in the pharmaceutical or food industries. Recently a large number of compounds with antioxidant properties have been reported. Because oxidative stress has been associated with several disorders, including neurological disorders, researchers have been looking at natural metabolites for their reduction. Researchers have discovered that a few of these compounds have an important inhibitory effect against the enzyme acetylcholinesterase (AChE), which is related to several neurological disorders [2]. The history of human and animal nutrition and well-being is long and connected, and it contains fungal biomass and metabolites.

Many different types of proteins are produced by filamentous and microfungi , lipids, vitamin minerals, trace elements, pigment dyes, antibiotics, pharmaceuticals, and more bioactive substances. For example, fungi have been utilized for producing for more than 50 years, alternative industrially significant enzymes and microbial biomass proteins. In the beginning by-products and derivatives which were rich in carbohydrates were converted into protein-rich fungal biomass. The

biomass was then prepared into a vegetarian substance further for food application. There has also been a notable increase in the number of papers on the synthesis of lipids from microbial sources in the worldwide literature during the past decade such as single cell oils (SCOs) produced by so-called oleaginous" microorganisms, including "oleaginous "fungi like zygomycetes species e.g. *Cunninghamella echinulata* and *Mortierella isabellina* [3]. Fungi could be a major source of polyunsaturated fatty acids (PUFAs), such as gamma-linolenic acid (GLA) and arachidonic acid (ARA), are polyunsaturated fatty acids [3].

Aging is an unavoidable biological process. However, people do not wish to grow older because of the associated risk factors of several chronic diseases [4]. The process of aging is additionally impacted by elements like chronic inflammation (CR), oxidative stress, unhealthy lifestyle, and environmental exposures [5]. This process is impacted by genetic factors, lifestyle choices, and environmental factors including xenobiotic populations, infection pathogens, UV radiation, dietary toxins, and more. The ageing process and senescence are associated including a range of internal and outside signs and symptoms, including wrinkles, atherosclerosis, diabetes, neurological conditions, and cancer [6]. While genetic and environmental factors impact healthy ageing, diet, and the gut microbiota are essential to the timely senescence process. Ageing is connected to differences in gut microbiota that are often associated with changes in the gastrointestinal tract and dietary patterns. Antioxidants have the potential to this process and prolong a healthy lifespan by inhibiting the development of free radicals or by reducing the amount of oxidative stress.

The two basic strategies for extending a healthy lifespan are genetic or pharmaceutical management and lifestyle change [7]. Essential nutrients including specific vitamins, minerals, vital amino acids (both essential and branched-chain) probiotics, plant metabolites, and polyunsaturated fatty acids (PFUs) like terpenoids and polyphenols can delay ageing and promote healthy ageing [8]. Ageing is impacted by a variety of lifestyle factors that are under human control, including nutrition and exercise. The most efficient is calorie restriction (CR). known antiaging process, it prolongs the lifespan of, mice, fruit flies, worms, yeast, and

humans as well. Also, CR increases a healthy lifespan by preventing the development of several diseases associated with ageing, such as diabetes, cancer, heart disease, and neurodegeneration. In model organisms, a variety of compounds isolated from plants and fungi have been shown to increase longevity and prevent diseases associated with aging [8].

The compounds derived from fungi and plants regulate similar cellular and physiological pathways as calorie restriction (CR). These include pathways linked to insulin and insulin-like growth factors such as sirtuins and the mammalian target of rapamycin. The control of these aging-associated pathways initiates several cellular processes, such as autophagy, DNA repair, and reactive oxygen species neutralization. When considered as a whole these cellular processes are thought to improve the body's responses to stress and delay the onset of chronic diseases. Edible mushrooms have been shown in several studies to have a variety of health benefits, including antiaging, antiviral, anti-inflammatory, antioxidant, immune boosting, lipid-lowering, antioxidant, and anticancer properties [9]. Many mytochemicals with antioxidant qualities may be found in edible and medicinal mushrooms, including phenols, flavonoids, polysaccharides, vitamins, carotenoids, ergothioneine, and other compounds. The antiaging properties of the medicinal fungus *Tricholo malobayense* could be credited to a variety of bioactive compounds.

The polysaccharide TLH-3 extracted from the fresh fruiting body of *Tricholo malobayense* shows d-galactose-induced anti-ageing potential in mice model [10]. Currently, many compounds with anti-ageing activity have been discovered. Several metabolite compounds have been studied for their ability to reduce or decrease the process of ageing. While research is ongoing the field is constantly evolving with some reported notable fungal metabolite compounds Resveratrol, Ergothioneine (EGT), and Cyclosporine, with a strong impact on antiaging. It has been shown that the hydrophilic compound ergothioneine (EGT) with a unique transporter called organic citation transporter 1 (OCNT1), had antiaging effects. Apart from to having antioxidative benefits, EGT has also been shown to have anti-inflammatory, anti-neurodegenerative, and anti-senescence properties [11]. Further insight to explore more compounds with anti-aging potential still needs to be

explored and the current project has been designed with the same purpose.

1.1 Problem Statement

Aging is a natural phenomenon with no specific solution to avoid it but the onset of early aging has become increasingly dominant these days. The impact of lifestyle, diseases, and stress is causing early aging. People are digging into various unnatural therapies to reverse the physical non-aesthetic as well as physiological implications rather than exploring natural bioactive compounds with the same potential.

1.2 Hypothesis

The fungal metabolites might have an active role in the reduction of early aging.

1.3 Aim and Objectives

This research aims to explore potential fungal metabolites showing antioxidant properties to control early aging.

This research entails the following objectives:

- To screen fungal metabolites with anti-inflammatory and antioxidant properties.
- To analyze the interaction between specific fungal metabolites at the desired target.
- To identify the impact of docked metabolites as inhibitory molecules against aging.

Chapter 2

Literature Review

2.1 Bioactive Fungal Metabolites

A metabolite is a compound that plants create for either their defense or survival processes (secondary metabolites), which are typically synthesized from the former, or for their growth and development (primary metabolites). Certain families, genera, or species of plants only produce certain secondary metabolites. Metabolites from another source, isolation of new drugs followed by their identification and explanation through their chemical structures. Once the therapeutic benefits of these isolated organic compounds have been shown by bioassays they are known as bioactive metabolites. The bioactive secondary metabolites act as the basis for the development of new pharmacological drugs after structure changes and further bioassay. Several of these metabolites have been demonstrated through research to be effective as antidiabetic, antibacterial, antioxidant, anticancer, and even insect-killing agents. Many plants with bacterial and fungal endosymbionts have been shown to possess bioactive chemicals. Known as mycotoxins, poisonous fungal metabolites are a class of bioactive molecules that have been identified from plants that have been infected by fungus [12].

2.2 Contaminants of Fungi

2.2.1 Mycotoxins

Mycotoxins are toxic secondary metabolites produced by fungi that can cause disease and death in humans and other animals. These are natural compounds with low molecular weights that exhibit chemical and toxicological heterogeneity. Mycotoxins are found in food items such as coffee beans, nuts, spices, cereals, and dried fruits Food items like cereals, almonds, spices, dried fruit, apples, and coffee beans can contain mycotoxins.

Mycotoxins have the potential to impact all organ systems, although they typically selectively target certain organ systems. Mycotoxicosis is associated with several diseases, such as nephropathy, several types of cancer, autoimmune hepatitis, hepatic diseases, hemorrhagic syndromes, and immune and neurological disorders [13].

2.2.2 Ochratoxins

Ochratoxins are naturally occurring mycotoxins that are present in many worldwide food products such as cereal grains, dried fruits, wines, and coffee contain mycotoxins. Cereal grain, dried fruits, wines, and coffee are some of these products. *Penicillium verrucosum*, *Aspergillums ochraceus*, *A. carbonarius*, and *A. niger* are among the several fungi that produce it. The most effective development of temperatures and water activity levels of these fungi differ and cause the contamination of different agricultural products.

It is harmful to human health, because of its nephrotoxicity; hepatotoxicity, carcinogenicity, teratogenicity, and immunosuppression. Ochratoxins A (OTA) structurally consists of adihydrocoumarin moiety linked with L phenylalanine via amide bounds. Although there are several significant variations regarding certain stages in the biosynthetic process, Ochratoxins A (OTA) biosynthesis has been proposed theoretically [14].

2.2.3 Ergot Alkaloids

The plant pathogen *clavicles* is the primary source of the class of mycotoxins known as ergot alkaloids (EAs). *Clavicles purpurea* is very important as it is the main source of EAs which have the ability to infect over 400 types of monocotyledonous plants. The primary crops impacted by EAs include rye, barley, wheat, millet, oats, and triticale, with rye having the highest incidence of fungal infection. The twelve major EAs are ergometrine (Em), ergotamine (Et), ergocristine (Ecr), ergokryptine (Ekr), ergosine (Es), and ergocornine (Eco). Egometrinine (Emn), egocristinine (Ecrn), ergotaminine (Etn), ergocroninine (Econ), egokryptinine (Ekrn), and ergosinine (Esn1) are their molecules. For the safety of consumers, it's important to monitor these dangerous substances since foods like cereal are necessary components in a variety of foods, like bread, pasta, cookies, baby food, and confections. Ergot alkaloids (EAs) continue to raise concerns regarding the recent rise in these compounds' contamination of food, which has an impact on both human and animal health [15].

2.2.4 Aflatoxins

These polyketides also consider mycotoxins, to several pathological conditions in both plants and animals. The four main forms of mycotoxins that are the most well-known are Alfa toxins G1, G2, B1, and B2. Researchers have identified B1 as the most highly toxic and carcinogenic of the four primary aflatoxins.

2.2.5 Fumonisins

Fumonisins represent a class of toxins that present a significant threat to the well-being of both food and animals, after aflatoxins. The toxicity of fumonisins is significant and frequently co-occurs with Mycotoxicosis (specifically referring to mycotoxins like aflatoxins). These diseases result in significant financial damage to the cattle and poultry breeding sector and pose a hazard to human well-being. Consequently, numerous studies have been investigating techniques to manage and

control fumonisins toxicity. Fumonisins tend to easily contaminate various grains, such as maize and rice, resulting in harmful effects on the liver and kidneys of numerous animal species that consume these grains and potentially leading to the development of tumors. Furthermore, the toxicity of fumonisins has been related to the development of human esophageal cancer and neural tube defect syndrome. As a result, fumonisins have emerged as a prominent area of research, following aflatoxin. Fumonisins are a type of secondary metabolite that is soluble in water. They are mostly produced by *Fusarium verticillioides*, *Fusarium proliferates*, and other species of *fusarium* [16].

2.3 Types of Fungal Metabolites

Like secondary plant metabolites, fungal secondary metabolites are generally produced once active growth stops. The synthesis of fungal secondary metabolites can occur via various pathways within the fungus. Based on their chemical makeup or place of biosynthesis, these routes are typically categorized into four types. Terpenes, polyketides, non-ribosomal peptides, and indole alkaloids are some of these classes.

2.3.1 Polyketides

Type I polyketide synthases (PKSs) are used in this system to join acetyl- and malonyl-coenzyme A (CoA) during the condensation process. The group includes aflatoxins and statins. Due to a variety of characteristics, including the number of reduction reactions, iteration processes, kind of elongation unit utilized, and potential for polyketide chain cyclization, this group exhibits a significant amount of variation. The fungal species Penicillium, Fusarium, and Alternaria primarily produce these polyketide metabolites. Certain secondary metabolites can reduce cholesterol, lowering agents. The cholesterol-lowering effect of a substance called the polyketides a drug derived from the fungi Aspergillums Terries and Meniscus rubber demonstrated [17].

2.3.2 Benzopyrones

The main phytotoxic benzopyrones produced by fungi are benzo-α and benzo-γ pyrones. Benzo-α-pyrones are also known as isocoumarin derivatives fungi [18].

2.3.3 Dibenzopyrone

Dibenzo α and dibenzo γ are the two primary types of phytotoxic dibenzopyrones found in fungi. Dibenzo-α-pyrones are a type of coumarin derivatives derived from heptaketides that have a bonded tricyclic nucleus. A diverse range of biological activities, including cytotoxicity, phytotoxicity, and antibacterial properties, are exhibited by several fungal dibenzo-α-pyrones. The structural characteristic has examined the Alternaria fungus produces dibenzo-α-pyrones, which are phytotoxic [19].

2.3.4 Benzophenones

Benzophenones are identified as xanthine derivatives and differentiated by a common phenol-carbonyl-phenol structure. The A-ring is produced by the shikimic acid pathway, while the B-rings are produced by the acetate-melonate cycle. *Daldinia concentrica* yielded two benzophenones, namely daldinalds A and B (Fungus species) in a rice seedlings experiment, both metabolites inhibited root development. *Fimetariella rabenhorstii* is a fungal species associated with oak decline, particularly in Iran was utilized to isolate moniliphenone and rabenzophenone, also known as chloromoniliphenone. The activity was evident through the induction of necrosis, with diameters typically falling within the range of 0.2–0.7 cm, as observed through the leaf cutting assay conducted on both tomato and oak leaves. Additionally, these two benzophenones were extracted from a solid culture of *Alternaria sonchi*, the sowthistle (*Sonchus spp.*) leaf pathogen. An assay using perforated leaf discs revealed that both metabolites were toxic to the leaves of swothistle (*Sonchus arvensis*) and couch-grass (*Elytrigia repens*) [20].

2.3.5 Naphthopyrones

Phytotoxic naphthopyrones are produced by fungi. that are classified a bis-naphthopyrones. Bisnaphtha-γ-pyrones include a class of polyketides found in fungi that exhibit many biologicalactions, including cytotoxicity, anticancer effects, antibacterial properties, and phytotoxicity. These compounds are classified as diametric naphtha-γ-pyrones. In this work, *Ustilaginoidea virens* (teleomorph: *Villosiclava virens*), the pathogen that causes rice false smut diseases, was identified from a solid rice culture. Specifically, isochaetochromin, B2, and ustilaginoidins E, F, and O were identified. The elongation of rice seedling radicles demonstrated a moderate degree of inhibitory effect. Among the many compounds examined, ustilaginoidin F had the most pronounced efficacy against rice seeds After then, four additional bisnaptho-β-pyrones, particularly ustilaginoidins B, I, R, and U, were taken out of rice false smut balls. Four additional bisnaptho-β-pyrones, particularly ustilaginoidins B, I, R, and U, were later isolated from rice false smut balls. These compounds demonstrated the ability to inhibit the growth of rice seedling radicles and germs [21].

2.3.6 Perylenequinonoids

A class of aromatic polyketides known as perilenequinonoids can be identified by phentacyclic conjugated chromophores. Fungal perylenequinones, or photoactivity phytotoxins, cause damage to host plant cells by absorbing light energy and generating reactive oxygen species.

2.3.7 Aromatics Macrolides

The class of fungal polyketides known as aromatic macrolides is characterized by the presence of a macrolide core structure that is linked in to an aromatic ring. Benzenediol lactones are the typical metabolites. These compounds show a variety of biological actions, such as cytotoxicity, phytotoxicity, and nematicidal properties [22].

2.3.8 Aromatic Polyketides

The properties of aromatic polyketides are involved polyketides aromatic structure type II polyketide synthases (PKSs) are widely used in the synthesis of aromatic polyketides. Through a series of stages, they produce a variety of polyketide chains. In these processes, precursor compounds condense with extender units, and then reduction, cyclization, aromatization, and further modification reactions occur. Aromatic polyketidesinclude fungus-derived phytotoxic polyketides including aromatic macrolides,anthraquinones, Perylenequinonoids, azaphilones, naphthalene's, benzopyrones,dibenzopyrones, benzophenones, naphthopyrones [22].

2.3.9 Non-ribosomal Peptides

Non-ribosomal peptide synthases (NRPSs) use a combination of proteinogenic and non-proteinogenic amino acids to synthesis non-ribosomal peptides. The production of this particular family of peptides without the requirement of mRNA. Examples include the immunosuppressive pharmaceutical agent cyclosporine, synthesized and used in the treatment of recipients of organ transplants, by *Tolypocladium nave* [23].

2.3.10 Terpenes

Trichothecenes, aristolochenes, carotenoids, gibberellins, and indole-diterpenes are all included in this group of fungal metabolites. Isoprene units (C5) are composed of up of terpenes that show both saturated and unsaturated states, as well as many alterations that give variety to this class. They can have a linear or cyclic structure. According to how many isoprene components they contain, sesquiterpenes (C15), hemiterpenes (C5), monoterpenes (C10), sesterterpenes (C25), triterpenes (C30), and sesquiterpenes (C15) are the various categories. The mevalonic acid synthesis is the pathway used by fungi to produce terpenes, which leads to the formation of isopentenyl, diphosphate, and its isomer, diamethylallyl diphosphate

Numerous enzymatic modifications, including redox reactions, glycosylation, alkylation, decarboxylation, and rearrangements, may be responsible for the group's diverse spectrum of structural and physical expression [24].

2.3.11 Indole Alkaloids

The two main precursors used in the production of indole alkaloids are dimethylallyl phosphate and tryptophan. In addition to tryptophan, amino acids may be used as precursors. Claviceps purpurea and related species contain ergot alkaloids, a well-studied class of indole alkaloids differentiated by the presence of an indole ring. The alkaloid ergot can decrease blood pressure through the process of vasodilation. Additionally, they also have inhibitory effects on noradrenaline and sclerotic by modulating the sympathetic nervous system. Ergots stimulate the contraction of the uterine muscles, which might result in an abortion. *Aspergillus fumigates* synthesise the alkaloids fumaclavines and fumitremorgens from tryptophan [25].

2.3.12 Cyclosporin A

Cyclosporine A, which is produced from *Trichoderma polysporum*, was first discovered to have antifungal effects. However, it is now known to possess strong immunosuppressive properties. This strategy is used to reduce the possibility of rejection in recipients of organ transplants. Gibberellins, which are generated from *Gibberella fujikuroi*, are an example of a secondary metabolite that has hormonal properties. Gibberellins, are used, for example, to produce seedless grapes to increase vegetable output accelerate barley malting, improve malt quality, and regulate stem elongation, flowering, and seed germination. *Gibberella sp.* (*Gibberella zeae*) also produces the hormone estrogen zearelanone, which is used to improve nutrition and growth in sheep and cattle Aflatoxin production by secondary metabolites may have adverse impacts that result in some disorders. These include both acute and chronic conditions, such as cancer, weakened immune systems, and in extreme situations, death [26].

2.4 Metabolic Homeostasis and Aging

Metabolism serves as a source of energy for cellular processes, facilitating the production of molecules involved in signaling transmission and the synthesis of essential cellular components. The well-known free radical hypothesis of aging proposed the idea. Several compounds, including resveratrol, astaxanthin, and gallic acid, have been found to enhance longevity and improve age-related diseases by effectively scavenging free radicals [27] The regulation of metabolism is strongly linked with nutrition sensing pathways, such as serotonin, adenosine monophosphate activates protein kinase (AMPK) pathway, and insulin-like growth (IGF) signaling (IIS) pathway target of rapamycin (TOR) signaling [28]. The signaling pathways are in responsible for identifying nutrients or metabolites in order to control the amounts of nicotinamide adenine dinucleotide (NAD), glucose, amino acids, and cAMP. The processes of growth metabolism and aging are regulated by these pathways.

2.4.1 Rapamycin

Rapamycin, is a macrolide, was obtained from *Streptomyces hygroscopic* us, an actinomycete, through the analysis of soil samples collected from easter Island. The initial identification of rapamycin as a novel antifungal drug was followed by further findings of its immunosuppressive and anticancer properties, by inhibiting the mechanistic target of rapamycin (mTOR). Serine/threonine protein kinase, or PI3K phosphatidyl 3 kinase, is the classification given to the mammalian target of rapamycin (mTOR). It regulates numerous biological processes such as metabolism, growth, proliferation, and aging, and it is vital for these processes [28].

2.4.2 Sappanone A

Caesalpinia sappan is the source of sappanone A, an isoflavone that has a major binding affinity for the protein inosine monophosphate dehydrogenase 2 (IMPDH2)

IMPDH2 is linked to ageing. In addition, the GFP-labeled DAF-16's molecular localization was detected, and the HSP-90 proteins' connection was simulated using molecular docking techniques. By changing the IIS pathway, sappanone A, at a 50μM concentration, can increase the life of *C. elegans* and postpone the senescence process.

2.4.3 Nicotinamide Adenine Dinucleotide (NAD), Nicotinamide Riboside (NR) and Nicotinamide Mononucleotide (NMN)

An essential pyridine nucleotide, nicotinamide adenine dinucleotide (NAD+, 30) is involved in several important biological processes, such as DNA repair, oxidative phosphorylation, and the control of gene expression via epigenetic mechanisms. Reduced NAD+ levels may play a major role in accelerating ageing and shortening lifespan and health span due to the correlation between NAD levels and enhanced life and health span as well as their effects on ATP production, intracellular calcium signal transduction, and immune function. A deacetylase linked to lifespan and one that was dependent on NAD for the detection of sirtunine represent whole new research directions in the field of ageing research [29]. It was discovered that giving NR to mice with metabolic impairment was linked to increased SIRT1 expression, lower oxidative stress, and better mitochondrial function [30].

2.5 Role of Fungal Metabolites

2.5.1 Resveratrol

Because the aging population is a major risk factor for numerous chronic diseases, the fast growth in its number has caused serious worries. Foods high in resveratrol, such as peanuts, blueberries, grapes, and red wine, have a variety of bioactive qualities. Research has shown that it has anti-aging, anti-inflammatory, anti-cancer,

anti-diabetes mellitus, anti-obesity, neuroprotective, and cardiovascular protective properties. The main ways that resveratrol prevents aging are through reducing inflammation, controlling apoptosis, boosting the management of oxidative stress, and improving mitochondrial function. Resveratrol could be an effective and safe compound for the prevention and treatment of aging and age-related diseases [31]. Studies have shown that resveratrol has antioxidant, anti-inflammatory, and metal-chelating qualities; however, the exact processes by which it bestows these varied advantages on various diseases are yet unknown. More research is showing that resveratrol can activate the deacetylase sirtuin 1 (SIRT1) in addition to its antioxidant and anti-inflammatory effects. In recent years, SIRT1 has become a promising therapeutic target to treat degenerative disorders associated with aging.

Research conducted on neural cells showed that resveratrol, functioning as a SIRT1 activator, protected SK-N-BE cells from oxidative stress and fatal effects of alpha-syncline and amyloid-beta (Aβ) peptide. Mammals that are calorie-restricted have been found to exist longer, and this effect is linked to SIRT1 gene activation. By enhancing SIRT1 activity, mitochondrial biogenesis, and proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α) deacetylation, resveratrol seems to imitate calorie restriction. These enzymes, in turn, are connected to longevity genes that are crucial for regulating the body's maintenance and repair processes, the regulation of the vintage system, and potentially effective therapeutic methods for neurodegenerative diseases [31].

- **Anti-inflammatory Response of Resveratrol**: Progress in medicine has facilitated the process of aging in humans. Given that the average lifespan in rich countries is already 70 years, it is estimated that by 2030, 20% of the world's population will be over 65. On the other hand, a significant and slow physiological decrease is often linked to aging. Aging is a significant risk factor in the development of cardiovascular disease. Over the past 10 years, resveratrol (RV) has attracted a lot of interest. Red grape, peanut, and blackberry skins are the main sources of this naturally occurring polyphenol [32].

- **Resveratrol's Function in Delaying the Progress of Aging**: The anti-aging characteristics of natural compounds and phytochemicals originating from plant sources have been carefully investigated with particular attention given to the role of resveratrol in slowing the development of aging. The aging process is accelerated by excessive oxidative stress, which leads to damage to DNA, mitochondria, and proteostasis [32]. It is vital to enhance the cellular capacity, to maintain the equilibrium between reactive oxygen species (ROS) and antioxidants in such instances. Many phytochemicals included in food have the capacity to scavenge reactive oxygen species (ROS).

2.5.2 Curcumin

Strong antioxidant properties of the substance protect cells from lipid peroxidation, protein carbonylation, and mitochondrial permeability transition [33]. However, this particular polyphenolic compound exhibits anti-inflammatory properties. The purpose of this study is to learn more about the signaling pathways that govern lifespan regulation and to explore the impact of curcumin on aging processes. Dietary interventions and caloric restriction could serve as physiological measurements for extending lifespan by lowering the metabolic rate, boosting the ability of cells, tissues, and organ systems for functional reserve and signal transduction control [33].

Curcumin's impact on the main aging signaling pathways: The insulin/insulin-like growth factor (IGF) signaling (IIS), the serine/threonine kinase mechanistic target of rapamycin complex (motors), and the protein kinase A (PKA) pathways are the main evolutionary conserved signaling pathways among the various signaling cascades through aging that are known to affect the longevity of organisms [34]. The impact of curcumin signaling pathways on the process of ageing. Because curcumin stimulates the PI3K/AKT pathway, FOXOs are transferred into the nucleus and FOXO-dependent gene expression is activated. This reduces oxidative stress and, as a result, lengthens the life span. Additionally, curcumin can lengthen life by modifying apoptotic and inflammatory proteins such

Bcl2, NF-κB, Bad, caspase 9, and fast. Another method that curcumin lengthens life is via inhibiting the mTORC1 pathway as shown in figure 2.1 [34].

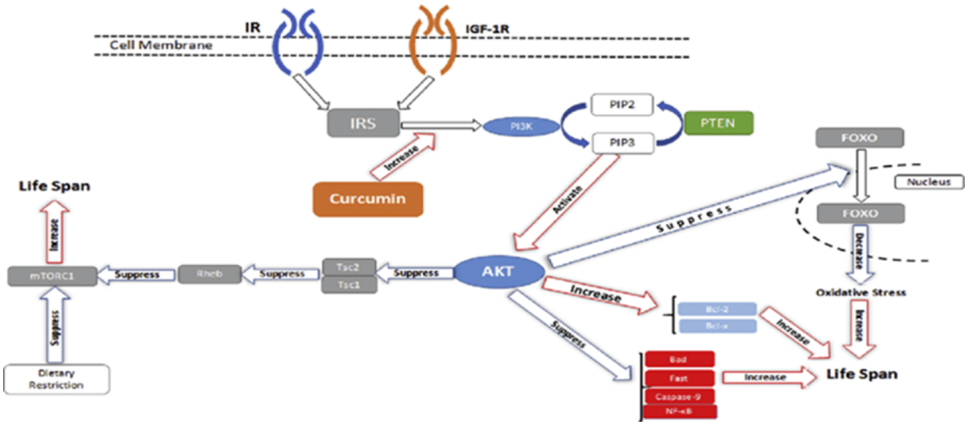


FIGURE 2.1: Effect of curcumin signaling cascades in the aging process

2.5.3 Ergothioneine

Anti-aging benefits are demonstrated by ergothioneine, a hydrophilic molecule that binds with the particular transporter Organic citation transporter 1 (OCTN1). Apart from its antioxidant qualities, EGT is also known for its anti-inflammatory, anti-neurodegenerative, and anti-senescence activities. Ergothioneine (EGT) is an amino acid with hydrophilic properties that is synthesized only by specific bacteria and fungi present in nature. The compound in question is a thiol-thione tautomer's derivative of hercynine. In both solution and physiological conditions, it primarily resides in the thione form, which offers enhanced stability. Thus, EGT exhibits greater resistance to natural oxidation compare to other thiols like glutathione, that undergo rapid oxidation. Moreover, it has been observed that EGT exhibits enhanced effectiveness in aging free radicals compared to other common antioxidants, including glutathione, trolox (a derivative of vitamin E), and uric acid. Histidine is the source of the thio-amino acid ergothioneine (ERG). It is known for its significant antioxidant properties and is mostly synthesized by microorganisms, particularly edible mushrooms. Multiple in vivo studies have demonstrated that ERG exhibits significant neuroprotective effects in mice subjected to various

neurotoxic stimuli, (e.g., cisplatin, beta-amyloid, and D-galactose) by preventing brain lipid peroxidation, increasing GSH levels, and restoring Ache activity, thereby improving learning and memory deficits [35].

2.5.4 Kojic Acid

The aging process produces various alterations in the skin, such as oxidative stress, and hyperpigmentation. Kojic acids are an iron chelator used to cure skin aging. It works by inhibiting tyrosinase and promoting depigmentation. Kojic acid can act as a UV protective property, suppress hyperpigmentation in humans, and inhibit melanin production, through its tyrosine inhibitory effects. Kojic acid has the potential to be utilized as a chemo modulator to improve the effectiveness of commercially available antifungal drugs or fungicides [36].

2.5.5 Ganoderic Acid

G. lucidum is a component of the triterpenoid ganoderic acid (GAC1).The triterpenoid GAC1 is thought to be the strongest one present in *Ganoderma lucidum*. It can suppress the production of cytokines to a comparable degree [37]. Table 2.1 shows the functions and mechanisms of bioactive compounds.

TABLE 2.1: Table showing the function and mechanism of bioactive components

Function	**Mechanism**	**Bioactive Components**
Life span extension	Binding to TIR-1 and activating the rab-1/pmk-1 signaling pathway to induce the expression of DAF-2	RF3
	Increase hydroxyl and DPPH radical scavenging activities as well as metal chelating activity	G. lucidum polysaccharides I, II, III. IV
	Increase scavenging of hydroxyl radicals, reactions with free oxygen species or ROOH and increase metal chelating activity	GLP

Table 2.1 continued from previous page

Function	Mechanism	Bioactive Components
Antioxidant activity	Increase the production of NADPH, SOD, Mn-SOD, CAT, GSH and GSH-Px: protect the mitochondria in macrophages against t-BOOH induced injury; increase the oxidation of LDL	GLPP
	Induce the productions of SOD, CAT, GPx and GSH and inhibit protein and lipid peroxidation.	Total & lucidum triterpenes
	Increase the production of IL-1, IL-2 and IFN-y, increase the numbers of CD14-CD26 monocyte/macrophage, CD83 CD1a" dendritic cells and CD16°CD56" NK cells; Increase the cytotoxicity of CD56° NK cells	RF3
Immuno modulatory effect	Increase the proliferation of macrophages and their activation through increase in the production of NO.	G. lucidum polysaccharides I, II, III, IV
	Activate NF-KB pathway to decrease the production of IL-8 and MCP-1.	GLPP
	Inhibit the production of TNF-a, INF-y and the secretion of IL-17a.	GAC1
Promotion of stem / Progenitor cell survival	Increase the expression of CAM. IL-1, MCP-1, MIP-1. RANTES, Increase the secretion of BMP-2, IL-11 and aggrecan: Boost TPO- and GM-CSF-like functions	RF3

2.6 Industrial Applications of Fungal Secondary Metabolites

Pigments are one of the other financial uses for secondary metabolites from fungi. The orange-pink color of cooked crab shells and salmonid flesh is attributed to the presence of astaxanthin, a carotenoid derived by the yeast Phaffia rhodozyma.

In Russia, the fungus Blakeslee Tripura is utilized for the industrial production of carotene. This compound function is a pigment in several plant species, including carrots, and plays a crucial role in photosynthesis and photo protection processes. Fungal species such as *Mortierella isabellina* and *Mucor circinelloides* have the potential to accumulate polyunsaturated fatty acids, which play a crucial role in promoting cardiovascular health and reducing levels of "bad cholesterol." Applications of secondary metabolites can be observed in several industries such as agriculture, medicine, pharmaceuticals, and manufacturing. Some metabolites have been used due to their psychoactive properties, like as the ergot alkaloid lysergic acid diethylamide (LSD). However, fungal secondary metabolites exhibit significant variety of are utilized across various industries, where they fulfill essential functions [38]. Applications of fungal metabolites are given in table 2.2.

TABLE 2.2: Applications of fungal metabolites

Fungal Metabolite	**Class**	**Applications**
Alfatoxins	Polyketides	Carcinogenic
Cyclosporine	Non-ribosomal peptide	Immunosuppressant
Ergots	Indole alkaloids	Hypotensive; induce contractions
Gibberellins	Terpenes	Plant growth hormone
Penicillins	Non-ribosomal peptide	Antibiotic
Statins	Polyketides	Hypocholesterolemic

2.7 Natural Food Additives from Fungi

There is a movement to replace synthetic food additives with natural ones due to increasing research that connects taking natural compounds to health benefits. Fungal metabolites feature many properties that have been explored for such replacement. This section focuses on the potential of fungal metabolites as food additives, showing their production functions, adaptation as coloring agents, and current developments and challenges in this field. Fungi offer a variety of food additives and technological enhancers, such as organic acids, colorants, and fatty acids, including certain ω-3 and ω-6 class fatty acids crucial for human metabolism.

Citric acid, derived from Aspergillums niger, and fumaric acid, obtained from Rhizopus oryzae, are ideal food additives that are commercially manufactured. The compounds that can be obtained from fungi, citric acid, and glycolic acid are the most widely produced on a commercial basis [39].

2.8 Sustainable Production of Fungal Metabolites

Development must occur in concert with the growing need for novel drugs, nutraceuticals, food additives, preventive medicines, and other naturally derived health-related products. Strategies to increase the amount that can be produced of bioactive metabolites derived from plants could improve slowly both plant seasonality and a lengthy growing period might have an impact on productivity. As a consequence, research has been focused on increasing the synthesis of bioactive compounds by the application of microorganism-based methods, for example, metabolic engineering.

Development must occur in concert with the growing need for novel drugs, nutraceuticals, food additives, preventive medicines, and other naturally derived health-related products. Strategies to increase the amount that can be produced of bioactive metabolites derived from plants could improve slowly both plant seasonality and a lengthy growing period might have an impact on productivity. As a consequence, research has been focused on increasing the synthesis of bioactive compounds by the application of microorganism-based methods, for example, metabolic engineering. Implementing changes to the method by which fungi are produced is an effective method of improving biomass and bioactive compound yield. Furthermore, there is a particular focus on entophytic fungi, particularly those capable of synthesizing compounds from their host plants as shown in table 2.3. This study presents a selection of many unique. examples of bioactive

substances that entophytic fungi can create once the ideal circumstances for fermentation are found by obtaining 224 patents associated with metabolites produced by endophytic fungi. These applications were applied to various industries such as agriculture, biotechnology, pharmaceuticals, and food industries, Most of the species used for these applications were *Aspergillus, Fusarium, Trichoderma, Penicillium, and Phomopsis* [40].

TABLE 2.3: Fungal species and their target parameters

Fungal Species	Host Plant	Target Compound	Health Benefit	Methodology	Target Parameter
A.terreus	Coconut tree	L - Asparaginase	Treatment of acute lymphocytic leukemia	Factorial experimental design Increase of scale (5-1 bioreactor system).	pH, temperature, incolum concentration
F.solani	*Chenomorpha fragrans*	Campto thecin	Anticancer	Box-Behnken design using one factor at a time method	Carbon and nitrogen sources, ethanol concentration, pH,temperature, incubation period
Peniclillium bilalae	*Phoenix dactylifera*	Acidic protease	Increasing in food digestibility	Response surface methodology. Plackett-Burman design.	Temperature, initial pH, metal ions, carbon and nitrogen source,incubation period.
P.ostreatos	No			Response surface methodology	Nutrients, particles size of the solid substrate, temperature, incubation period

2.9 Role and Regulation of Fungal Secondary Metabolism During Interaction

Low molecular weight compounds that are not necessary for growth are known as secondary metabolites, or SMs. Fungi can function as information networks or as a means of defence since they can connect with a wide variety of living things. In both plant and animal diseases, structural markers (SMs) are sometimes referred to as virulence factors. This function most likely developed as a defence mechanism against potential fungal targets such as nematodes, amoebae, and other invertebrates. The role and function of SMs in the interactions between fungus and microbes, plants, and animals are explained in the section that follows [41].

2.10 Secondary Metabolites Application

Low-molecular-weight substances known as secondary metabolites (SMs) are not necessary for growth. Because fungus communicate with so many different organisms, they can provide protection or act as information networks. Structural markers (SMs) are commonly referred to as virulence factors in both plant and animal infections. Most likely, that function changed as a defense mechanism against invertebrates that may eat on fungus, such as nematodes and amoebae. The section that follows describes the function and importance of SMs in the interactions between fungi and microorganisms, plants, and animals [42].

2.11 Benefits & Research Applications of Fungal Metabolites

Fungal metabolites have a wide range of pharmacological activities that might change significantly along with their variety of structures. But there are a few things in this field that need to be addressed, such as how much of an impact occurs in vivo effects. The prevention and treatment of non-transfusion-dependent

cardiomyopathy (NTCD) and a diverse array of advantageous biological effects exhibited by fungal metabolites. Some of these effects provided a number of possible compounds for the development of new drugs. A.terreus produces terrein a secondary metabolite, in significant amounts a crude extract of 537.26 ±23.42 g/kg. In vitro studies have reported terrain's anti-inflammatory and antioxidant properties. The A. terreus crude extract possesses significant therapeutic characteristics and exhibits a high initial yield, hence enabling its large-scale manufacturing and technological advancement for the prevention of certain age-related non-transferable cardiovascular diseases (NTCD) [43].

2.12 Physiological Disorders and Their Impact on Early Ageing

2.12.1 Schizophrenia

Approximately 1 percent of the global population suffers from schizophrenia, a constant psychiatric disorder. This particular condition typically shows during the early stages of Psychotic symptoms, which include positive, negative, and cognitive symptoms, which are often present in late adolescence or adulthood [44]. The symptoms are positive and do not occur in healthy people. but appear in people with schizophrenia. This condition's symptoms include auditory and visual hallucinations, paranoia, delusions, and significant cognitive disorders. The negative symptoms commonly observed in Individuals without schizophrenia, such as apathy, poor communication skills, social disengagement, lack of enjoyment, and habitual acts are reduced or absent in patients with schizophrenia. The condition known as schizophrenia is distinguished by the presence of psychotic symptoms and deficiencies in memory. Researchers have found that resveratrol has the potential to be used in the treatment of schizophrenia because of its neuroprotective properties. Resveratrol's antipsychotic properties were examined in a recent study including people with schizophrenia [44].

2.13 Antioxidant Compounds of Mushrooms as Neuroprotective Agents

Previous studies have indicated that mushroom antioxidant components have the potential to age-related neurological diseases that may be prevented by boosting antioxidant defenses and reducing oxidative stress [45]. However, it is, that mushroom polysaccharides have received the majority of research funding in studies done so far on the neuroprotective properties of mushrooms [45].

2.14 Mushroom Effects and Their Anti-Aging Properties

The composition of mushrooms and their potential anti-aging benefits have been the focus of several studies. Mushrooms include a range of components such as polysaccharides, phenolic, terpenoid, lipids, vitamins, and mineral. These components have been shown to have antioxidant, antiwrinkle, and antiaging activities [45]. However, it is important to note that the anti-aging effects of mushrooms mainly target the process of skin aging and age- related diseases. Antioxidants found in mushrooms include phenolic compounds, polysaccharides, and ergothioneine. These compounds can rapidly absorb free radicals and reduce the effects of oxidative stress [45].

2.15 Regulation of Genetic Pathways and Genes Involved in Aging

Multiple genetic factors, such as Cyclin-dependent kinase inhibitor 1 (p21), cellular tumour antigen p53 (p53), sirtuin1 (SIRT1), and the 66-kDa isoform of ShcA (p66Shc), along with numerous pathways, for example, pathways It is known that aging and lifespan are related to the IIS pathway and the rat sarcoma/protein

kinase A (Ras/PKA) pathway. One of the components known to prevent the start of several age-related disorders, including as cancer, neurodegeneration, and cardiovascular disease, is SIRT1.

2.16 Regulation of Genetic Pathways and Genes Involved in Aging

Ageing and longevity have been linked to multiple genetic factors, such as Sirtuin1 (SIRT1), cellular tumour antigen p53 (p53), cyclin-dependent kinase inhibitor 1 (p21), and the 66-kDa isoform of ShcA (p66Shc), as well as multiple pathways, including the Ras/PKA pathway and the IIS pathway. For example, SIRT1 is known to have a protective role against the onset of several age-related illnesses, including as cancer, neurodegeneration, and cardiovascular disease [46].

2.17 Insulin-like Growth Factor Pathway

Insulin-like growth factor (IGF)-I have significant mitogenic and differentiating effects on almost all cell types and has an impact on protein metabolism control. IGF-I receptors, which have two extracellular α-subunits with hormone binding sites and two membrane-spanning β-subunits encoding an intracellular tyrosine kinase, mediate the physiological actions of IGF-I and are responsible for the biological effects of the protein. Tyrosine residues on a variety of substrates, including IRS and Shc proteins, are phosphorylated as a result of receptor kinase activation following hormone interaction [47].

According to the early tyrosine phosphorylation processes help IGF-I signals reach a complex network of serine/threonine kinases and intracellular lipids. These kinases are essential for tissue differentiation regulation, cell division, and apoptosis prevention. Through their participation in IGF-I signaling, the previously indicated homologs of pro-aging genes in lower organisms-IGFIR, PI3K, Ras, Akt,

Tor, and S6K-play a critical role as growth and survival mediators in mammalian cells. That a number of genes and the pathways that link them are thought to be essential for increasing stress sensitivity and ageing in animals. Furthermore, by preventing the activation of apoptotic pathways, these genes may offer protection as shown in figure 2.2 [47].

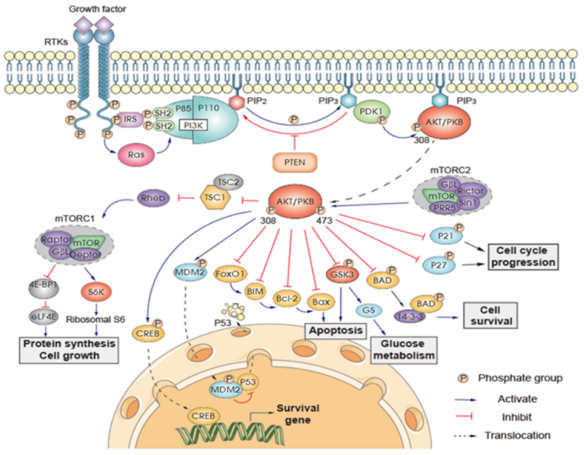


FIGURE 2.2: Insulin-like growth factor pathway

2.18 Protein Kinase B (AkT) Signaling

In response to external stimuli, the PI3K-Akt pathway is an intracellular signal transduction process that supports angiogenesis, growth, metabolism, and cell survival. The phosphorylation of serine and/or threonine residues on several substrates downstream helps in the process. The main proteins involved are Akt/Protein Kinase B and phosphatidylinositol 3-kinase (PI3K). PKB/Akt research began in 1977. discovery of an ATK8 virus by Steal et al. and colleagues, which included a previously unknown cancer. The oncogenic sequence produced from cells was extracted and designated as Akt.

In 1991, PKB/Akt was discovered to be a new phospho-protein kinase with broad expression when three separate research teams discovered genes linked to it. Further research on PKB/Akt's function in many cellular processes was made possible

by these results. Cantley's team also succeeded in 1990 in isolating phosphatidylinositol 3-kinase (PI3K). In 1995, Roth et al. and associates subsequently demonstrated that insulin causes Akt to become active. Research has since demonstrated how important membrane phospholipids generated by PI3K are for PKB/Akt activation [48].

2.18.1 PI3K-AKT Composition

Receptor tyrosine kinases (RTKs), phosphati dylinositol 3 - kinase (PI3K), phosphati dylinositol- 4, 5 - bisphosphate (PIP2), phosphati dylinositol - 3, 4, 5 - trisphosphate (PIP3), and AKT/protein kinase B are the main molecules involved in this signaling pathway. RTKs function as high-affinity cell surface receptors for a variety of polypeptide, cytokine, and hormone growth factors. This specific receptor possesses three unique functional domains: the transmembrane domain, the intracellular tyrosine kinase domain, and the extracellular ligand binding domain. Receptor tyrosine kinases (RTKs) dimerize when two of its monomers come into contact with one another after growth factors attach to them.

The intracellular tyrosine kinase domain is activated as a result of this dimerization process, and each monomer is then auto phosphorylated. The hydroxyl group at position three on the phosphatidylinositol inositol ring can be phosphorylated by a kinase known as PI3K (figure 2.2). The two distinct domains that make up the PI3K protein are the catalytic domain (P110) and the regulatory domain (P85). PI3K activation often results from either indirect activation mediated by adapter molecules like insulin receptor substrate (IRS) proteins or direct stimulation by the regulatory subunit linked to the active receptor. A RAS protein that binds GTP can activate PI3K. PIP2 and PIP3 are both trace amounts of phospholipids found in cell membranes.

In general, PIP3 functions as docking phospholipids, binding certain domains to facilitate the transit of selected proteins to the cell membrane and the subsequent activation of signalling pathways. In the PI3K-AKT pathway, the phosphate group at position 3 of PIP3 may bind to both the AKT and PDK1 proteins.

Through the selection of AKT protein to the plasma membrane facilitated by this binding mechanism, PDK1 is able to attach to and phosphorylate T308 inside the activation site. Whole Akt activation is achieved by either DNA-PK or mTORC2 phosphorylating Akt at S473 in the carboxy-terminal hydrophobic region [49].

2.18.2 PI3K-AKT Regulation

Since the PI3K-Akt pathway has so many downstream effects, it needs to be properly regulated. As Figure 2.3 shows, the PIP3 level and the inactivation of AKT protein are the two targets that can be negatively regulated. The PIP3 level can be down regulated by tensin homolog, protein phosphatase, and AKT protein because they can convert PIP3 into PIP2.Two negative regulatory proteins, protein phosphatase2A (PP2A) and phosphatase PHLPP, Akt is dephosphorylates at Thr308 and Ser473 respectively. TNFα and PPARα/ι, which are peroxisome proliferator-activated receptors, are both regulated by NF-κB, a transcription factor that is activated by Akt. The pathway also consists of feedback mechanisms. These factors, in order, to suppress the expression of PTEN as a positive reaction mechanism. The feedback chain that is negative is facilitated by the activation of mTORC1 and S6K1.S6K1 has the capability to phosphorylated IRS1 several serine sites which slow its binding to RTKs and consequently suppresses the activation of PI3K [50].

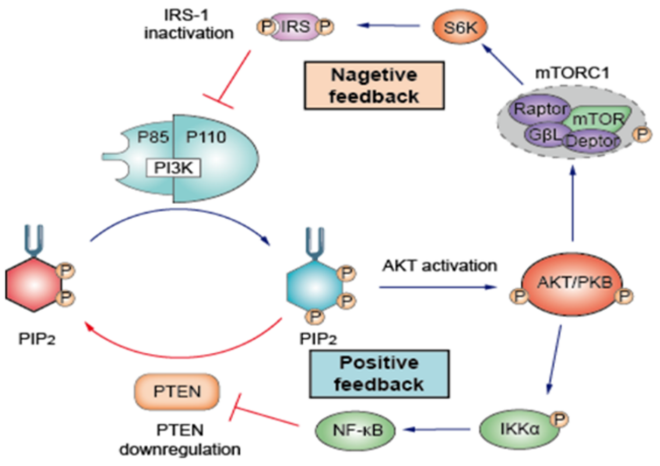


FIGURE 2.3: PI3K-AKT Regulation

2.18.3 Downstream Signaling

Akt has the ability to transfer from the nucleus and cytoplasm to the plasma membrane, where a significant number of its substrates are found. There are two possible outcomes of Akt phosphorylation: stimulatory or inhibitory.resulting in the suppression or enhancement of target protein function. The regulation of several cellular processes by Akt is dependent upon the specific target protein. In this analysis, we will examine numerous key effects of the PI3K-Akt pathway [51].

2.18.4 Cell Apoptosis/Survival

Proapoptotic proteins and activities are inhibited by Akt activation, which increases cellular survival. It suppresses the expression or activity of the Bim, Bax, and Bcl-2 family of proteins. Additionally, Akt exerts inhibitory effects on the production of many proteins containing only BH3 by modifying transcription factors, including p53 and FOXO. An oncogene called P53 helps in the process of cellular death. Akt can promote the degradation of p53 using phosphorylating MDM2. The Akt protein can phosphorylate GSK3, an enzyme variation in a stable regulatory region at the N-terminus, resulting in the inactivation of the kinase. This process ultimately regulates apoptosis and glucose metabolism through the modulation of GSK3 [49].

2.18.5 Protein Synthesis and Cell Growth

One of Akt's primary roles is to stimulate cell division by blocking TSC2, which triggers mTOR complex 1 (mTORC1). An evolutionarily conserved strategy for regulating cell growth is the regulation of translation initiation and ribosome production, with mTORC1 being a key player in this process. Activation of S6K and eukaryotic initiation factor 4E (eIF4E)-binding protein 1 (4E-BP1) can be achieved by Akt. After then, ribosomal S6 is stimulated by S6K activation, which improves protein synthesis and encourages cell division.

2.18.6 Cell Cycle and Proliferation

The protein P21/Waf1/Cip1 keeps the cell in a state of inactivity. In a comparable way, P27 and Kip2 contribute to cell stability throughout the G1 phase. P21/Waf1/Cip1 and P27/Kip2 can be phosphorylated by Akt protein, which counteracts their anti-proliferative actions and encourages them to remain in the cell. Therefore, it could make it easier for the cell to enter the cell cycle and then proliferate. The PI3K-AKT signaling pathway's main function is to encourage cell division and expansion while inhibiting cell death [47].

2.19 Molecular Docking

Finding a ligand's optimal arrangement within a target binding site can be done through a process called molecular docking. The binding affinity between the ligand and the target protein is assessed using a specific scoring function. In the binding area of the target protein, molecular docking also helps to determine the proper ligand conformation. The three-dimensional structures of the ligands and target proteins provide the input for docking. One popular approach in structure-based drug discovery is the use of a target protein's three-dimensional structure.

To assess the binding affinity between the ligand and the target proteins as well as the exact location of the ligand inside the binding site, a specialized scoring function is used. Additionally, it helps in the identification of novel small molecular compounds, revealing the essential properties, such is a high affinity for binding the target protein and sufficient absorption, distribution, metabolism, and excretion, which help in the selection of lead compound for the target [30].

- The docking process requires a three-dimensional structure of protein which is downloaded from protein data bank.
- Minimum size of molecules or compounds or virtual compounds that contain a database is required.

- In addition, a computational structure is needed for calculating the scoring process and performing the process of docking.

Protein and ligand docking is one of the key areas of molecular docking, which obtained high popularity and appreciation due to its role in structure-based drug design. Molecular dynamics, distance geometry method genetics algorithm, etc. are the most widely used algorithms in molecular docking and the most frequently software used for molecular docking are Auto Dock vina, Auto Dock, CB Dock, and ICM, etc. The target proteins and potential ligands selected for this research are given in table 2.4.

TABLE 2.4: Ligands and target proteins

Targets Proteins	Ligands
IGF1R	Ergothioneine
PI3K	Kojic acid
Akt	Ganoderic acid

Chapter 3

Materials and Methods

This chapter provides a comprehensive overview of the methodology (Figure 3.1) adopted for the insilico analysis of the fungal metabolites as a tool towards Anti-agin treatment.

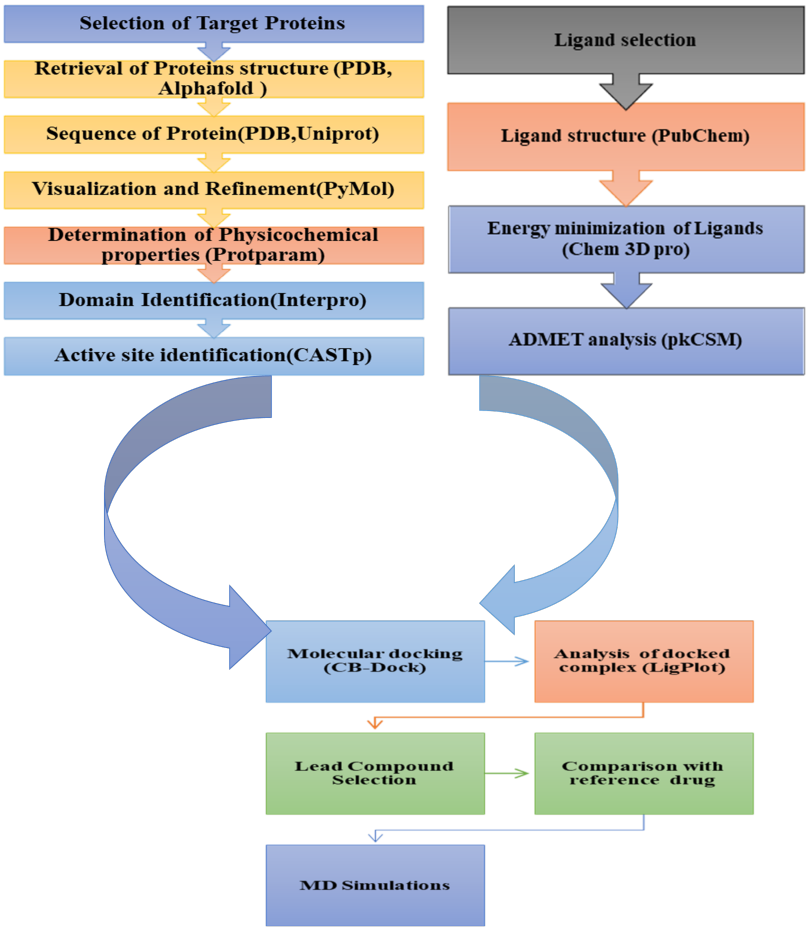


FIGURE 3.1: Technical Route of Current Study

3.1 Route Cause of Aging

In case of aging, no specific treatment is recommended as aging is a natural phenomenon with no specific solution to avoid it. Lifestyle, diseases, and stress are major causes of early aging. People are using different unnatural therapies to slow the process of aging but they may cause physiological implications. Alternative sources are required to lessen the extent of this problem [52]. IGF1R, PI3K, and AkT are potential proteins responsible for the ageing mechanism. Ergothioneine, kojic acid, and ganoderic acid can be potential metabolites to control early aging [53].

3.2 Selection of Target Proteins

The main purpose of the selection of the respective proteins is that they are essential for regulating the differentiation of cells, signaling, and metabolism. The IGF1R, PI3K, and AkT were selected based on their potential to control aging [54].

3.3 3D Structure Prediction of Proteins

The 3D structures can be predicted through PDB www.rcsb.org. Alternatively, I-TASSER can be used if some structures are not available on PDB. I-TASSER zhanglab.ccmb.med.umich.edu/I-TASSER stands for Interactive Threading Assembly Refinement. This software is available online and it predicts the three-dimensional structure and function of proteins. First of all, it identifies the structural model of the PDB through various strategies which include the atomic models of full length and they are built by using simulations of the different threading fragments [55]. The 3D structure of proteins is also predicted by the I-TASSER service and this server gives us five 3D structures of proteins so based on C-score we can select the best 3D structure of the protein [56]. Alphafold alphafold.com is another reliable source for the prediction of the 3D structure of proteins [57].

3.4 Retrieval of Protein FASTA Sequence

The FASTA sequence of the selected target proteins (IGF1R, PI3K, AkT) was taken from the protein sequence database UniProt www.uniprot.org. Alternatively, PDB or NCBI www.ncbi.nlm.nih.gov databases can also be used for sequence retrieval of target proteins [58].

3.5 Analysis of Physicochemical Properties of Target Proteins

The function of the proteins is significantly influenced by their physicochemical characteristics. ProtParam expasy tool web.expasy.org/protparam was used for the prediction of these features of IGF1R, PI3K, and AkT. ProtParam was used to calculate the number of negatively charged residues (Asp+ Glu) and positively charged residues (Arg+ Lys), theoretical pI, molecular weight, aliphatic index, grand average of hydrophobicity, instability index, Ext coefficient (Cys included), and Ext coefficient (Cys not included) [59].

3.6 Protein Structure Analysis and Refinement by Use of PyMol

PyMOL pymol.org is an open-source molecular graphics program that has been used extensively worldwide for the three-dimensional examination and representation of several proteins and small compounds including nucleic acids, densities of different electrons and varying surfaces, and also the trajectories. It is also used for editing the molecules, tracing the ray, and also to make animations and movies. This is software that is based on Python and also contains many plugin tools to enhance its use and facilitate drug targeting and designing by the use of

PyMol software. The excess components linked to the protein must be deleted after downloading the protein structure which was done by the use of an open-source system PyMol [60].

3.7 Functional Domain Identification of Targeted Proteins

Interpro www.interpro.com is a database that was utilized to determine the targeted proteins IGF1R, PI3K, and AkT's functional domains [61]. Sequence, structure, and relationships are all involved in conserved domains.

3.8 Active Site Identification

The area in which the target protein's active site is located is where the ligand exhibits the greatest or maximal interaction with the protein. Amino acids have a major role in the ligand-protein complex building process. CASTp sts.bioe.uic.edu software was used for the detection of protein binding pockets [62].

3.9 Selection of Active Metabolic Ligands

After an extensive literature review, those ligands were selected that have previously shown some antiaging properties. These include ergothioneine, kojic acid, and ganoderic acid.

3.10 Retrieval of Chemical Structure of Ligands

PubChem pubchem.ncbi.nlm.nih.gov is the world's largest repository of easily accessible chemical information databases. So the chemical compounds that were

selected as potential ligands were taken from the PubChem database in SDF format [63]. If in case the selected ligand structure is not available then our next attempt would be to download the canonical smiles from PubChem and then insert them in the software Chem Draw after obtaining the 3D (Dimensional) structure repeat the energy minimization step using Chem3D ultra. At the end pdb format will be selected to save the energy-minimized structure of the ligand.

3.11 Energy Minimization of Ligands

Ligands energy was minimized by using Chem3D ultra. It is a necessary step to refine the ligands before performing docking otherwise, there will be unreliable docking scores [64].

3.12 Virtual Screening of Ligands by Application of Lipinski Rule of Five

An essential criterion for determining whether ligands are likely to be drugs is the Lipinski rule. Certain chemicals are likely to be utilized as active pharmaceuticals in humans if they adhere to the lipinski rule of five. pkCSM omictools.com/pkcsm-tool is an online tool (Pharmacokinetics parameters were checked by using canonical smiles of particular ligands from pdb) and also helps to check whether ligands obey lipinski rule or not [65]. The rules are described as under:

- The log P value should be in the range of five.
- Maximum number of H-bond acceptor should be limited to ten.
- Maximum number of H-bond donor should be limited to five.
- The molecular weight should be below five hundred grams.
- Rotatable bonds should be limited to five.

3.13 Ligands ADMET Analysis

After filtering the ligands by applying the lipinski rule, the next step of the study was the prediction of pharmacokinetic and toxicity properties. pkCSM omictools.com/pkcsm-tool was used Phmacokinetics Parameters were checked by using canonical smiles for particular ligands from PDB. The weak candidates of the drug would be eliminated during ADMET analysis.

The remaining candidates can be selected as potential drugs against the disease. The target proteins were used to optimize the ADMET (Absorption, Distribution, Metabolism, excretion, and toxicity) associated with the human body [66].

3.14 Molecular Docking

For performing the molecular docking between the protein and the ligand, CB-dock (Cavity detection guided blind docking) was used. DockCB clab.labshare.cn finds the sites of docking automatically. CB-Dock is a method of protein and ligand docking that indicates the sites of bonding, the size, and the center calculated. The box size is adjusted according to the ligand and then docking is performed. The docking is performed through AutoDock Vina. Its accuracy ratio is greater because the docking process is more focused on cavity binding [67]. We uploaded the proteins to do docking, use the 3D structure in pdb format and the ligand's 3D structure in SDF format. After this docking is performed, the result would be 5 different poses of interaction.

Its accuracy ratio is greater because the docking process is more focused on cavity binding [67]. We uploaded the proteins to do docking, use the 3D structure in pdb format and the ligand's 3D structure in SDF format. After this docking is performed, the result would be 5 different poses of interaction.

To select the best pose, we would look at the lowest docking score which is given in KJ/m-1 CB- Dock will provide an interactive 3D visualization of results in 5

different poses. Based on the lowest vina score expressed in (kJ/m1), the optimal position was chosen [68].

3.15 Analysis of Docked Complexes via Ligplot

The interaction between the ligand and the active pockets of the protein was computed in order to interpret docking results. The two kinds interaction between the ligand and the active pockets of the protein that were examined are hydrophobic and hydrogen bonding.

Ligplot Plus (v.1.4.5) was used to analyze the protein-ligand interactions. The protein-ligand interactions for the designated ligands in the PDB file are automatically schematically diagrammed by this application [69].

3.16 Lead Compound Identification

The most active inhibitor was found after a thorough examination of docking scores, pharmacokinetic studies, toxicity features, and protein and ligand interactions. Our lead compound was the one that followed all these parameters (docking scores, pharmacokinetic studies, toxicity features, and protein and ligand interactions).

3.17 Reference Anti-aging Drug Selection

The purpose of this step is to identify the commercial drugs that are already in use for antiaging disease treatment purposes. KEGG www.genome.jp/kegg/. Drug Bank databases were used for the identification of the commercial drugs that are already in use for anti aging disease treatment purposes.

Drug Bank go.drugbank.com databases were used for this purpose because they provide details about drugs and their pathways [70].

3.18 Comparison between Lead Compound and Reference Drug

Docking values, molecular interactions, and pharmacokinetic features were compared between the reference anti-aging drug and the suggested lead molecule.

3.19 Molecular Dynamic (MD) Simulation Analysis

MD The best docking poses were subjected to molecular dynamic simulations with AMBER20 to improve optimization and knowledge of how the atoms interact. The protein topology file was generated using the AMBER suit's ff14SB force field, whereas the ligand topologies were generated using the GAFF force field. Following neutralization with Na+ ions, the systems were solvated in a cubic TIP3P water box. To reduce the possibility of steric conflicts, each atom in the complexes was subjected to a 0.1 kcal/mol restriction, and energy reduction was done over 5,000 steps using the steepest descent approach and the conjugate gradient. All atoms covalently bound to hydrogen were subjected to the SHAKE algorithm, which permitted an integration time step of 2 fs, to reach equilibrium. Once equilibrium was reached for each system, an MD run lasting 100 ns was started. A MD run lasting 100 ns was initiated when each system had attained equilibrium. The complexes' radius of gyration (Rg), root mean square fluctuation (RMSF), and root mean square deviation (RMSD) were examined throughout this period. The QtGrace was used to plot the graphs displaying the studied parameters sourceforge.net/QtGrace. simulation Analysis

Chapter 4

Results and Discussion

4.1 Selection of 3D Structure and Refinement of Target Protein

3D structures of target proteins IGF1R, PI3K and AkT were taken from PDB under pdb IDs 1IGR, 4OVV and 1O6K respectively. Using PyMol, the protein structures were created by eliminating any ligands and water molecules. To obtain the stable conformation After the ligands, the absent polar hydrogens were added, and other atoms were removed to prevent overlaps and modified file was saved in PDB format. The refined structures are shown in figure 4.1 4.2and 4.3 below.

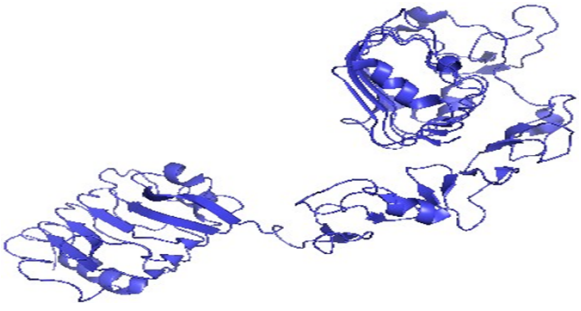


FIGURE 4.1: Structure of IGF1R

IGF1R is a multifunctional receptor with key roles in growth, development, metabolism, cell survival, differentiation, and disease, particularly cancer. Its wide-ranging effects are mediated through complex intracellular signaling pathways [71].

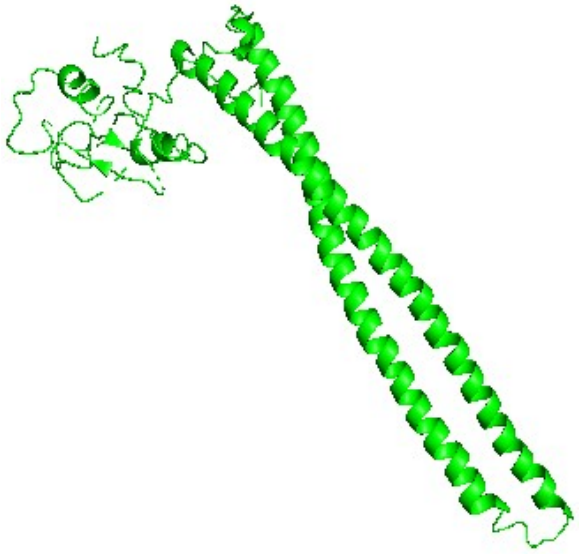


FIGURE 4.2: Structure of PI3K

PI3Ks are pivotal enzymes in cellular signaling, affecting cell growth, metabolism, survival, migration, differentiation, protein synthesis, autophagy, immune responses, and vascular functions. Their dysregulation is associated with a range of diseases, including as immunological disorders, diabetes, and cancer [72].

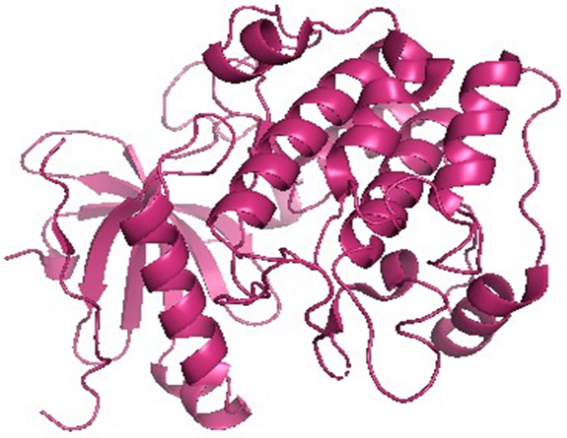


FIGURE 4.3: Structure of AkT

AKT is a key regulator of numerous cellular processes, including growth, proliferation, metabolism, survival, migration, angiogenesis, differentiation, autophagy, immune responses, and DNA repair. Its dysregulation is implicated in various diseases, particularly cancer, where it often contributes to tumor progression and resistance to therapy [73]. SIRT1 and mTOR receptor structures were predicted and refined by the same method in another aging-related research [74].

4.2 Primary Sequence Retrieval

Using UniProt, the chosen proteins' FASTA sequences were obtained. The FASTA sequence of IGF1R, PI3K, and AkT was downloaded from uniport under accession number P08069, Q8NEB8, and Q9Y243 with Chain A, 1-456, Chain B 327-593 and Chain A 146-476 amino acids residues length respectively as shown in figure 4.4.

>1IGR_1|Chain A|INSULIN-LIKE GROWTH FACTOR RECEPTOR 1|Homo sapiens
(9606)EICGPGIDIRNDYQQLKRLENCTVIEGYLHILLISKAEDYRSYRFPKLTVITEYLLLFRVAGLESLGDLFPNLTVIRGWKLFY
NYALVIFEMTNLKDIGLYNLRNITRGAIRIEKNADLCYLSTVDWSLILDAVSNNYIVGNKPPKECGDLCPGTMEEKPMCEKTTIN
NEYNYRCWTTNRCQKMCPSTCGKRACTENNECCHPECLGSCSAPDNDTACVACRHYYYAGVCVPACPPNTYRFEGWRCVD
RDFCANILSAESSDSEGFVIHDGECMQECPSGFIRNGSQSMYCIPCEGPCPKVCEEEKKTKTIDSVTSAQMLQGCTIFKGNLLI
NIRRGNNIASELENFMGLIEVVTGYVKIRHSHALVSLSFLKNLRLILGEEQLEGNYSFYVLDNQNLQQLWDWDHRNLTIKAGK
MYFAFNPKLCVSEIYRMEEVTGTKGRQSKGDINTRNNGERASCESDVDDDDKEQKLISEEDLN
>4OVV_2|Chain B|Phosphatidylinositol 3-kinase regulatory subunit alpha|Homo sapiens (9606)
MNNNMSLQDAEWYWGDISREEVNEKLRDTADGTFLVRDASTKMHGDYTLTLRKGGNNKLIKIFHRDGKYGFSDPLTFSSVV
ELINHYRNESLAQYNPKLDVKLLYPVSKYQQDQVVKEDNIEAVGKKLHEYNTQFQEKSREYDRLYEEYTRTSQEIQMKRTAIEA
FNETIKIFEEQCQTQERYSKEYIEKFKREGNEKEIQRIMHNYDKLKSRISEIIDSRRRLEEDLKKQAAEYREIDKRMNSIKPDLIQLR
KTRDQYLMWLTQKGVRQKKLNEWLGN
>1O6K_1|Chain A|RAC-BETA SERINE/THREONINE PROTEIN KINASE|HOMO SAPIENS
(9606)KVTMNDFDYLKLLGKGTFGKVILVREKATGRYYAMKILRKEVIIAKDEVAHTVTESRVLQNTRHPFLTALKYAFQTHDRL
CFVMEYANGGELFFHLSRERVFTEERARFYGAEIVSALEYLHSRDVVYRDIKLENLMLDKDGHIKITDFGLCKEGISDGATMKT
FCGTPEYLAPEVLEDNDYGRAVDWWGLGVVMYEMMCGRLPFYNQDHERLFELILMEEIRFPRTLSPEAKSLLAGLLKKDPK
QRLGGGPSDAKEVMEHRFFLSINWQDVVQKKLLPPFKPQVTSEVDTRYFDDEFTAQSITITPPDRYDSLGLLELDQRTHFPQF
DYSASIRE

FIGURE 4.4: FASTA sequence of IGF1R, PI3K and AkT

4.3 Physicochemical Characterization of Protein

Scientists utilize an online tool called ProtParam to forecast a variety of parameters, including the molecular and structural properties of specific proteins [75]. Table 4.1 lists the physiochemical characteristics of P13K, AkT, and IGF1R.

TABLE 4.1: The physicochemical properties of IGF1R, PI3K and AkT

Target Proteins	MW	PI	NR	PR	Ext Co1.	Ext Co2.	Instability Index	Aliphatic Index	GRAVY
IGF1R	54334.86	5.24	65	52	67655	65780	39.40	80.15	-0.367
PI3K	33610.89	8.61	48	51	45840	45840	36.42	70.93	-1.114

Table 4.1 continued from previous page

Target Proteins	MW	PI	NR	PR	Ext Co1.	Ext Co2.	Instability Index	Aliphatic Index	GRAVY
AKT	38987.70	5.80	52	45	39100	38850	30.14	83.84	-0.361

The aliphatic index indicates the aliphatic composition of a protein. The protein's thermostability is shown by the high aliphatic index value. Protein residues with both positive and negative charges are included in molecular weight. Low GRAVY shows better interaction with water molecules.

The total number of negatively charged residues and the molecular weight (Asp +ve Glu), total number of positively charged residues (Arg +ve Lys), and theoretical isoelectric point (when protein is neutral and free of charge) are represented by the symbols MW, pl, NR, and PR. Grand average of hydropathicity is represented by GRAVY, extinction coefficients are represented by Ext. Co1 when all pairings of Cys residues form cystines, and extinction coefficients are represented by Ext.

Co2 when all Cys residues are reduced [76]. Khotimah et al performed the same studies on the antiaging protein Nrf2-keap 1 and predicted physicochemical properties of it [77].

4.4 Functional Domain Identification of Proteins

In order to ascertain the domains and functional sites of specific proteins, Interpro was utilized. It is a useful tool for functional study of protein sequences. Sequence, structure, and relationships are all involved in conserved domains.

Proteins can have many functional domains, each of which carries out a distinct task. Sequence, structure, and relationships are all involved in conserved domains. The functional domain of a protein is the active region that engages in interactions between proteins and other substances [78].

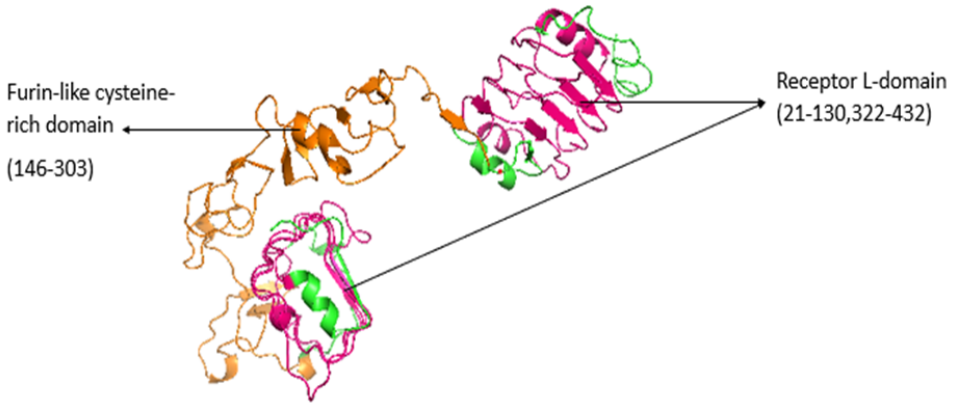


FIGURE 4.5: Domains of IGF1R

Figure 4.5 shows the functional domains of the protein IGF1R. It is 456aa long protein consisting of two domains. Orange colour is showing Furin-like cysteine-rich domain start at residue 146 and end at 303, while the pink one is Receptor L-domain start at residue 21 and 322 and end at residue 130 and 432.

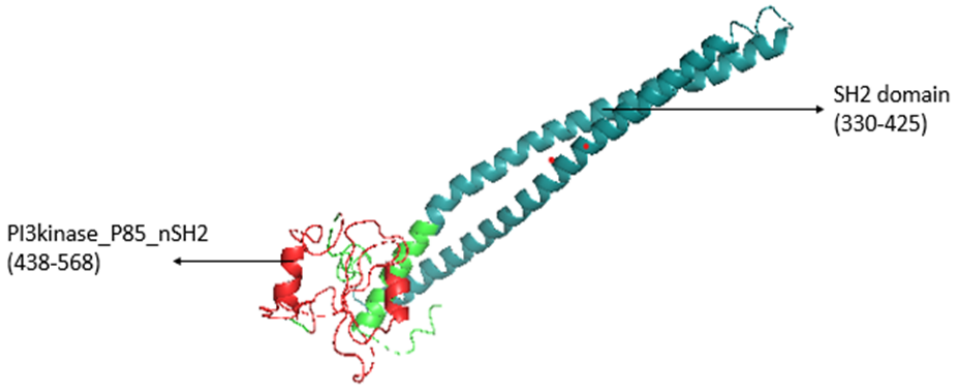


FIGURE 4.6: Domains of PI3K

PI3K protein also consists of two domains as shown in figure 4.6. One is blue colour SH2 domain start at residue 330 and end at residue 425, while the red one is phosphatidylinositol 3-kinase regulatory subunit p85 related, inter-SH2 domain start at residue 438 and end at residue 568.

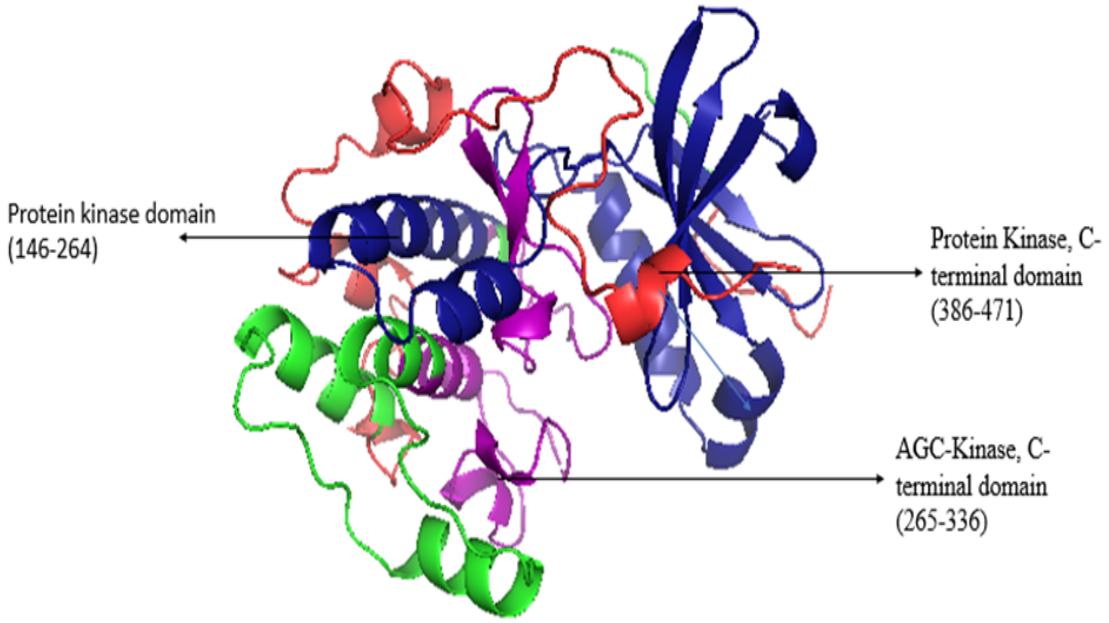


FIGURE 4.7: Domains of AkT

AkT protein consists of three domains as shown in figure 4.7. Protein kinase domain is shown in blue colour starting at residue 146 and ending at 264, AGC-Kinase, C-terminal domain is shown in purple colour starts from residue 265 and ends at 336 while red colour.

C-terminal domain is shown in purple colour starts from residue 265 and ends at 336 while red colour is showing Protein Kinase, C-terminal domain start at residue 386 and end at residue 471. Collagen 1 protein domains were analysed by using same software by setiawan et al [79].

4.5 Active Site Identification

The CASTp software, which determines the number of pockets that can be bound and gives details on their surface area and volume, was utilized to determine the active sites of the protein [80].

Figure 4.8 below illustrates the areas and volumes of target proteins IGF1R, PI3K, and AkT. The red area depicts the active sites available for a particular protein.

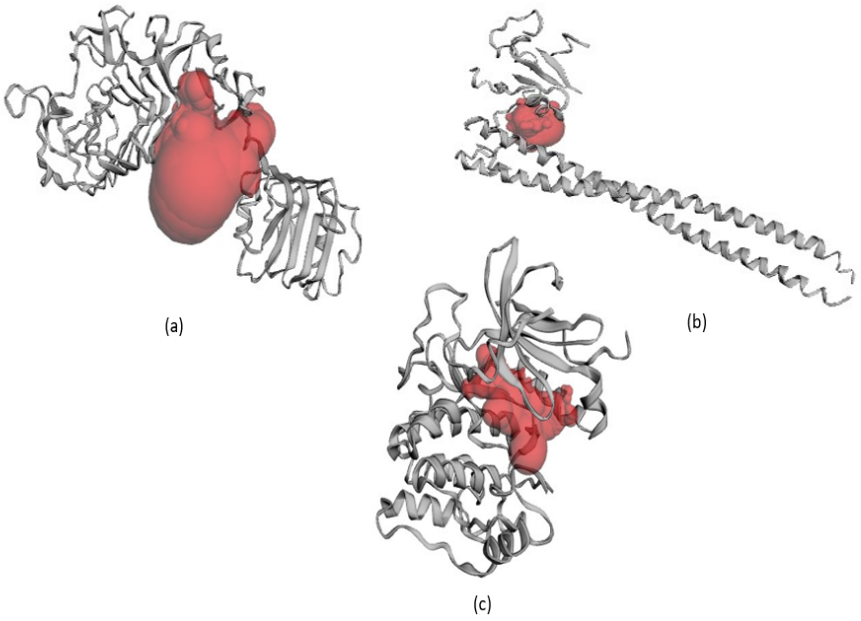


FIGURE 4.8: Active sites of (a) IGF1R (b) PI3K (c) AkT

CASTp data depicts different numbers of pockets for each protein. According to CASTp data, the IGF1R consists of 36 pockets while PI3K and AkT consist of 11 pockets each. The binding pocket is the region where ligands can bind. The number of pockets with size and volume is already shown in above table 4.2.

TABLE 4.2: Area and volume of binding pockets of IGF1R, PI3K, and AkT

Pocket ID	**IGF1R**		**PI3K**		**AkT**	
	Area	Volume	Area	Volume	Area	Volume
1	25748.990	19184.533	11664.986	8416.106	21105.685	13055.998
2	14.322	7.906	1.644	2.124	4.438	8.519
3	2.888	3.829	2.625	2.124	2.668	4.615
4	7.546	3.398	3.302	2.041	2.805	1.330
5	3.390	1.779	2.497	1.391	3.398	0.717
6	8.212	1.367	2.612	0.644	2.004	0.598
7	4.580	1.311	1.519	0.434	0.785	0.181
8	6.226	1.135	0.459	0.064	0.889	0.101
9	4.619	1.068	0.144	0.006	0.821	0.054
10	4.780	0.962	0.001	0.000	0.432	0.012

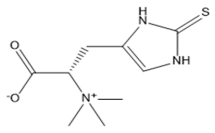
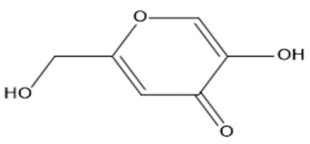
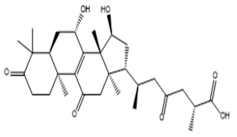
In the case of IGF1R the largest binding pocket has a surface area 25748.990 while its volume is 19184.533. For PI3K the largest pocket surface area is 11664.986 while

its volume is 8416.106. In the case of AkT the largest binding pocket surface area is 21105.685 while its volume is 13055.998. Rahagir et al found information about mTOR, SIRT1 and AMPK proteins by using CASTp [74].

4.6 Retrieval of Chemical Structure of the Ligands

The ligand to be selected should be on the best resolution structure with that based on crystal-chemical class and their binding affinities. With that what matters is the conformational selection of the ligand. A ligand preferentially binds to one of the conformers in this selection process, boosting its numbers in comparison to the overall population and fortifying it of that protein. The largest chemical databank in the world, PubChem, was searched for ligands, or fungal compounds [81]. These ligands' 3D structures were extracted in SDF format from the PubChem database. Resveratrol, polydatin, and quercetin were selected as bioactive metabolites by Fatima et al and followed the same methodology for ligand acquisition [82]. Table 4.3 shows all the selected ligands with the information regarding their structure.

TABLE 4.3: Chemical structure of ligands

S.No	**Ligands Name**	**Molecular Formula**	**Structure**
1	Ergothioneine	$C_9H_{15}N_3O_2S$	
2	Kojic acid	$C_6H_6O_4$	
3	Ganoderic acid	$C_{30}H_{44}O_7$	

4.7 Energy Minimization of Ligands

After downloading the structures of the ligands that were selected the next step that was performed was minimizing the energy of these ligands. This step is an important one as we can't use simply the downloaded structure as the ligands are unstable and it can directly affect the docking vina scores. The refined structures of ligands obtained after energy minimization are given in figure 4.9.

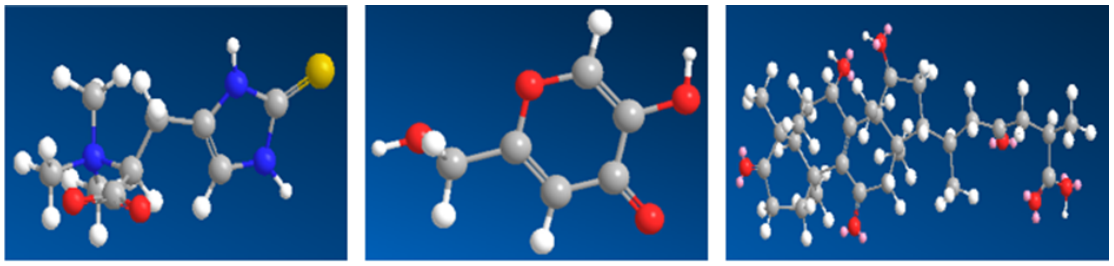


FIGURE 4.9: Energy minimization of ligands (a) Ergothioneine (b) Kojic acid (c) Ganoderic acid

4.8 Virtual Screening of Ligands

For compounds to be separated as both drug-like and nondrug-like virtual screening and pharmacokinetic properties are followed. The Lipinski rule deals with certain parameters like Molecular weight which should be ≤ 500, log P ≤ 5, H-bond donors ≤ 5, H-bond acceptors ≤ 10, and rotatable bonds ≤ 5. These rules are to be followed by orally active compounds. The drug-like is dependent on the mode of administration. A compound is considered a drug when it follows 3 or more rules and If a compound breaks more than two rules then its absorption is not good [83]. Virtual screening of ligands is shown is table 4.4.

TABLE 4.4: Virtual screening of ligands

Ligands	Log P Value	Molecular Weight	H-bond Donor	H-bond Acceptor	Rotatable Bonds
Ergothioneine	-0.56051	229.305	2	3	4
Kojic acid	-0.1623	142.11	2	4	1

Table 4.4 continued from previous page

Ligands	Log P Value	Molecular Weight	H-bond Donor	H-bond Acceptor	Rotatable Bonds
Ganoderic acid	4.1315	497.65	3	5	5

Table 4.4 shows that all ligands follow the lipinski rule. The log P value of all ligands is below 5 and the molecular weight of all is also below 500. The hydrogen bond donor, acceptor, and rotatable bonds are also in range for all ligands.

4.9 ADMET Analysis of Ligands

A second investigation was conducted utilizing the online program pkCSM to Determine the ADMET properties of ligands as a measure of pharmacokinetics after the lipinski rule. There are two general words in pharmacology: pharmacodynamics and pharmacokinetics. Within the field of pharmacology, pharmacodynamics examines how medications affect the body. In pharmacokinetics, we investigate how medications are absorbed, distributed, metabolized, and excreted [84].

4.9.1 Absorption Properties of Ligands

The $CaCO_2$ solubility helps in predicting the absorption of the drugs which are administered orally. Value >0.90 (log Papp in 10-6 cm/s) is considered as high $CaCO_2$ permeability. The water solubility of the ligands is given as log mol/L. this indicates the compound solubility in water at 25 C. Hence the lipid-soluble drugs will be less soluble than the water-soluble drugs. Intestinal absorption indicates the value or proportion of the compound that will be absorbed into the intestines. A value less than 30% is considered poorly absorbed [85].

P-glycoprotein is an ABC transporter that functions to extrude toxins or other xenobiotics from the cells by acting as a biological barrier. P-glycoprotein inhibition can be a therapeutic target or it can act in contradiction. Skin permeability is important for developing transdermal drugs. Any compound with a value >

-2.5 has a low skin permeability [86]. The absorption properties of ergothioneine, kojic acid, and ganoderic acid are given in table 4.5.

TABLE 4.5: Absorption properties of ligands

ADMET Properties		**Ergothioneine**	**Kojic Acid**	**Ganoderic Acid**
Absorption	Water solubility	-1.455	-1.752	-3.984
	$CaCO_2$ Permeability	0.863	0.637	0.608
	Intestinal absorption (human)	93.938	93.152	64.393
	Skin permeability	-2.735	-3.157	-2.737
	P-glycoprotein substrate	No	Yes	Yes
	P-glycoprotein I inhibitor	No	Yes	No
	P-glycoprotein II inhibitor	No	Yes	Yes

Water solubility and skin absorption for all ligands is low while $CaCO_2$ permeability is normal, intestinal absorption of all ligands is more than 90% except ganoderic acid. Skin permeability for all ligands is low. If a compound is positive for Pgp substrate then its means that it can be easily pumped out of the cells to reduce its absorption.

4.9.2 Distribution Properties of Ligands

The theoretical volume or VDss indicates the entire dosage of the medication that must be dispersed evenly to produce a concentration similar to that of blood plasma. The medication is more widely disseminated in the tissues than in the plasma if the VDss value is more than 2.81 L/kg. The Volume Distribution of Steady State (VDss) will be low if the value is below 0.71 L/kg. Many drugs in the plasma exist in an equilibrium between a bounded and an unbounded state of the serum proteins. As a drug binds more to the serum proteins it will have less efficiency of diffusion to cellular membranes [87].

The blood-brain barrier reduces the amount of exogenous substances that can reach the brain directly while protecting it. If a compound has a value of logBB >0.3 then it will easily cross the BBB barrier hence been effective and if it is

logBB<-1 then it is poorly distributed. Compounds with a value of logPS>-2 penetrate the CNS whereas value logPS<-3 does not penetrate the CNS [88]. Table 4.6 shows the distribution properties of ergothioneine, kojic acid and ganoderic acid. The table indicates all ligands have safe range which is given below.

TABLE 4.6: Distribution properties of ligands

ADMET Properties		**Ergothioneine**	**Kojic Acid**	**Ganoderic Acid**
Distribution	VDss (human)	-0.451	-0.086	-0.599
	Fraction unbound (human) Fu	0.695	0.817	0.182
	BBB permeability log BB	-0.714	0.077	-0.911
	CNS permeability log PS	-3.054	-2.976	-3

The parameters through which the distribution properties are determined include VDss which is in the given range to be distributed in the blood and the tissues. The values of the fraction unbound of these ligands show that out of the total dose, this fraction will not be bounded to the protein. All these ligands mentioned in above table cannot cross the blood brain barriers and the CNS.

4.9.3 Metabolism Properties of Ligands

The enzyme cytochrome P450 is in charge of the liver's detoxification process. Many drugs get deactivated by this enzyme but certain drugs is capable of activating. this enzyme's inhibitors can directly affect the metabolism of the drug hence should not be used. Similarly, CYP2D6 and CYP3A4 are responsible for the drugs' metabolism.

Inhibition of these affects the pharmacokinetics of the drug in use [89]. The prediction of the metabolism of ligands is given below. Table 4.7 shows the metabolic properties of ergothioneine, kojic acid, and ganoderic acid.

TABLE 4.7: Metabolism properties of ligands

ADMET Properties		**Ergothioneine**	**Kojic Acid**	**Ganoderic Acid**
Metabolism	CYP2D6 substrate	No	No	No
	CYP3A4 substrate	No	No	Yes

Table 4.7 continued from previous page

ADMET Properties		Ergothioneine	Kojic Acid	Ganoderic Acid
	CYP1A2 inhibitor	No	No	No
	CYP2C19 inhibitor	No	No	No
	CYP2C9 inhibitor	No	No	No
	CYP2D6 inhibitor	No	No	No
	CYP3A4 inhibitor	No	No	No

All three ligands mentioned are neither the CYP2D6 substrates nor they are CYP2C19, CYP2C9, CYP2D6, and CYP3A4 inhibitors whereas the rest parameters are shown in the table. Table 4.7 shows the metabolic properties of ergothioneine, kojic acid, and ganoderic acid. It indicates that all the three ligands mentioned are not CYP2D6, or CYP3A4 substrate except ganoderic acid. Whereas these ligands are not CYP2C19 inhibitior, CYP2C9 inhibitior, CYP2D6 inhibitior, CYP3A4 inhibitior and CYP1A2 inhibitior.

4.9.4 Excretion Properties of Ligands

Two organs are involved in drug excretion, the liver, which is engaged in biliary excretion, and the kidneys, which are involved in renal excretion. Excretion may also include other organs, such as the lungs in the case of volatile or gaseous substances. Moreover, drugs can be expelled through tears, saliva, and perspiration. The excretion values of the ligands are given in table 4.8.

TABLE 4.8: Excretion properties of ligands

ADMET Properties		Ergothioneine	Kojic Acid	Ganoderic Acid
Excretion	Total Clearance	0.571	0.638	0.242
	Renal OCT2 substrate	No	No	No

The Renal OCT2 substrate acts as a transporter that helps in clearing the drugs and other compounds. Total clearance indicates hepatic clearance which means the drug is metabolized and renal clearance indicates the drug is excreted [90]. Since none of these ligands are renal OCT2 substrates, the body would not be

able to eliminate them, as the above table illustrates and hence the total clearance values are given accordingly.

4.9.5 Toxicity Properties of Ligands

An online program called pkCSM is used to check the ADMET i.e. absorption, distribution, metabolism, excretion, and toxicity values of drugs and bioactive substances. By using this tool we will determine the toxicity of the ligands selected for this different methods are used to test whether a given ligand is toxic or not. AMES toxicity test is used to test the mutagenic potential of the compound by using bacteria. If it shows a positive response, then the ligand is mutagenic which can also act as a carcinogen. The toxicity of *T. Pyriformis* (protozoa bacterium) is used as a toxic endpoint in the *T. Pyriformis* toxicity method. Any value >-0.5 log ug/L is considered toxic. The values predicted in the Minnow toxicity test are used to represent the concentration at which the compound could cause the death of 50% of the minnows. The value below 0.5 mM is regarded as acute toxic [91]. In the oral rat chronic toxicity test, the concentration of the drug that necessitates a certain amount of treatment time is related to the expected log value of the lowest observed adverse impact, expressed in log mg/kg bw/day. A hepatotoxicity test predicts that if a compound could affect liver functioning or not. A hazardous substance's maximum tolerated dose (MRTD) indicates how dangerous it is to a certain person. In phase 1 clinical studies, ligand toxicity information helps guide the initial recommended dosage of a medication. The logarithmic representation of the MRTD value is log mg/kg/day. If a chemical's value is less than or equal to 0.477 log (mg/kg/day), it is said to have a low MRTD; if it is more than 0.477 log (mg/kg/day), it is said to have a high MRTD [92]. The toxicity values of all ligands are given in table 4.9.

TABLE 4.9: Toxicity values of ligands

ADMET Properties		**Ergothioneine**	**Kojic Acid**	**Ganoderic Acid**
Toxicity	AMES toxicity	No	No	No

Table 4.9 continued from previous page

ADMET Properties	Ergothioneine	Kojic Acid	Ganoderic Acid
Max recommended tolerated dose (human)	0.628	0.748	0.147
hERG I inhibitor	No	No	No
hERG II inhibitor	No	No	No
Oral rat acute toxicity (LD50)	2.331	2.037	2.622
Oral rat chronic toxicity (LOAEL)	1.327	1.613	1.85
Hepatotoxicity	No	No	No
Skin sensitization	No	No	No
T.Pyriformis toxicity	0.285	-0.219	0.285
Minnow toxicity	2.701	3.178	1.645

No inhibition of hERG I or hERG II was seen in any ligand. None of the ligands demonstrated hepatotoxicity, cutaneous sensitivity, or AMES toxicity. skin sensitivity. Every ligand's MRTD value is within the range. T. pyriformis activity was seen in all ligands at least 0.5 log µg/L. All ligands had minnow toxicity values above the acceptable threshold of 0.5 mM. A computational study of madecassic acid, madecassoside, and asiatic acid was carried out to predict the adsorption, distribution, metabolism, excretion, toxicity, and lipinski rule parameters by using pkCSM [77].

4.10 Molecular Docking

To carry out docking, the three-dimensional structures of the protein and ligands are used. An online blind auto docking program called CB dock is utilized for this. CB Dock computes the cavity sizes and predicts the protein binding locations. CB Dock provides us with the top five possess and receptor models upon docking. Based on the cavity size and the vina score, the optimal position was chosen among these five [93]. Figure 4.10 shows the best docking complexes of ligands and proteins.

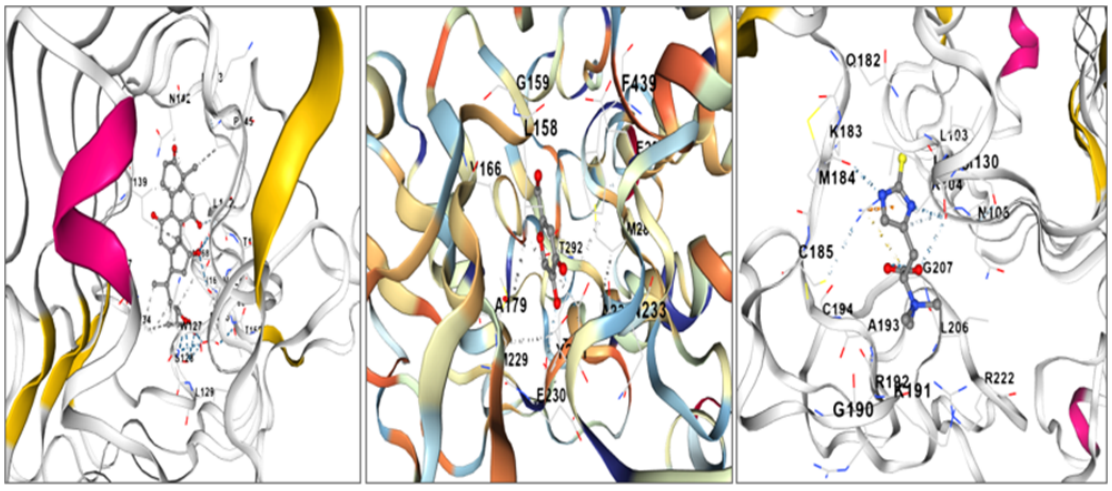


FIGURE 4.10: Dock complexes (a) Ganoderic acid-IGF1R (b) Kojic acid-AkT (c) Ergothioneine-IGF1R

Molecular docking is performed by using IGF1R, PI3K, and AkT as the receptor proteins and the 3 ligands i.e. ergothioneine, kojic acid, and ganoderic acid are selected. The ligands are in SDF format, while the protein is in PDB format. After verifying the input files, CB Dock uses Open Babel and MGL tools to transform them into files in the pdbqt format. Next, CB dock determines the receptor's cavities as well as the diameters and centers of the top five cavities. The protein-ligand interaction's high-affinity score determines which of the five optimal conformations is the best [94]. The scores obtained after the docking of proteins and ligands are shown in tables 4.10.

TABLE 4.10: Docking score of ligand-protein complexes

Target Proteins	**Ligands**		
	Ergothioneine	Kojic Acid	Ganoderic Acid
IGF1R	-5.6	-5.0	-8.8
PI3K	-5.4	-5.0	-8.2
AkT	-5.4	-5.3	-8.0

Table 4.10 shows the docking result of three selected ligands that are ergothioneine, kojic acid, and ganoderic acid. It shows that ganoderic acid has the highest binding score of -8.8, with IGF1R protein. Kojic acid has a binding score of -5.3 with AkT protein whereas ergothioneine has a binding score of -5.6 with IGF1R. Kojic acid

has a binding score of -5.3 with AkT protein whereas ergothioneine has a binding score of -5.6 with IGF1R. A similar methodology was adopted by Dhamodiran et al for docking of cycloleonuripeptide| _B-AMPK$\alpha 2\beta 1$, R734_AMPK$\alpha 2\beta 1$ and cycloleonuripeptide _B-AMPK$\gamma 1$. The best one was cycloleonuripeptide| _B-AMPK$\alpha 2\beta 1$ complex with a docking score of -9.91 [95].

4.11 Analysis of Docked Complexes via Ligplot

To understand docking data, how the ligand and protein interact in active pockets was estimated. Hydrogen bonding and hydrophobic bonding interactions were the two types of interactions that were investigated. Ligplot Plus (v.1.4.5) was used to analyze the interactions between proteins and ligands. By using Ligplot the interaction of the active conformation of ligands and the target protein has been identified [96].

To understand docking data, how the ligand and protein interact in active pockets was estimated. Hydrogen bonding and hydrophobic bonding interactions were the two types of interactions that were investigated. Ligplot Plus (v.1.4.5) was used to analyze the interactions between proteins and ligands. By using Ligplot the interaction of the active conformation of ligands and the target protein has been identified. The saved conformations for the ligand-receptor complex of each molecule were analyzed in detail. The saved conformations for the ligand-receptor complex of each molecule were analyzed in detail. This program creates schematic representations of the protein-ligand interactions between the specified ligands in the PDB file automatically. The docked files were uploaded in PDB format to get hydrogen and hydrophobic bonding. The docked files were uploaded in PDB format to get hydrogen and hydrophobic bonding. A significant number of hydrophobic and hydrogen bond interactions were observed between the 3 ligands and the 3 target proteins [97]. Ligand-receptor complex shows strong hydrogen bonding and hydrophobic interactions. The following diagrams show the ligand-receptor interactions.

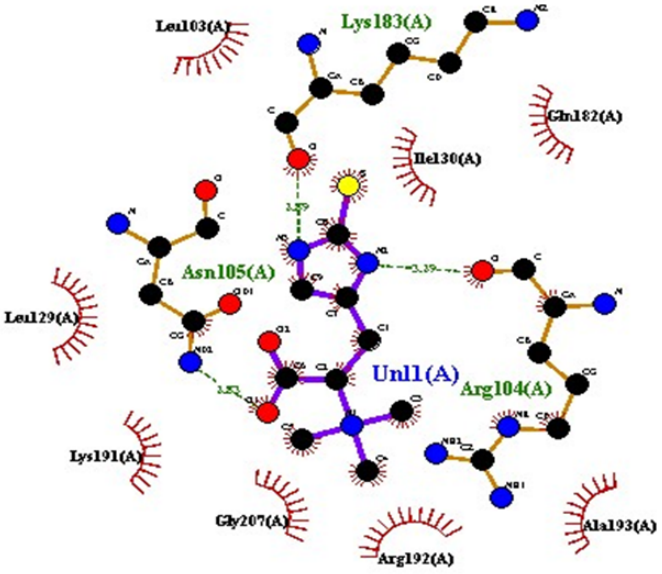


FIGURE 4.11: Interaction of ergothioneine with IGF1R

Above figure shows the interaction of ergothioneine with IGF1R. It shows that there are three hydrogen bonds and eight hydrophobic interactions.

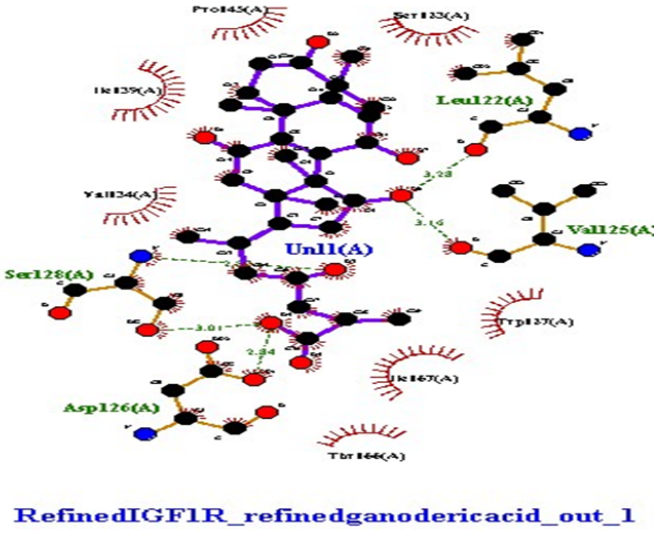


FIGURE 4.12: Interaction of ganoderic acid with IGF1R

Figure 4.12 shows the interaction between ganoderic acid and IGFIR. It shows that there are four hydrogen bonds and seven hydrophobic interactions.

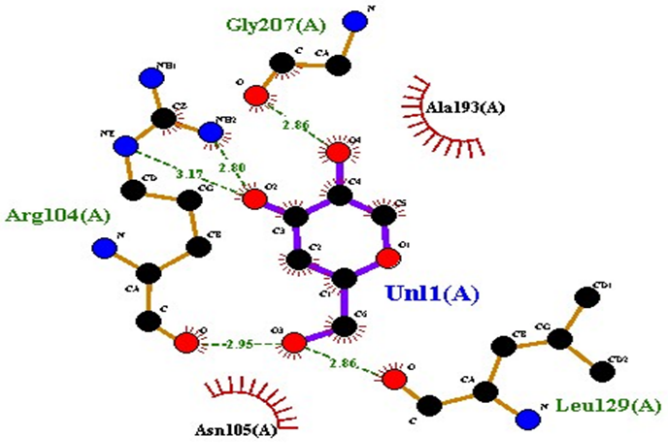


FIGURE 4.13: Interaction of kojic acid with IGF1R

Figure 4.13 shows the interaction between kojic acid and IGFIR. It shows that there are five hydrogen bonds and two hydrophobic interactions.

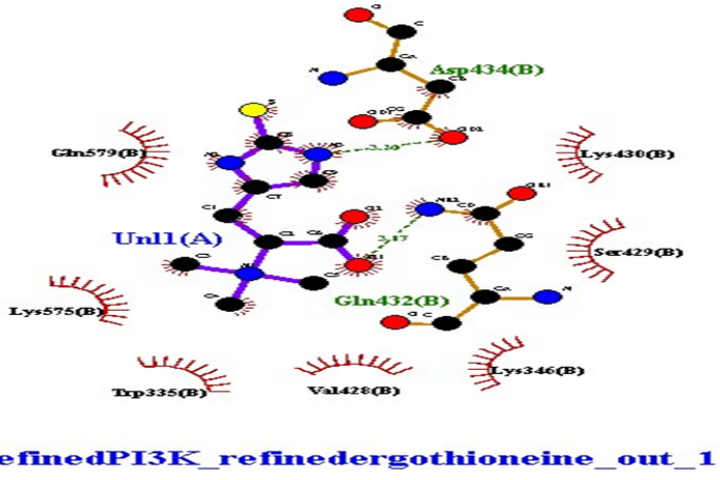


FIGURE 4.14: Interaction of ergothioneine with PI3K

Figure 4.14 shows the interaction between ergothioneine and PI3K. It shows that there are two hydrogen bonds and seven hydrophobic interactions.

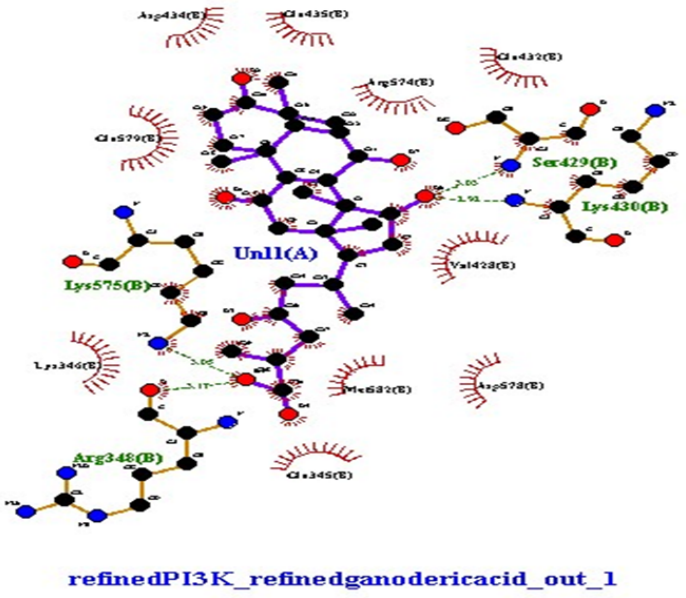


FIGURE 4.15: Interaction of ganoderic acid with PI3K

Figure 4.15 shows the interaction between ganoderic acid and PI3K. It shows that there are four hydrogen bonds and ten hydrophobic interactions.

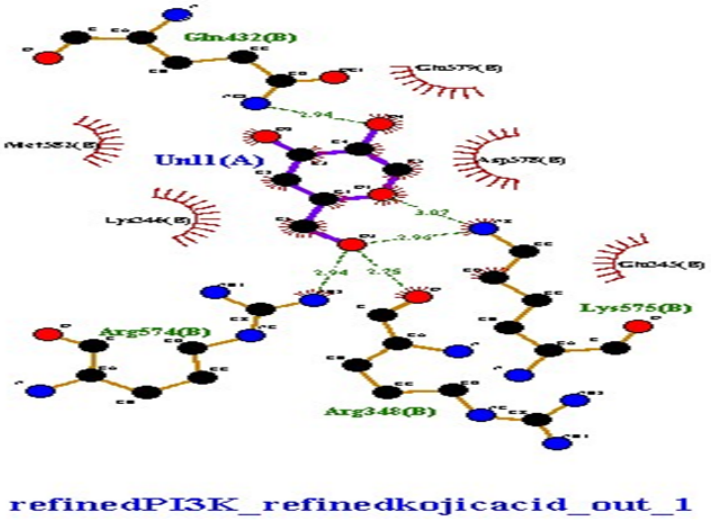


FIGURE 4.16: Interaction of kojic acid with PI3K

Figure 4.16 shows the interaction between kojic acid and PI3K. It shows that there are five hydrogen bonds and five hydrophobic interactions.

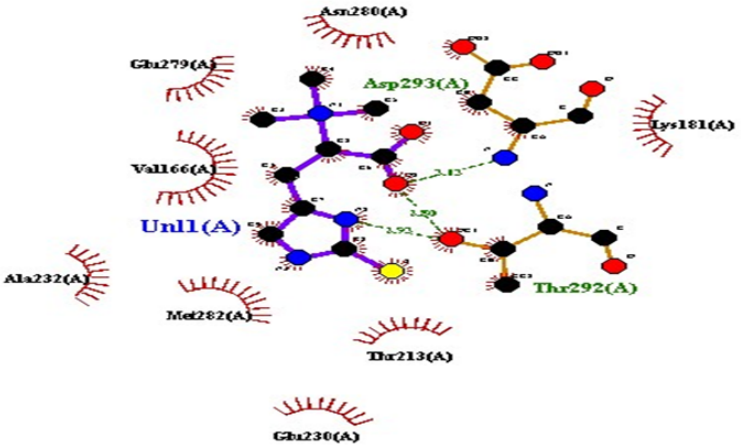


FIGURE 4.17: Interaction of ergothioneine with AkT

Above figure shows the interaction between ergothioneine and AkT. It shows that there are three hydrogen bonds and eight hydrophobic interactions.

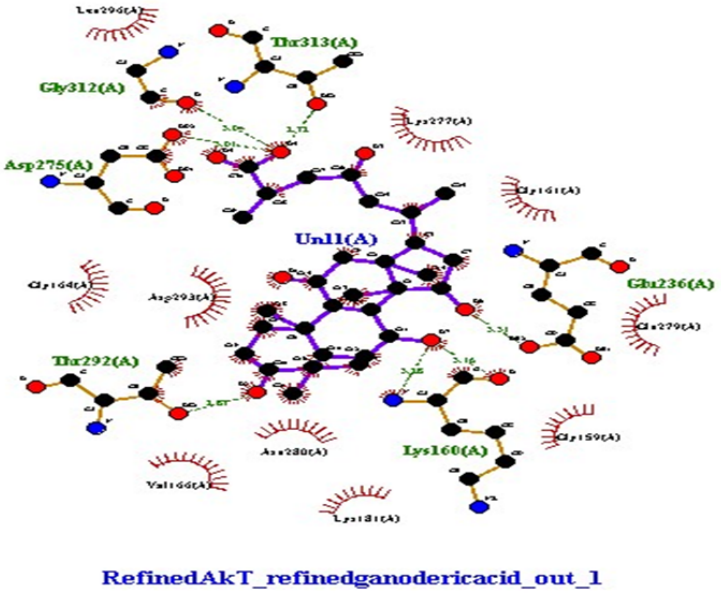


FIGURE 4.18: Interaction of ganoderic acid with AkT

Figure 4.18 shows the interaction between ganoderic acid and AkT. It shows that there are seven hydrogen bonds and ten hydrophobic interactions.

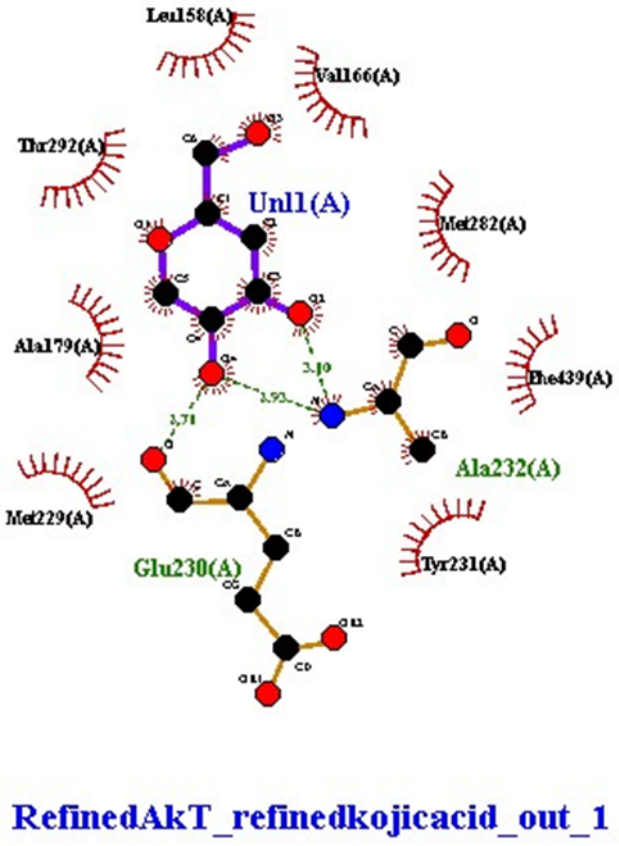


FIGURE 4.19: Interaction of kojic acid with AkT

Figure 4.19 shows the interaction between kojic acid and AkT. It shows that there are eight hydrophobic interactions and three hydrogen bonds. The complete detail of these interactions along with name of amino acids and hydrogen bond distance is shown in table 4.11.

TABLE 4.11: Docking interaction analysis

S. No	**Docking Complex**	**Binding Energy**	**No of HBs**	**Amino Acids**	**H-bonding Distance**	**Hydrophobic Interactions**
1	Ergothioneine - IGF1R	-5.6	3	Asn105	3.39	Leu103
				Lys183	3.39	Leu129
				Arg103	3.33	Lys191
						Gly207
						Arg192
						Ala193
						Ile130

Table 4.11 continued from previous page

S. No	Docking Complex	Binding Energy	No of HBs	Amino Acids	H-bonding Distance	Hydrophobic Interactions
						Gln182
2	Ganoderic Acid - IGF1R	-8.8	4	Leu122	3.28	Ser133
				Val125	3.16	Pro145
				Ser128	3.01	Ile139
				Asp126	3.84	Val134
						Tyr166
						Ile167
						Trp137
3	Kojic Acid - IGF1R	-5	5	Gly207	2.86	Ala193
				Arg104	2.8	Asn105
				Arg104	3.17	
				Leu129	2.86	
				Leu129	2.95	
4	Ergothioneine - PI3K	-5.4	2	Asp434	3.3	Gln579
				Gln432	3.17	Lys575
						Trp335
						Val428
						Lys346
						Ser429
						Lys430
5	Ganoderic Acid - PI3K	-8.2	4	Ser429	3.03	Gly435
				Lys430	3.91	Arg574
				Lys575	3.05	Glu435
				Arg348	3.17	Arg434
						Glu579
						Val428
						Arg578
						Met682
						Glu345
						Lys346

Table 4.11 continued from previous page

S. No	Docking Complex	Binding Energy	No of HBs	Amino Acids	H-bonding Distance	Hydrophobic Interactions
6	Kojic Acid - PI3K	-5	5	Gln432	2.94	Glu579
				Lys575	3.02	Asn578
				Lys575	2.96	Glu345
				Arg348	2.25	Met332
				Arg574	2.94	Lys346
7	Ergothioneine - AkT	-5.4	3	Asp293	3.13	Asn280
				Thr292	3.3	Lys181
				Thr292	3.93	Thr213
						Met282
						Ala232
						Val166
						Glu279
8	Ganoderic Acid - AkT	-8	7	Thr313	3.72	Leu296
				Gly312	3.03	Lys277
				Asp275	3.01	Gly161
				Glu236	3.31	Glu279
				Lys160	3.16	Gly159
				Lys160	3.35	Lys181
				Thr292	3.67	Val166
						Arg293
						Gly164
						Arg280
9	Kojic Acid - AkT	-5.3	3	Ala232	3.10	Leu158
				Ala232	3.93	Val166
				Glu230	3.71	Met282
						Phe439
						Tyr231
						Met229
						Ala179
						Thr292

Table 4.11 below shows the details of hydrogen and hydrophobic interactions between the receptor protein and selected ligands. The values show that ganoderic acid forms the highest hydrogen bonds i.e. seven while the highest hydrophobic interactions are also shown by ganoderic acid. Ergothioneine and kojic acid also form a considerable number of hydrophobic interactions and hydrogen bonding but their number is less than ganoderic acid. Bisht et al predicted the 3D conformation and 2D interaction plots of best-docked peptides and ligands in the protein binding site. Ligands exhibited comparatively interesting interactions with the receptors, further validating the docking methodology [98].

4.12 Lead Compound Identification

The ligands' pharmacokinetic and physiochemical properties determine their fate as for being drug or non-drug compounds. Lipinski's rule is the first filter and pharmacokinetics is the second filter for this identification. All ligands were seen obeying the lipinski rule of five so they all get selected for docking. The next knockout stage is pharmacokinetic screening. In this screening ganoderic acid was selected as it showed the best ADMET values over ergothioniene and kojic acid concerning moderate water solubility, good intestinal absorption, and minimal toxicity. Number of hydrogen bonds and docking score and hydrophobic interactions of ganoderic acid are also good than other ligands, so ganoderic acid was selected as lead compound.

4.13 Reference Anti-aging Drug Identification

Metformin is chosen as a reference drug because of its multiple uses and efficacy against aging mechanisms. Metformin's primary anti-aging mechanism of action is related to its ability to activate the AMP-activated protein kinase (AMPK) pathway [99]. AMPK is a cellular energy sensor that, when activated by metformin, triggers a series of beneficial effects, including improved mitochondrial function,

reduced cellular senescence, modulation of longevity pathways like mTOR, and anti-inflammatory properties, all of which contribute to delay the onset of diseases associated with aging and promoting healthy aging, as demonstrated in various animal studies and some human clinical trials, making metformin a promising possibility for further research and development as a treatment for aging [100].

4.14 Metformin and Lead Compound Comparison

To identify the better treatment for aging and the best fungal bioactive metabolite for controlling aging mechanisms, a comparison between metformin and ganoderic acid was done. The comparison was being performed through parameters like ADMET properties, lipinski rule and docking complexes analysis.

4.15 Metformin Structure Prediction

First of all metformin structure was downloaded in SDF format from PubChem. Then its energy was minimized by using chem3D pro to get the refined structure. The chemical formula of metformin is C4H11N5 and its refined structure of metformin is given in figure 4.20.

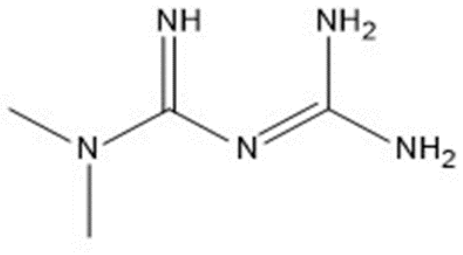


FIGURE 4.20: Structure of metformin

4.16 Lipinski Rule Comparison

The metformin and ganoderic acid were compared to observe their response to the lipinski rule. To evaluate their pharmacokinetic properties to assess their bioavailability, safety, efficacy, and drug-likeness. The comparison is given in table 4.12.

TABLE 4.12: Comparison of lipinski rule

Ligands	**Log P Value**	**Molecular Weight**	**H-bond Donor**	**H-bond Acceptor**	**Rotatable Bonds**
Metformin	-1.24383	129.167	3	1	0
Ganoderic acid	4.1315	497.65	3	5	5

Table 4.12 shows that metformin log P value is negative, it also has no rotatable bonds while ganodaric acid has followed all lipinski rules.

4.17 ADMET Properties Comparison

To identify a better drug candidate, the absorption, distribution, metabolism, excretion, and toxicity properties of the medication and the lead chemical were compared using the ADMET properties.

4.17.1 Absorption Properties Comparison

The comparison between metformin and ganoderic acid for checking absorbance models is given in table 4.13.

TABLE 4.13: Absorption properties comparison

ADMET Properties		**Metformin**	**Ganoderic Acid**
Absorption	Water solubility	-2.707	-3.984
	$CaCO_2$ Permeability	-0.339	0.608

Table 4.13 continued from previous page

ADMET Properties		Metformin	Ganoderic Acid
	Intestinal absorption(human)	59.401	64.393
	Skin permeability	-2.735	-2.737
	P-glycoprotein substrate	Yes	Yes
	P-glycoprotein I inhibitor	No	No
	P-glycoprotein II inhibitor	No	Yes

Absorbed in the stomach of ganoderic acid is more than metformin. The skin permeability value of both is in range. The substrate model for P-glycoprotein is not well absorbed, since P-glycoprotein serves as a biological barrier and an ABC transporter, the P-glycoprotein substrate model is important.

4.17.2 Distribution Properties Comparison

The comparison between the distribution properties of metformin and ganoderic acid is given in table 4.14.

TABLE 4.14: Distribution properties comparison

ADMET Properties		Metformin	Ganoderic Acid
Distribution	VDss (human)	-0.232	-0.599
	Fraction unbound (human) Fu	0.811	0.182
	BBB permeability log BB	-0.946	-0.911
	CNS permeability log PS	-2.238	-3

The above table shows the comparative distribution properties of metformin and ganoderic acid. Other parameters are in range except for CNS permeability. The CNS, or central nervous system, model is predicated on the idea that drugs with a log PS value more than -2 may readily permeate the CNS, but those with a log PS value less than -3 cannot. Because of its low value, metformin cannot enter the central nervous system.

4.17.3 Metabolism Properties Comparison

The comparison between the metabolism properties of metformin and ganoderic acid is given in table 4.15.

TABLE 4.15: Metabolic properties comparison

ADMET Properties		**Metformin**	**Ganoderic Acid**
Metabolism	CYP2D6 substrate	No	No
	CYP3A4 substrate	No	Yes
	CYP1A2 inhibitor	No	No
	CYP2C19 inhibitor	No	No
	CYP2C9 inhibitor	No	No
	CYP2D6 inhibitor	No	No
	CYP3A4 inhibitor	No	No

Mostly located in the liver, cytochrome P450 is an enzyme involved in detoxification because it oxidizes foreign substances to make them easier for the body to eliminate. It either deactivates or activates some medicines. Therefore, determining whether a chemical is a P450 substrate or not, as well as if it is a P450 inhibitor, is crucial. Table 4.15 illustrates how the metabolic characteristics of the medication and lead chemicals are almost identical.

4.17.4 Excretion Properties Comparison

The comparison between the excretion properties of metformin and ganoderic acid is given in table 4.16.

TABLE 4.16: Excretion properties comparison

ADMET Properties		**Metformin**	**Ganoderic Acid**
Excretion	Total Clerance	0.1	0.242
	Renal OCT2 substrate	No	No

The value of total clearance is a combination of hepatic and renal clearance and is important so that the dose rates of the drugs can be assessed. Ganoderic acid has

more total clearance than metformin. The renal OCT2 (organic cation transporter 2) model is the second one, and it aids in the renal clearance of medications and other substances. Concerning inhibitors, one may experience negative effects from being an OCT2 substrate. The medication and the main ingredient are not substrates of renal OCT2.

4.17.5 Toxicity Properties Comparison

The comparison between the toxicity properties of metformin and ganoderic acid is given in table 4.17.

TABLE 4.17: Toxicity properties comparison

ADMET Properties		**Metformin**	**Ganoderic Acid**
Toxicity	AMES toxicity	Yes	No
	Max tolerated dose (human)	0.902	0.147
	hERG I inhibitor	No	No
	hERG II inhibitor	No	No
	Oral rat acute toxicity (LD50)	2.453	2.622
	Oral rat chronic toxicity (LOAEL)	2.158	1.85
	Hepatotoxicity	No	No
	Skin sensitization	Yes	No
	T.Pyriformis toxicity	0.25	0.285
	Minnow toxicity	3.972	1.645

Nine models are used to assess the toxicity of the lead ingredient and the standard medication. Metformin can cause cancer because it is mutagenic, according to AMES toxicity model 1. According to the second maximum tolerated dosage model, a value is deemed low if it is equal to or less than 0.477 log mg/kg/day, whereas a higher value is deemed high. The chart indicates that the tolerable dosage of ganoderic acid is minimal. The third model involves hERG I and II inhibitors, none of which is an inhibitor. The relative toxicity is evaluated using the fourth model of oral rat acute toxicity.

Model 5 of oral rat chronic toxicity provides the lowest dosage values that might have a negative outcome. Hepatotoxicity Model 6 indicates that a medicine may

harm the liver. The table demonstrates that ganoderic acid and metformin are not hepatotoxic. The number seven is used to verify the dermal goods model's sensitivity to the skin. The skin has exhibited sensitivity to metformin. To test for toxicity, Model 8 employs T. Pyriformis, whereas Model 9 utilizes minnows. The relative toxicity values of ganoderic acid and metformin are displayed in table 4.17.

4.18 Docking Score Comparison

The target proteins IGF1R, PI3K, and AkT were docked with both the lead and standard compounds, and the docking result provided us the highest binding score. The dock complexes of metformin against IGF1R, PI3K, and AkT are shown in figure 4.21.

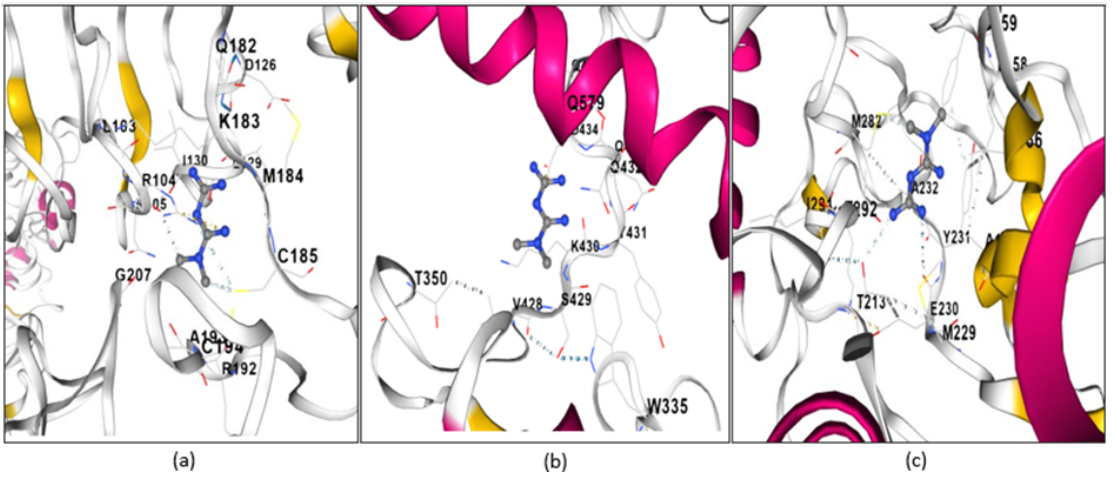


FIGURE 4.21: Docking complexes of (a) metformin-IGF1R (b) metformin-PI3K (c) metformin-AkT

Table 4.18 shows the docking score comparison of the standard drug metformin and lead compound ganoderic acid.

TABLE 4.18: Docking comparison of metformin and ganoderic acid

Target Proteins	**Ligands**	
	Metformin	Ganoderic Acid
IGF1R	-4.6	-8.8

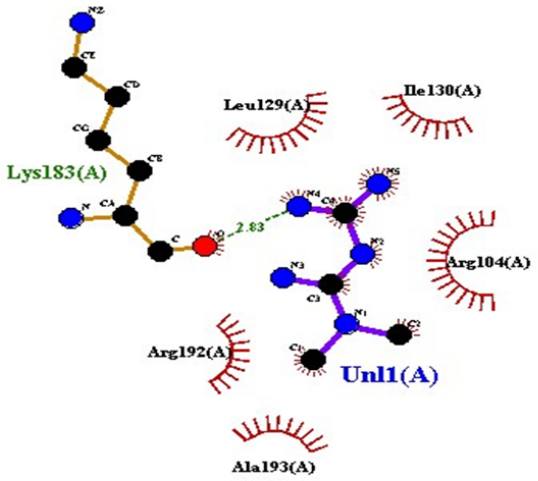
Table 4.18 continued from previous page

Target Proteins	Ligands	
PI3K	-4.8	-8.2
AkT	-4.9	-8.0

As can be shown in table 4.18, the vina score of the lead compound ganoderic acid is significantly greater than that of the generic medication metformin. The docking scores of the metformin against target proteins IGF1R, PI3K, and AkT are -4.6, -4.8 and -4.9 respectively while for ganoderic acid these scores are -8.8, -8.0 and -8.0. These results show that lead compound ganoderic acid can bind with target proteins IGF1R, PI3K and AkT more efficiently than the of standard drug metformin.

4.19 Docking Analysis Comparison

Based on the quantity of hydrogen bonds, hydrophobic interactions, interacting amino acids, and steric interactions, ligplot evaluates the docking results. The following figures show the docking analysis of metformin with ganoderic acid.



RefinedIGF1R_refinedmetformin_out_4

FIGURE 4.22: Docking interaction of metformin-IGF1R

Figure 4.22 shows the docking interaction of metformin with IGF1R. It shows that there is single hydrogen bonds while there are five hydrophobic interactions.

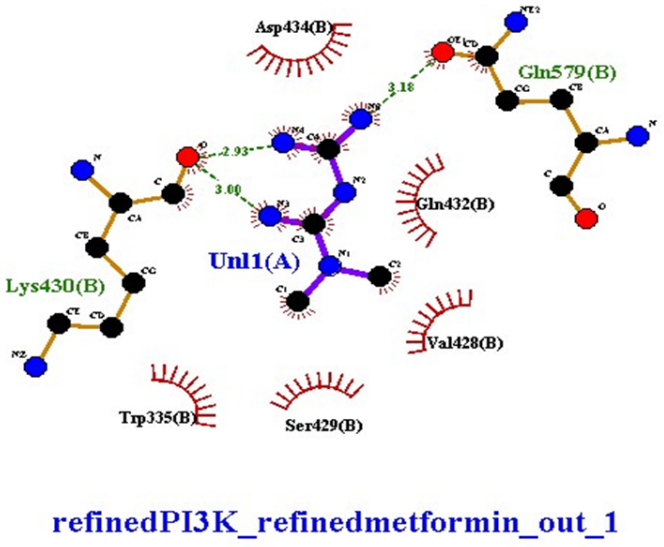


FIGURE 4.23: Docking interaction of metformin-PI3K

Figure 4.23 shows the docking interaction of metformin with PI3K. It shows that there are three hydrogen bonds and five hydrophobic interactions.

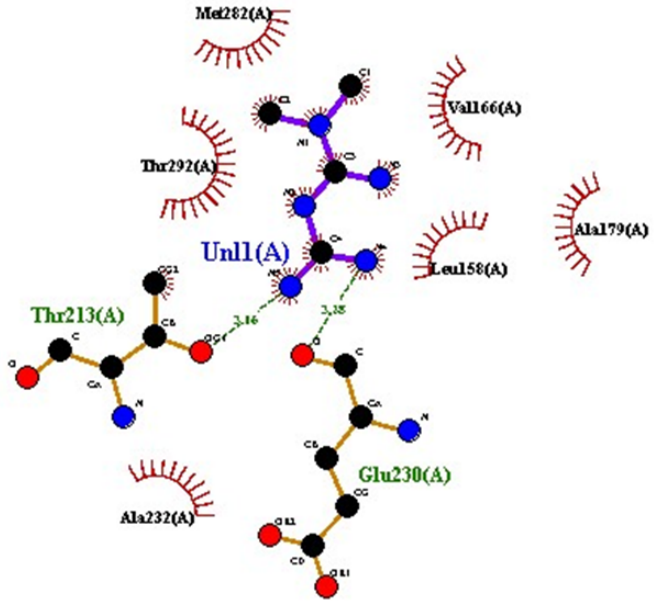


FIGURE 4.24: Docking interaction of metformin-AkT

Figure 4.24 shows the interaction of metformin with AkT. It shows that there are six hydrophobic interactions and two hydrogen bonds. The complete details of the docking analysis of standard drug metformin and lead compound ganoderic acid are shown in table 4.19.

TABLE 4.19: Docking analysis comparison

S.No	Docking complex	Binding energy	No of HBs	Amino acids	H-bonding distance	Hydrophobic Interactions
1	Metformin-IGF1R	-4.6	1	Lys183	2.83	Leu129
						Ile130
						Arg104
						Ala193
						Arg192
2	Ganoderic acid-IGF1R	-8.8	4	Leu122	3.28	Ser133
				Val125	3.16	Pro145
				Ser128	3.01	Ile139
				Asp126	3.84	Val134
						Tyr166
						Ile167
						Trp137
3	Metformin-PI3K	-4.8	3	Lys430	2.93	Asp434
				Lys430	3	Gln432
				Gln579	3.18	Val428
						Ser429
						Trp335
4	Ganoderic acid-PI3K	-8.2	4	Ser429	3.03	Gly435
				Lys430	3.91	Arg574
				Lys575	3.05	Glu435
				Arg348	3.17	Arg434
						Glu579
						Val428
						Arg578
						Met682
						Glu345

Table 4.19 continued from previous page

S.No	Docking complex	Binding energy	No of HBs	Amino acids	H-bonding distance	Hydrophobic Interactions
						Lys346
5	Metformin-AkT	-4.9	2	Thr213	3.16	Val166
				Glu230	3.25	Ala179
						Leu158
						Ala232
						Thr292
						Met282
6	Ganoderic acid-AkT	-8	7	Thr313	3.72	Leu296
				Gly312	3.03	Lys277
				Asp275	3.01	Gly161
				Glu236	3.31	Glu279
				Lys160	3.16	Gly159
				Lys160	3.35	Lys181
				Thr292	3.67	Val166
						Arg293
						Gly164
						Arg280

Table 4.19 shows the comparison of docking analysis of standard drug metformin with lead compound ganoderic acid. A comparison shows that the metformin-IGF1R complex consists of one hydrogen bond and five hydrophobic interactions while ganoderic acid-IGF1R complex includes seven hydrophobic interactions and four hydrogen bonds. The metformin-PI3K complex consists of three hydrogen bonds and five hydrophobic interactions while ganoderic acid-PI3K complex consists of it includes 10 hydrophobic interactions and four hydrogen bonds.

Metformin-AkT complex consists of two hydrogen bonds and six hydrophobic interactions while ganoderic acid-AkT complex consists of seven hydrogen bonds and ten hydrophobic interactions. An overall comparison shows that lead compound metformin showed the best physicochemical and pharmacokinetic properties over

reference drug metformin. The number of hydrogen bonds, hydrophobic interactions, and docking score of ganoderic acid are also good as compared to the reference drug metformin. It shows that ganoderic acid can act as a promising anti-aging therapeutic candidate in the future.

4.20 Results of Molecular Dynamic (MD) Simulation

A hundred nanoseconds molecular dynamics simulation was used to examine the best configuration of the ligands under study, which was found by molecular docking at the AKT protein. The protein's initial structure, at 0 ps, was overlaid along the trajectories files to get the RMSD values.

4.20.1 Root Mean Square Deviation

The AKT-Ganodericacid docked complex had an average RMSD value of 8.61 Å, whereas the AKT-Ergothioneine complex had an average RMSD value of 10.33. Both complexes occupied the binding pocket and exhibited structural stability throughout the simulation, with the AKT-Ganoderic acid complex showing relatively better stability based on the lower RMSD value Figure 4.25.

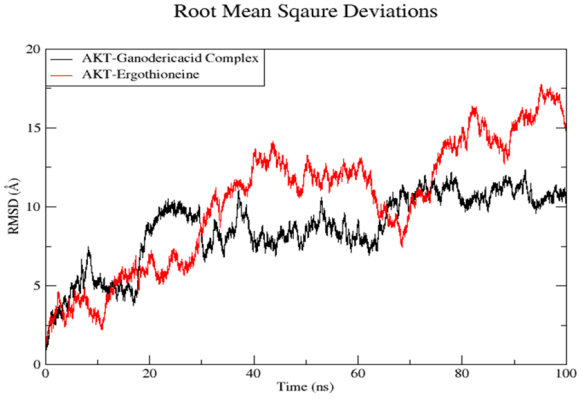


FIGURE 4.25: Trajectories Analysis RMSD of Backbone atoms of AKT protein

4.21 Ligands Mean Square Deviation

Observations revealed that AKT - Ergothioneine fluctuated more than AKT - Ganodericacid. Examining snapshots that were taken at different intervals made it clear that the protein's altered conformation was the source of the changes in RMSD trend, even though every compound occupied the binding pocket and remained stable throughout the simulation run (Figure 4.26). The estimated average RMSD values for Ganodericacid and Ergothioneine were 1.24 and 1.38 Å, respectively.

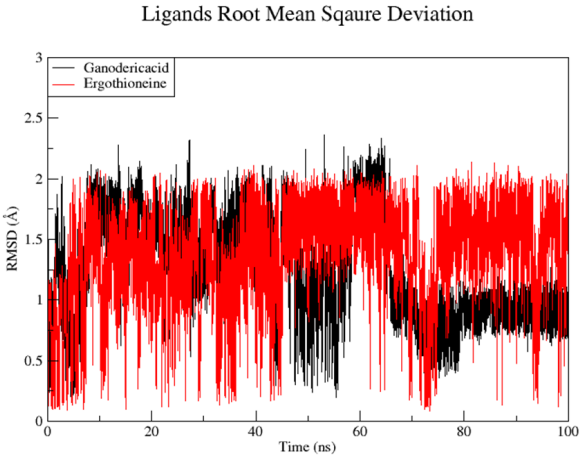


FIGURE 4.26: Trajectories Analysis RMSD of ligands bound with AKT

4.22 Root Mean Square Fluctuations

Furthermore, RMSF graphs were generated, which depict the adaptability of amino acid residues during the simulation periods. The complexes including ganodericacid and ergothioneine with AKT protein had an average RMSF value of 23.17 Åand 40.0 Å, respectively. According to the RMSF values, as shown in Figure 4.27 trajectories Analysis of Root mean square Deviation ergothioneine fluctuated less from their original conformation than ganodericacid. This might imply that the amino acids are static, which could indicate that the studied compounds are stable in the protein's binding region (Figure 4.27).

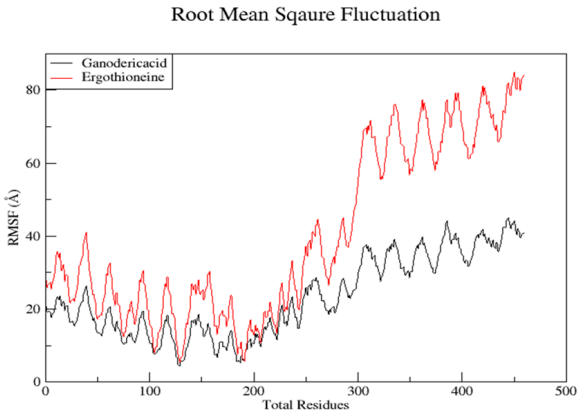


FIGURE 4.27: Trajectories Analysis Root Mean Square Fluctuation (RMSF) of Backbone atoms of AKT protein

4.23 Radius of Gyration

Additionally, the radius of gyration (Rg) values of the protein and ligand complexes were calculated to conduct an additional evaluation of the ligands' compactness and stability (Figure 4.28). Ganodericacid and Ergothioneine with AKT had average Rg values of 60.20 and 66.51 Å, indicating compactness and stability of the complexes examined.

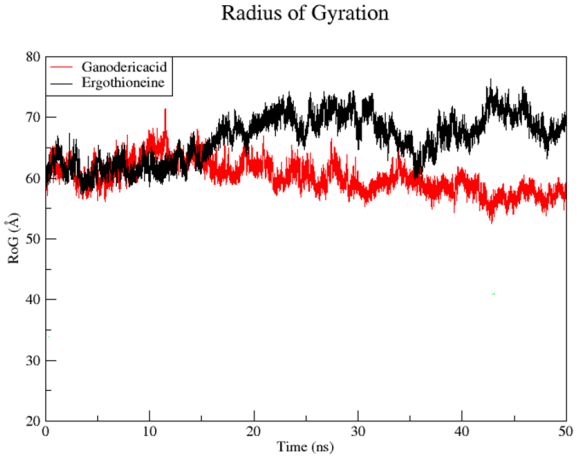


FIGURE 4.28: Trajectories Analysis Radius of gyration of Docked Complexes

Chapter 5

Conclusion and Future Prospects

This research is based on the anti-aging characteristics of fungal metabolites, including ergothioneine, kojic acid, and ganoderic acid, which indicates a prospective avenue for therapeutic implementation [101]. According to molecular docking studies, ganoderic acid has a considerable affinity for important ageing-related proteins like Akt, PI3K, and IGF1R [102]. Molecular dynamic simulations have also approved its interaction with best docked poses of AKT in terms of root mean square deviation (RMSD), root mean square fluctuation (RMSF), and radius of gyration (Rg). Numerous hydrogen bonds and hydrophobic contacts define these interactions, indicating a strong and stable relationship that is essential for blocking aging-related processes. Given their strong binding, ganoderic acid may be able to control these pathways effectively, thereby delaying or even stopping some elements of the ageing process.

Furthermore, ganoderic acid's ADMET profile provides additional support to its prospective use as a medicinal substance. Oral administration of the chemical is advantageous due to its modest solubility in water and a good range of intestinal absorption. Moreover, it also exhibits minimal toxicity, rendering it a more secure substitute in contrast to other anti-aging therapies now accessible. Ganoderic acid exhibits better pharmacokinetic qualities and a higher binding affinity than metformin, a common anti-aging prescription. These findings highlight ganoderic acid's potential as a safer and more effective anti-aging treatment.

Overall the results of this investigation highlight ganoderic acid's considerable potential as an anti-aging treatment. Given its positive ADMET profile and robust interactions with the proteins PI3K, Akt, and IGF1R, the chemical may prove to be a powerful therapeutic agent in the fight against age-related issues and aging [103]. Ganoderic acid may be able to effectively influence important aging-related pathways, potentially providing a unique strategy for extending healthy lifespans and enhancing the quality of life in aging populations, based on the strong binding and interaction patterns seen in molecular docking experiments and molecular dynamic simulations. Ganoderic acid is a possible substitute for metformin in anti-aging therapy because of its higher binding affinity and interaction strength.

A multimodal strategy involving nutritional interventions, pharmaceutical therapies, and lifestyle alterations is necessary due to the complicated dynamics of ageing. With its wide range of bioactive characteristics, ganoderic acid is a good fit for this integrated strategy. The fact that it has broad health-promoting qualities, such as antioxidant and anti-inflammatory properties, in addition to targeting particular biochemical pathways, is very significant. These attributes are vital in reducing the complex impacts of aging.

Going forward, it is critical to confirm these encouraging in silico results with comprehensive studies both in vivo and in vitro. In order to confirm the safety profile and effectiveness of ganoderic acid as reported in computational research, experimental validation utilizing cellular and animal models will be essential. To gain a satisfactory knowledge of ganoderic acid's therapeutic potential, research on the precise molecular processes by which it interacts with the PI3K, Akt, and IGF1R pathways is necessary.

Furthermore, investigating ganoderic acid's synergistic effects with other naturally occurring anti-aging substances may improve its effectiveness. Expanding the medicinal applications of ganoderic acid could lead to more potent anti-aging compositions when combined with other bioactive compounds. To assess the safety, effectiveness, and ideal dosage of ganoderic acid in humans, clinical trials are necessary. The practical use of laboratory results will be greatly aided by these trials, opening the door to novel and potent anti-aging treatments.

The development of sophisticated drug delivery systems is necessary to enhance the targeted activity and bioavailability of ganoderic acid. By using methods like controlled-release formulations and nanoencapsulation, ganoderic acid's medicinal effects might be maximized and its effective distribution to the body's intended locations could be ensured. In addition, a more thorough screening of fungal metabolites ought to be carried out to find more substances that might have anti-aging qualities. This all-encompassing strategy may increase the number of natural anti-aging agents in the toolbox, providing a multitude of opportunities for therapeutic advancement.

To reach the maximum potential of ganoderic acid as a natural anti-aging agent, it is advised to carry out extensive research both in vivo and in vitro to verify its effects, look into the intricate molecular mechanisms, consider the compounds' synergistic effects, start clinical trials to evaluate safety and efficacy, create sophisticated drug delivery systems to improve bioavailability and carry out more thorough screenings of fungal metabolites to find additional compounds with potential anti-aging properties. These guidelines will enable the full exploration of ganoderic acid's potential and open the door to novel treatment approaches in the battle against aging and age-related illnesses.

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