

In-Vitro Antioxidant and In Silico Molecular Docking Studies Of Aqueous and Ethanolic Extract of *Biophytum Poterioides* Leaves

P.Aadhavan (560021521501), S.Aarthi (560021521502),
V.Abarna (560021521503), E.Abinaya (560021521504),
A.Afra banu (560021521505)

*Under the Guidance of MRS. R.NIRMALA M.PHARM.,
Associate professor, Department of Pharmaceutical Chemistry*

*School of Pharmacy Practice
DHANALAKSHMI SRINIVASAN COLLEGE OF PHARMACY
PERAMBALUR-621212*

Prof, Dr.S.MOHAMED HALITH

*M. Pharm, IPh. D..AIDCHN
Principal*

Dhanalakshmi Srinivasan College of Pharmacy, Perambalur-621212.

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I. INTRODUCTION

Herbal medicines are naturally occurring, plant-derived substances that are used to treat illnesses within local or regional healing practices. These products are complex mixtures of organic chemicals that may come from any raw or processed part of a plant. Herbal treatments are the most popular form of traditional medicine, and are highly lucrative in the international marketplace. The medicinal plants contribute to 80% of the raw materials used in the preparation of drug. Herbal medicine also known as herbalism or botanical medicine, is a medical system based on the use of plants or plant extracts that may be eaten or applied to the skin. Since ancient times, herbal medicine has been used by many different cultures throughout the world for many treatments like malaria, warts, bowel disorders, heart conditions and chronic pain, come from pharmacists and doctors learning about folk knowledge. [1]

Phytochemistry

Phytochemicals (from Greek phyto, meaning "plant") are chemical compound produced by plant have been used as traditional medicine. Medicinal plants have immense therapeutic properties due to presence of some biological active compound. Indian sub-continent has a long history of using plant as traditional medicine. It plays an

significant role in preventing and treating of human diseases. It is evident from research that the plants have the potentiality of its medicinal value because it is a rich sources of phytochemical ingredient.

Phytochemical profile

Phytochemicals are secondary plant metabolites can be classified based on the chemical composition (containing nitrogen or not), chemical structure (for example, having rings, containing a sugar), the biosynthetic pathway (e.g., phenylpropanoid, which produces tannins) or their solubility in various solvents. Investigative studies have demonstrated that many of plants contain secondary metabolites can be divided into three chemically distinct namely alkaloids, terpenes and phenolics that could be potential sources for several effectiveness. [2]

Phytochemistry is the study of the chemicals produced by plants, particularly the secondary metabolites, synthesized as a measure for self defence against insects, pests, pathogens, herbivores, ultraviolet exposure and environmental hazards. Phytochemistry takes into account the structural composition of this metabolites, the biosynthetic pathways, functions, mechanisms of action in the living system as well as its medicinal, industrial, and commercial application. The proper understanding of phytochemicals is essential for drug discovery and

for the development of novel therapeutic agents against major diseases.^[3]

Plants are diverse widely distributed from lands, rocks hills, mountains to marine environments. There are over 400000 species of plants in the world (Pitman and Jorgensen,2002), out of which only a small fraction of about 35000-70000 species of plant have been screened for their medicinal use (Veeresham, 2012). The medicinal potentials of phytochemicals are exhibited from the least primitive to higher plants. According to Fabricant and Farnsworth (2001), about 80% of 122 plant derived drugs are related to their original traditional uses. Reportedly as at the dawn of 21st century, 11% of the 252 drugs considered as basic and essential by the World Health Organization (WHO) were exclusive of following plant origin(Veeresham, 2012).

Plant parts such as the leaves, flowers, stems, barks, roots and seeds that are prone to insects, pests, microbial attacks, and the harsh environment have more amounts of phytochemicals than other parts of the plants. Supportive evidence is the variation that exists in the same species of plants grown in the harsh environment and those in areas with less environmental stress.

The **Ethnobotanical** studies of medicinal plants for the treatment of disease have existed since antiquity. For instance, the discovery of quinine marked the first successful use of chemical compounds to treat infectious disease (David and Jacoby, 2005). This was considered as the most important medical discovery of the 17th century (Achan et al.,2011). But in practical terms, the use of the quinine source, that is, the bark of the cinchona (quinaquina) tree dated back as at the 16th century. However the beginning of the isolation of plant chemical compounds marked the early stages of modern phytochemistry.

For instance, phytochemistry is an important part of Systematic Botany, Taxonomy, Ethno botany, conservation biology, Plant Genetic and Metabolomics, Evolutionary Science and Plant Pathology. The field of pharmacy and Pharmacognosy, Complementary and Alternative medicine, Ethno medicine, Biochemistry, Microbiology, Bioinformatics and Computational chemistry employs the knowledge of phytochemistry in the discovery of bioactive compounds.

The field of biotechnology and process engineering, nutrition and food science, organic chemistry, employs the knowledge of phytochemistry in the production of natural products with increase phytochemical yield. In the control of environmental pollution, the knowledge of phytochemistry is essential in applying bioremediation techniques such as phytoremediation to mop up harmful substances.

IMPORTANCE OF PHYTOCHEMISTRY

The knowledge of phytochemistry is essential in the:

1. Search for the discovery of new drugs and repurposing of existing ones
2. Characterization and standardization of traditional herbal drugs in the crude form
3. Assessment of the toxicity levels of plants
4. Understanding of plant physiology, biosynthetic pathways, and Metabolomics
5. Identification and classification of plants
6. Study of inter and intra specific chemical variability within plant
7. Biotechnology and genetic engineering for the optimization and synthesis of classic compound
8. Plant pathology
9. Development of environmental friendly, bio-fungicides, insecticides, pesticides.
10. Food preservation

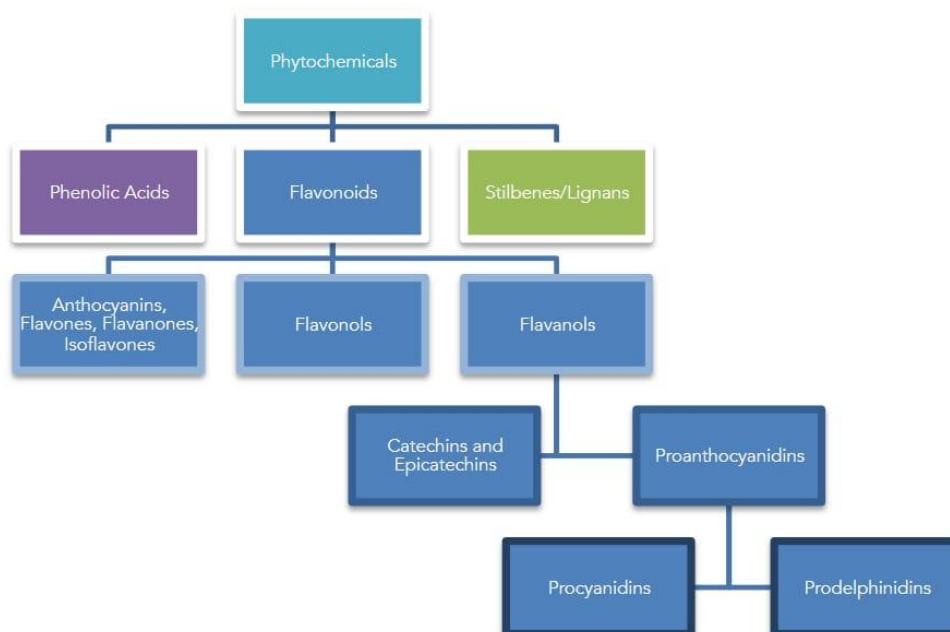


Figure 1: Types of Phytochemicals

Phytochemicals have been in existence since time immemorial and are known to be responsible for the organoleptic properties (color, taste, flavor, aroma, and odor) of plants, such as the smell of garlic.

In general, the plant chemicals that protect plant cell from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack or called as phytochemicals.

These compounds are known as secondary plants metabolites and have biological properties such as anti-oxidant activity, anti-microbial effect, modulation detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation modulation of hormone metabolism and anticancer property. There are more than thousand known and many unknown phytochemicals. It is well known that plants produce these chemicals to protect themselves, but recent researches demonstrate that many phytochemicals can also protect human against diseases.

In wide ranging dietary phytochemicals are the found in fruits, vegetables, legumes, whole grains, nuts, seeds, fungi, herbs and spices. Broccoli, cabbage, carrots, onion, garlic, whole wheat bread, tomatoes, grapes, cherries, straw berries, raspberries, beans, legumes, and soy foods are common source.

The exact classification of phytochemicals could have not been performed so far, because of the wide variety of them. In recent year phytochemicals

are classified as primary or secondary constituents, depending on their role in plant metabolism.

Primary constituents include the common sugars, amino acids, proteins, purine and pyrimidines of nucleic acids, chlorophylls etc. Secondary constituents are the remaining plant chemicals such as alkaloids, terpenes, flavonoids, lignans, plant steroids, curcumines, saponin, phenolics, flavonoids and glycosides. Literature survey indicate that phenolic are the most numerous and structurally diverse plant phytoconstituents.

In India, plant of therapeutic potential are widely used by all section of people both as folk medicine in different indigenous system of medicine like Siddha, Ayurveda, and Unani and also as processed product of pharmaceutical industry. ^[4]

II. Literature Review

1. A. A. Hamid et. al., (2010) - Antioxidants protect cells from damage caused by free radicals and are essential for the survival of plants and animals. They are found in foods like fruits, vegetables, meat,

and fish, and have important biochemical, pharmacological, and therapeutic roles.^[5]

2. Vivek Kumar Gupta et. al., (2006)- Oxygen free radicals cause damage to biomembranes and DNA, leading to tissue injury and various diseases, while antioxidants help neutralize these harmful effects. Due to health concerns about synthetic antioxidants like BHT and BHA, research has focused on safe, natural plant-based antioxidants.^[6]

3. Boris V.Nemzer et. al., (2019)- Epidemiological studies show that antioxidant-rich foods help reduce the risk of chronic diseases and premature aging. Natural antioxidants like epigallocatechin gallate, quercetin, curcumin, resveratrol, and lycopene exhibit strong antioxidant activity and provide significant health benefits.^[7]

4. Rajdeep Mundiara et. al., (2017)- Antioxidants in fruits and vegetables protect cells from damage caused by free radicals and reactive oxygen species (ROS), thereby reducing the risk of chronic diseases and aging. Nutrients like vitamins A, C, E, β -carotene, lycopene, and resveratrol enhance immunity and help prevent cardiovascular diseases and cancer.^[8]

5. Emad A. Shalaby et.al., (2013)- The human antioxidant defense system includes enzymatic antioxidants like catalase, superoxide dismutase, and glutathione S-transferase, and non-enzymatic antioxidants such as ascorbic acid and tocopherol that neutralize reactive oxygen species. Research focuses on evaluating natural and synthetic antioxidants using various in vitro assays to understand their mechanisms, advantages, and effectiveness in preventing oxidation.^[9]

6. Mortada Mohamed El-Sayed et. al.,(2015)- The study evaluated phytochemical constituents and antioxidant activity of *Salix mucronata* leaf extracts, finding that 85% methanol extract showed high phenolic and flavonoid content with strong antioxidant activity. The ethyl acetate fraction exhibited the highest antioxidant potential, indicating that the plant is a rich natural source of antioxidants with strong correlation to total phenolic content.^[10]

7. Asep Bayu Dani Nandianto et. al., (2019)- This study explains how Fourier Transform Infrared (FTIR) spectroscopy can be used to identify chemical bonds and organic components such as proteins, carbohydrates, and lipids. It provides a step-by-step guide to interpreting FTIR peaks and compares the results with literature to serve as a reference for analyzing biochemical and organic materials.^[11]

8. Idi Nuhu et. al., (2024)- Gas chromatography–mass spectrometry (GC-MS)

analysis of *Albizia gumifera* leaf extracts identified 44 bioactive compounds, with ethanol and water extracts showing the highest yields. Phytochemical screening revealed alkaloids, flavonoids, phenols, and other constituents, indicating its strong medicinal and nutraceutical potential.^[12]

9. Peter Folorunsho Ayodele et. al., (2023)- Molecular docking is a bioinformatics tool used in structure-based drug design to predict interactions between small molecules and target proteins, aiding drug discovery. This tutorial demonstrates a step-by-step procedure for using PyRx and BIOVIA Discovery Studio for molecular docking analysis.^[13]

10. Shamima Shultana et. al., (2021)- Drug design, also known as rational drug design, involves studying the molecular structure and shape of compounds to predict how they bind to specific biological targets. By understanding these interactions, drugs can be designed to selectively inhibit or activate biomolecules to produce therapeutic effects.^[14]

11. Kevizano Jacinta Zashumo et. al., (2022)- An in silico study designed a novel anti-obesity compound by modifying 2-methyl-1-phenylpropan-2-amine and evaluating its drug-likeness, ADMET properties, and toxicity using Molinspiration and ProTox II. The results showed good Lipinski compliance, favorable bioactivity, low predicted toxicity (Class III), and potential for further molecular docking studies as an obesity therapy agent.^[15]

12. Sugali Brahmani Bai et. al., (2022)- Due to rising antibiotic resistance, new 2,4,6-trisubstituted pyrimidine derivatives were synthesized and characterized, then evaluated for antibacterial activity. Some compounds, particularly 3 and 4, showed significant activity comparable to ciprofloxacin, while compound 5 demonstrated good predicted drug absorption.^[16]

13. Ganga GS et. al., (2024) -This study developed a green extraction method for apigenin from *Biophytum poterioides* using natural deep eutectic solvents and microwave-assisted extraction, achieving a sixfold higher yield than ethanol. The optimized method was efficient, eco-friendly, and showed strong antioxidant activity, supporting its suitability for large-scale sustainable extraction.^[17]

14. Santhosh Kumar ES et. al., (2019)- *Biophytum poterioides* (Oxalidaceae), an enigmatic species, erroneously has been considered as synonymous with *B. nervifolium* Thwaites in recent treatments. Upon examining the types and other related specimens we prefer to treat it as an independent species.^[18]

AIM

To evaluate the phytochemical profile and potential bioactivity of *Biophytum poterioides* using spectroscopic, chromatographic, and computational techniques.

III. OBJECTIVE

1. Phytochemical Profiling:

- Identify functional groups using FTIR spectroscopy
- Determine volatile compounds using GC-MS analysis

2. Antioxidant Activity:

- Assess antioxidant potential using DPPH, ABTS, or FRAP assays

3. Molecular Docking:

- Investigate interactions between [plant compounds] and target proteins (e.g., enzymes, receptors)
- Predict binding affinities and potential mechanisms of action.

Plan of work

1. Sample Preparation:

Extract and prepare samples for analysis

2. Spectroscopic and Chromatographic Analysis:

- FTIR: Identify functional groups
- GC-MS: Profile volatile compounds

3. Antioxidant Assays:

Conduct DPPH, ABTS, or FRAP assays

4. Molecular Docking:

Study interactions with target proteins

5. Data Analysis and Interpretation:

Integrate results, discuss implications.

ANTIOXIDANT

Antioxidants are essential for the survival of all living organisms, including plants and animals. They prevent oxidative damage caused by reactive oxygen species (ROS) such as singlet oxygen, superoxide radicals, and hydrogen peroxide. Although oxygen is vital for life, its partial reduction produces these

reactive species, leading to oxidative stress, which damages biomolecules like DNA, proteins, and lipids. This oxidative damage is linked to several diseases such as arthritis, cancer, atherosclerosis, and aging.

Plants and animals possess natural defense mechanisms to combat free radicals. Free radicals are atoms or molecules with unpaired electrons that initiate chain reactions, destabilizing other molecules. Antioxidants neutralize these radicals even at low concentrations, thereby protecting cells from oxidative damage.^[19]

In plants, antioxidants act as radical scavengers and form part of both enzymatic and non-enzymatic defense systems. The enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), ascorbate peroxidase (APX), and glutathione reductase (GR). Non-enzymatic antioxidants consist of ascorbic acid (vitamin C), tocopherol (vitamin E), carotenoids, and various phenolic compounds.

Natural antioxidants present in foods such as fruits, vegetables, spices, and herbs are gaining attention due to their safety and therapeutic potential. Synthetic antioxidants are being replaced with natural antioxidants, such as the Mediterranean diet, are associated with lower risks of cardiovascular and cerebrovascular diseases.

Plant-derived antioxidants, including flavonoids, tannins, phenols, and vitamins, play important roles as free radical scavengers, peroxide decomposers, and metal chelators. These compounds contribute significantly to human health by reducing oxidative stress and protecting against chronic diseases.

These components includes,

1. Nutrient-derived antioxidants - Vitamin C, vitamin E, carotenoids, glutathione, lipoic acid.
2. Antioxidant enzymes – Superoxide dismutase, glutathione peroxidase, glutathione reductase.
3. Metal-binding proteins – Ferritin, lactoferrin, albumin, ceruloplasmin.
4. Phytonutrients – Flavonoids and other plant-based compounds.^[20]

IMPORTANCE OF ANTIOXIDANT IN HUMAN HEALTH

Antioxidants can prevent or slow cell damage caused by free radicals, which are unstable molecules that the body produces as a reaction to environmental and other pressures. Free radicals can increase the risk of inflammation and various health

issues. They are sometimes called “free-radical scavengers.” The sources of antioxidants can be natural or artificial. Certain plant-based food are thought to be rich in antioxidant. Plant-based antioxidant are a kind of phytonutrient, or plant-based nutrient.

The body also produces trusted sources some antioxidants, known as endogenous antioxidant. Antioxidants that come from outside the body are called exogenous. Factors that increase the production of free radicals in the body can be internal, such as inflammation, or external, for example, pollution, UV exposure, and cigarette smoke. Oxidative stress has been linked trusted sources to heart diseases, immune deficiency, emphysema, Parkinson’s diseases, and other inflammatory or ischemic condition. Antioxidant are said to help neutralize free radicals in our bodies, and this is thought to boost overall health.^[21]

OXIDATIVE STRESS

Oxidative stress refers to the imbalance between the production of reactive species and antioxidant defense. According to Sies, oxidative stress is defined as “a disturbance in the pro oxidant-antioxidant balances in favor of the former, leading to serious damage”. In all living organisms, include humans, reactive oxygen species [ROS] and free radicals are produced during metabolic and immune system function. Molecular oxygen [O₂] has ability to un-pair and leave free radicals which are unstable and highly reactive leads to formation of ROS. These species play a dual role-beneficial at low concentrations by supporting biological functions such as phagocytosis and apoptosis, but harmful at high levels, causing cellular damage.

The human body possesses several defense mechanisms to neutralize the harmful effects of ROS. The principal defensive agents against oxidative damage are antioxidants, including endogenous enzymes like catalase, superoxide dismutase (SOD), and peroxidase, as well as small antioxidant molecules such as glutathione, thioredoxin, and glutaredoxin. When ROS production exceeds the capacity of these antioxidants, oxidative stress occurs, resulting in damage to vital biomolecules like DNA, proteins, lipids, and carbohydrates. This disruption affects normal cellular metabolism, growth, and function.

Various researches proves that oxidative stress responsible for the development or enhancement of human diseases, such as

- Ulcerative colitis

- Parkinson,s disease
- Alzheimer,s disease
- Atherosclerosis
- Major depression
- Cancer
- Diabetic nephropathy
- End stage renal disease
- Cardiovascular disease
- Mild cognitive impairment and
- Aging.

ROS such as superoxide radicals , hydroxyl radicals, and hydrogen peroxide are the primary agents responsible for oxidative damage. Among these, hydrogen peroxide is particularly significant due to its ability to penetrate cell membranes and its relatively longer lifespan. Persistent oxidative stress can cause severe molecular damage, leading to various degenerative and chronic diseases^[22]

FREE RADICAL

➤ A free radical can be defined as any molecular species capable of independent existence that contain as unpaired electron in an atomic orbital. The presence of an unpaired electron results in certain common properties that are shared by most radicals. Many radicals are unstable and highly reactive.

➤ They can either donate an electron to or accept an electron from other molecules, therefore behaving as oxidant or reductants.

➤ Free radical attack important macromolecule leading to cell damage and homeostatic disruption. Targets of free radical include all kind of molecule in the body. Among them, lipids, nucleic acid and proteins are the major targets^[23]

PRODUCTION OF FREE RADICALS IN THE HUMAN BODY

Production of free radical in the human body free radical and other ROS are derived either from normal essential metabolic in the human body or from external sources such as exposure to

- X-rays
- Ozone
- Cigarette smoking
- Air pollutants
- Industrial chemicals

Free radical formation occurs continuously in the cells as a consequence of both

- 1) Enzymatic and
- 2) Non enzymatic reactions.

Enzymatic reactions, which serve as source of free radicals, include those involved in the respiratory chain, in phagocytosis, in prostaglandin synthesis, and in the cytochrome P-450 system. Free radicals can also be formed in non-enzymatic reactions of oxygen with organic compounds as well as those initiated by ionizing reactions.

EFFECTS OF OXIDANT IN BODY TISSUES

- Excessive amounts of radicals can have deleterious effects on many molecule including protein, lipid, RNA DNA since they are very small and highly reactive.
- ROS can attack bases in nucleic acids, amino acids side chain in protein and double bonds in unsaturated fatty acids, in which OH is the strongest oxidant.
- In addition, intake of dietary antioxidant may help to maintain an adequate antioxidants status in the body.

OXIDATIVE DAMAGE TO DNA

Many experiments clearly provide evidences that DNA and RNA are susceptible to oxidative damage. It has been reported that especially in aging and cancer, DNA is considered as a major target. Oxidative nucleotide as glycol, DTG, and 8-hydroxy 2-deoxyguanosine is found to be increased during oxidative damage to DNA under UV radiation or free radical damage. It has been reported that mitochondrial DNA are more susceptible to oxidative damage that have role in many diseases including cancer. It has been suggested that 8- hydroxy 2-deoxyguanosine can be used as biological marker for oxidative stress.

OXIDATIVE DAMAGE TO PROTEINS

Proteins can be oxidatively modified in three ways; oxidative modification of specific amino acid, free radicals mediated peptide cleavage, and formation of protein cross- linkage due to reaction with lipid peroxidation products. Protein containing amino acid such as,

- Cysteine
- Methionine
- Arginine
- Histidine seen to the be most vulnerable to oxidation

Free radicals mediated protein modification increases susceptibility to enzymes proteolysis. Oxidative damage to protein products

may affect the activity of enzymes, receptor, and membrane transport. Oxidative damaged protein products may contain very reactive groups that may contribute to damage to membrane and many cellular functions.

LIPID PEROXIDATION

Lipid peroxidation occurs on polysaturated fatty acid located on the cell membranes and it further proceeds with radical chain reaction. Hydroxyl radical is thought to initiate ROS and remove hydrogen atom, thus producing lipid radical and further converted into diene conjugate.

Further by addition of oxygen it forms peroxy radical this highly reactive radical attacks another fatty acid forming lipid hydroperoxide [LOOH] and a new radical. Thus, lipid peroxidation is propagated. Due to lipid peroxidation a number of compounds are formed for example alkanes, malanoaldehyde, and isopronates.

CARDIOVASCULAR DISEASES

Heart disease continue to be the biggest killer, responsible for about half life of all the death. The oxidative events may affect cardiovascular disease therefore it has potential to provide enormous benefits to the health and lifespan. Poly unsaturated fatty acid occur as major part of the low density lipoproteins (LDL) in blood and oxidation of these lipid components in LDL play a vital role in atherosclerosis.

The three most important cell types in the vessel wall are

- Endothelial cells
- Smooth muscle cells
- Macrophage

Oxidized low-density lipoprotein (LDL) releases free radicals that cause lipid peroxidation, leading to blood vessel damage, foam cell formation, and plaque buildup – key features of atherosclerosis. Antioxidants such as β -carotene and vitamin E help prevent this oxidative damage and reduce the risk of cardiovascular diseases.

CARCINOGENESIS

Reactive oxygen and nitrogen species, such as superoxide anion, hydrogen peroxide, hydroxyl radical, and nitric oxide and their biological metabolites also play an important role in carcinogenesis.

ROS induce DNA damage, as the reaction of free radicals with DNA includes strand break base modification and DNA protein cross- links. [24]

ANTIOXIDANT ENZYMES

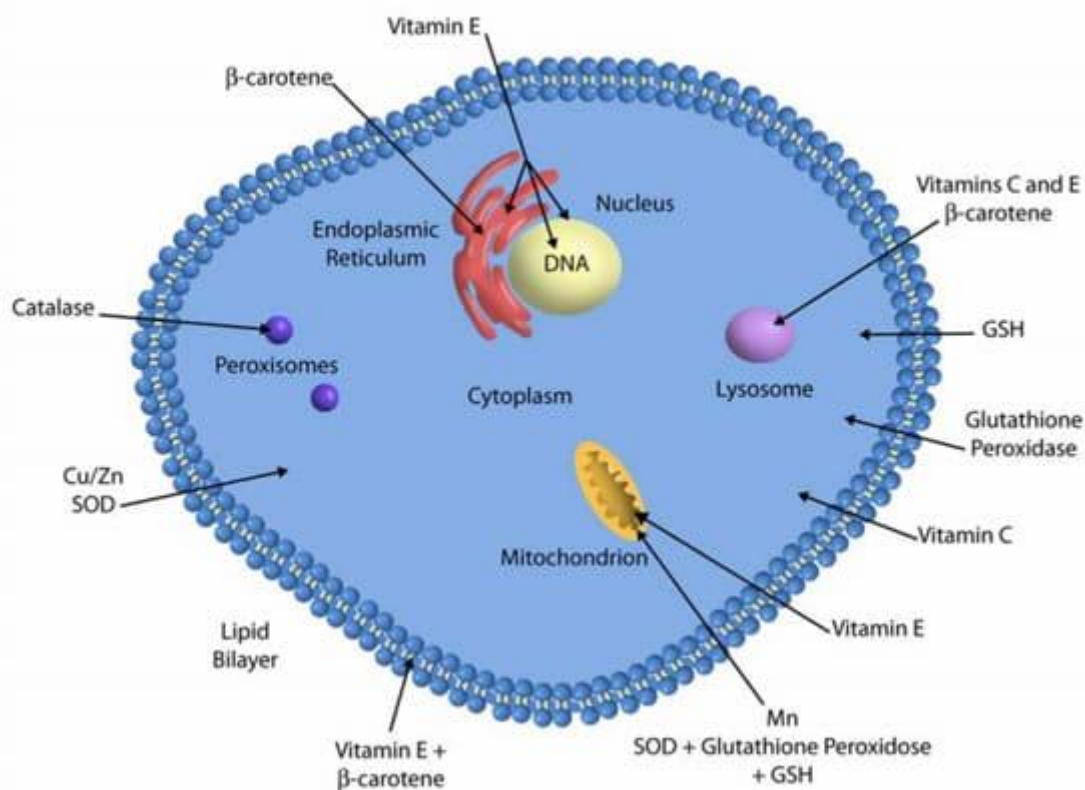
An antioxidant is a molecule which has the ability to prevent or slow the oxidation of macromolecule. The role of antioxidant is to lower or terminate these chain reaction by removing free radical or inhibiting other oxidation reaction by being oxidized themselves. So, antioxidant are often reducing agents such as polyphenols or thiols. Although oxidation reactions

are vital for cells, they have damaging effects; hence, plants and animals contain various antioxidant,

such as

- Vitamin C
- Vitamin E
- Glutathione
- β-carotene

FIGURE: 2 ANTIOXIDANT ENZYMES



The mechanisms which followed by antioxidant defense are:

- Blocking of free radical production
- Oxidants scavenging
- The converting toxic free radicals into less toxic substances
- Blocking the production of secondary toxic metabolites and mediators of inflammation
- Blocking of the chain propagation of the secondary oxidants
- Repairing the injured molecules
- Irritation and enhancing the endogenous antioxidant defense system.

All of these defense mechanisms act hand by for production of the body from oxidative stress. The antioxidant system in the human body consist of

powerful non- enzymatic and enzymatic antioxidants.

The antioxidant enzymes in all body cell consists of three major classes of antioxidants enzymes which are the

- Catalase,
- Superoxide dismutases (SOD),
- Glutathione peroxides (GPX)

Antioxidant enzymes play a crucial role in maintaining cellular homeostasis and protecting cells from oxidative species (ROS). These enzymes act as the body's defense mechanism against oxidative damage induced by pollutants, drugs, and other environmental stressors. Major antioxidant enzymes include superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST), and

glutathione reductase (GR), along with the thioredoxin system.

Superoxide dismutase (SOD) catalyzes the conversion of superoxide radicals into oxygen and hydrogen peroxide, preventing oxidative damage.

Catalase (CAT) then decomposes hydrogen peroxide into water and oxygen through catalytic and peroxidatic activities. It is one of the most efficient antioxidant enzymes, preventing hydrogen peroxide accumulation and protecting tissues from oxidative injury. Catalase is found in both eukaryotic and bacterial cells and is essential for preventing oxidative-mediated cell damage. Overexpression of catalase has been shown to protect against myocardial infarction and hypertension in animal studies.

Glutathione peroxidase (GPX) reduces hydrogen peroxide and lipid hydroperoxides into harmless compounds, while glutathione-S-transferase (GST) detoxifies cytotoxic and genotoxic substances, protecting tissues from oxidative injury. GST also contributes to the breakdown of lipid peroxides and detoxification of xenobiotics such as drugs, carcinogens, and pesticides.

Glutathione reductase (GR) catalyzes the reduction of oxidized glutathione (GSSG) to its reduced form (GSH), ensuring a stable antioxidant balance in cells. GR, a thermostable flavoprotein containing FAD as a cofactor, is vital for maintaining cellular defense against oxidative and chemical stress. Deficiency of GR leads to hemolysis and increased susceptibility to oxidative damage.

The thioredoxin system, consisting of thioredoxin and thioredoxin reductase, also scavenges ROS and supports redox balance. Together, these enzymatic antioxidants form an integrated defense network that detoxifies reactive intermediates, maintains cellular homeostasis, and prevents oxidative stress-related diseases.^[25]

CLASSIFICATION OF ANTIOXIDANT

Antioxidants are broadly grouped into two categories, i.e., (A) Natural antioxidants and (B) Synthetic antioxidants.

A. Natural antioxidants:

They are the chain breaking antioxidants, which react with lipid radicals and convert them into more stable products. They include retinoids (vitamin A), bioflavonoids (citrus), polyphenols (hydroxytyrosol), tocopherols (Vitamin E) and ascorbic acid (Vitamin C). They play a significant role in the prevention of cancer, heart disease, aging and immune

deficiency disease. The natural antioxidants are divided into endogenous and exogenous types.

• Endogenous antioxidants:

These are produced by the body and classified as (i) enzymatic and (ii) non-enzymatic.

(i) Enzymatic antioxidants:

Enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and glutathione reductase (GR) help remove free radicals by converting them into water. They require trace elements like zinc, copper, manganese, and iron as cofactors.

(ii) Non-enzymatic antioxidants:

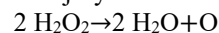
Non-enzymatic antioxidants interrupt free radical chain reactions and include reactions and include alpha-lipoic acid, coenzyme Q10 (CoQ10), glutathione, SOD, CAT, and GPX. These antioxidants convert free radicals into oxygen and water, thereby neutralizing them.

a. Superoxide dismutase (SOD):

SOD catalyzes the conversion of superoxide radicals into oxygen and hydrogen peroxide, repairing cell damage and reducing inflammation.

b. Catalase:

Catalase decomposes hydrogen peroxide into water and oxygen, preventing oxidative injury.



• Exogenous antioxidants:

They are obtained through diet, or supplements containing essential minerals copper, zinc, manganese, iron and selenium. They strengthen the immune system and support the body's endogenous antioxidant defenses.

B. Synthetic antioxidants:

Synthetic antioxidants are man-made chemical compounds approved for use in foods to prevent rancidity and spoilage. They are more potent but may have adverse effects with long-term use. These antioxidants, introduced in the 1940s, are mainly used in fats, oils, cosmetics, and packaged foods. Common examples include:

i. Butylated hydroxytoluene (BHT):

A mixture of isomeric organic compounds that prevent food spoilage and rancid odors in food cosmetics, and rubber products.

ii. Butylated hydroxytoluene (BHT):

A phenolic compound used to prevent oxidation in foods containing fats and oils, though excessive use may cause metabolic or behavioral effects in some individuals.^[26]

Table:1 Comparative properties of natural and synthetic antioxidant

Natural antioxidant	Synthetic antioxidant
They have multiple health benefits.	They may cause adverse effects on human body.
They are readily acceptable by the body.	They require more effect in metabolism.
They can be used as pigment (anthocyanins) or flavoring spices.	Their property is only to stabilize oil.
Being of plant origin they offer a variety of products.	They do not offer a variety of products.
They have antioxidant effects on human tissues.	They do not have antioxidant effect on human tissues.
They may offer the quality of functional food (nutraceuticals) to oils.	They do not offer nutritional quality.
These should be consumed raw (benefits ceased by cooking heat).	Cooking heat does not affect their quality (recommended for frying).

SOURCES OF ANTIOXIDANTS

Natural Antioxidants

Nutritional factors play an important role in preventing damage caused by free radicals. A diet rich in natural antioxidant boosts the body's defense, reduces the risk of free radical-related diseases and supports immune functions. Major dietary antioxidants include vitamin E (tocopherol), vitamin C, vitamin A, β -carotene and phenolic compounds.

- Vitamin A, C, and E are well-studied for their protective effects.
- Carotenoids such as lycopene and lutein remove singlet oxygen, reduce cancer risk, and protect the retina.

Selenium

Selenium is important in glutathione peroxidase (GSHPx), which protects cell membrane from oxidation and helps in hydrogen peroxide and lipid metabolism. It acts like vitamin E, functioning as an antioxidant, protecting cells from free radical, reducing the risk of cancer and cardiovascular diseases.

Phenolic compounds and Polyphenols

Phenolic compounds (like phenolic acids and flavonoids) have strong antioxidant activity depending on the number and position of hydroxyl groups. Common flavonoids includes quercetin, kaempferol, and apigenin. Polyphenols enhance the activity of other antioxidants such as vitamin C and lipid-soluble vitamins and are considered among the most effective antioxidants.

Sources

Natural antioxidant mainly comes from plants. Rich sources includes fruits, vegetables, cereals, legumes, tea, coffee, wine, beer, herbs and spices, Animal

products like milk, fish and eggs contain smaller amounts.

Fruits as a source of antioxidants

Fruits are rich in vitamins, minerals, and active compounds such as vitamin C, carotenoids, and polyphenols.

- Blackcurrants: High in vitamin C (120-215 mg/100 g) and anthocyanins.
- Strawberries: Contain vitamin C (35-104 mg/100g) and ellagic acid derivatives.
- Grapes: Contain resveratrol, catechins, and quercetin that help prevent atherosclerosis.
- Citrus fruits (oranges, lemons, grapefruits): Excellent vitamin C sources (40-50 mg/100g) flavanones and lycopene.
- Apples: Major sources of polyphenols (up to 5g/kg), mainly in the peel.

Vegetables as a source of antioxidants

Vegetables provide vitamins, carotenoids, polyphenols and flavonoids.

- Tomatoes and Red pepper: High in vitamin C, lycopene, and flavanols.
- Brassica vegetables (kale, cabbage, broccoli, brussels sprouts): Contain vitamin C, carotenoids and phenolic compounds.
- Onion: Provide quercetin and sulfur compounds.

Colored vegetables such as red cabbage, red onion, and purple carrots contain anthocyanins responsible for their color and antioxidant power.

Animal-Derived foods as a source of antioxidants

Animal-derived foods contain antioxidants like amino acids, peptides, and proteins.

- Methionine and cysteine (thiol groups) show strong antioxidant activity.
- Casein, whey proteins, and glutathione (GSH) protect against lipid oxidation and regenerate vitamin E.
- Carotenoids are stored in the liver and fat tissues, converted from β -carotene into vitamin A.

Beverages and spices as a source of antioxidants

Beverages such as coffee, tea, cocoa, red wine, and beer, and spices like clove, cinnamon, and ginger are rich source of antioxidants.

- Tea contains catechins, with green tea showing the highest activity.
- Coffee contains chlorogenic acid (up to 8%), and cocoa has 12-18% phenolics (mainly procyanidins).
- Red wine is rich in resveratrol, while beer contains phenolic acids and flavonols.
- Spices provide potent phenolics and are widely used across Asian and Mediterranean diets.

Antioxidants in Different Nation's Diets

The type and level of antioxidants in diets vary by country, depending on food habits.

- USA: Fruits and vegetables (26% polyphenols); oranges supply 25% of total antioxidants.
- Finland: High anthocyanin intake from berries like cranberries and bilberries.
- Netherlands: Tea, chocolate, and apples supply flavonols.
- Spain and France: Apples, grapes, and red wine provide high catechin and polyphenol intake (~1 g/day).
- Asia: Rich in isoflavonoids from soybean products^[27]

ANTIOXIDANT ASSAY

On the basis of chemical reactions involved in antioxidant analysis, there are two major categories

- Electron transfer (ET) reaction assay
- Hydrogen atom transfer (HAT) reaction assay

Electron Transfer Reaction Assay

Electron transfer reaction assay are typical assay that have been used widely all around the world. It involves one redox reaction with the oxidation as an indicator of the reaction endpoint, and as the probe for monitoring the reaction.

Electron transfer reaction antioxidant activity assay

1. Diphenyl-1-picrylhydrazyl (DPPH) Radical scavenging
2. Ferric ion reducing antioxidant power (FRAP)
3. ABTS Radical scavenging
4. Total phenolic assay by folic-ciocalteu reagent

Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activity

Principle

DPPH radical scavenging assay is a simple and easy method for measurement for measurement of antioxidant activity. The method involves reduction of the DPPH radical by antioxidant compound the radical's initially purple color to pale yellow, which can be monitored using a spectrophotometer. It should be noted that DPPH radicals are not reactive towards tyrosine (monohydric phenols), glucose (simple sugar), purines and pyrimidines (Molyneux,2004). Besides that, precipitation might occur between DPPH radicals and proteins. Different stoichiometry of reaction has been reported between DPPH radicals and various antioxidants. For example, two molecules of DPPH radicals can be reduced by one molecule of ascorbic acid (vitamin C). Ascorbic acid is a compound that able to donate two hydrogen atoms (H^+) to hydroxyl groups. Hence, the stoichiometry is 2:1.

Chemical required

- 2,2-Diphenyl- 1- picrylhydrazyl (DPPH), Tris-HCL solution (pH-7.4),
- Butylated hydroxytoluene (positive control),
- Solvent (ethanol/ methanol),
- Trolox (standard),
- Absolute alcohol (methanol/ethanol),
- Distilled water.

(Note: DPPH is not able to dissolve in water.)

General procedure for Determination of DPPH Radical Scavenging

1. Prepare 60 μ M of DPPH solution methanol.
2. Mix 3.9 ml of DPPH solution with 0.1 ml of the sample/extract/standard/control solution.
3. Monitor the reaction progress by measuring the absorbance of the mixture at 515 nm against a blank at 30 min or at every 15 min until the absorbance is stable.
4. Calculate the percentage of scavenging activity for the remaining DPPH over a negative control.

5. Calculate the antioxidant capacity using a linear regression line or a standard calibration curve prepared at different concentrations of Trolox.

Calculation

$$\text{Scavenging activity \%} = (A_0 - A_1/A_0)100^{[28]}$$

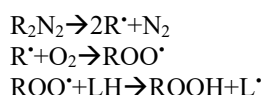
HYDROGEN ATOM TRANSFER (HAT) REACTION ASSAY

Hydrogen atom transfer-based are typical assays involving autoxidation, initiated by an azo compound. The reaction involves 4 major steps

- i. Initiation
- ii. Propagation
- iii. Inhibition
- iv. Termination

The steps are:

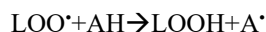
i. Initiation



ii. Propagation

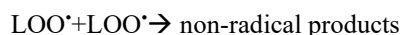


iii. Inhibition



iv. Termination

$A^{\cdot} + (n-1) LOO^{\cdot} \rightarrow$ non-radical products



Where R_2N_2 is an azo compound. LH is a substrate and AH is an antioxidant.

Hydrogen atom electron reaction antioxidant activity assay

1. Total radical-trapping antioxidant parameter (TRAP)
2. ABTS radical scavenging method
3. Hydroxyl radical scavenging activity
4. Oxygen radical absorbance capacity (ORAC)

ABTS assay

ABTS is a measure of antioxidant activity in contrast to antioxidant concentration which includes a proportion of biologically-inactive antioxidant. ABTS permits the measurement of antioxidant activity of mixtures of substance, hence helping to distinguish between additive and synergistic effects. The original assay is based on the activation of metmyoglobin with hydrogen peroxide in the

presence of ABTS to produce the radical cation either in the presence or absence of antioxidant. This concludes that the faster-reacting of the ferryl myoglobin radical. A more appropriate assay method is using a decolorization technique because the directly generated radical is stable prior to reacting with the putative antioxidant. This improved technique for the generation of ABTS involves the direct production of the blue/green ABTS chromophore via the reaction between ABTS and potassium persulfate which has absorption maxima at wavelength 645nm, 734nm and 815nm with the more commonly used maximum absorbance reported to be at 415nm.

The addition of antioxidant to the pre-formed radical cation reduces ABTS on a time-scale to a certain extent, depending on the antioxidant activity of the samples analyzed, the concentration of the antioxidant and the duration of each reaction. Thus, the extend of decolorization as percentage inhibition of the ABTS radical cation is determined as a function of both concentration and time and is calculated relative to the reactivity of Trolox standard under similar condition. A modification of the method utilizes to determine the antioxidant capacity of was also developed. For the evaluation of antioxidant activity, ABTS solution is diluted with ethanol (96%) to obtain an absorbance of 0.700(±0.020) at 734nm. 2ml of ABTS solution are mixed with 100µL of the sample solution in a cuvette and the decrease in the absorbance is measured after 6 minutes. The reagent blank is prepared by adding 100ml of ethanol instead of the samples. Ascorbic acid was used as the standard at different concentration (0-100mg/L) prepared in 96% ethanol and assayed under a similar produce as that conducted on the samples with the means of the three values expressed as mf ascorbic acid equivalent/ 100g.

ABTS assay is beneficial as it reduces labor time, material cost and sample volume. A some of the assays are adapted for a more convenient mass screening using quantitative spectrophotometer as well as applied in agriculture and food industries. Although this method has been reported and commercialized by CAYMAN, it does not incorporate any blank samples which could result in further inaccuracies in the measurement^[29]

APPLICATIONS OF ANTIOXIDANTS

Antioxidants neutralize free radicals both in vivo and in vitro, reducing oxidative stress, delaying aging, and preventing oxidation of food and drugs. They

have broad applications in the pharmaceutical, cosmetic, health food and food industries.

1. Pharmaceutical Industry

In recent decades, non-infectious chronic diseases such as cardiovascular diseases, cancer, and diabetes have become major health concerns. Excess free radicals in the body are a key cause of these diseases. Antioxidants show antibacterial and anticancer effects by eliminating free radical scavenging activity and inhibited cancer cell growth in breast, lung, colon, and prostate cancer models.

2. Cosmetic Industry

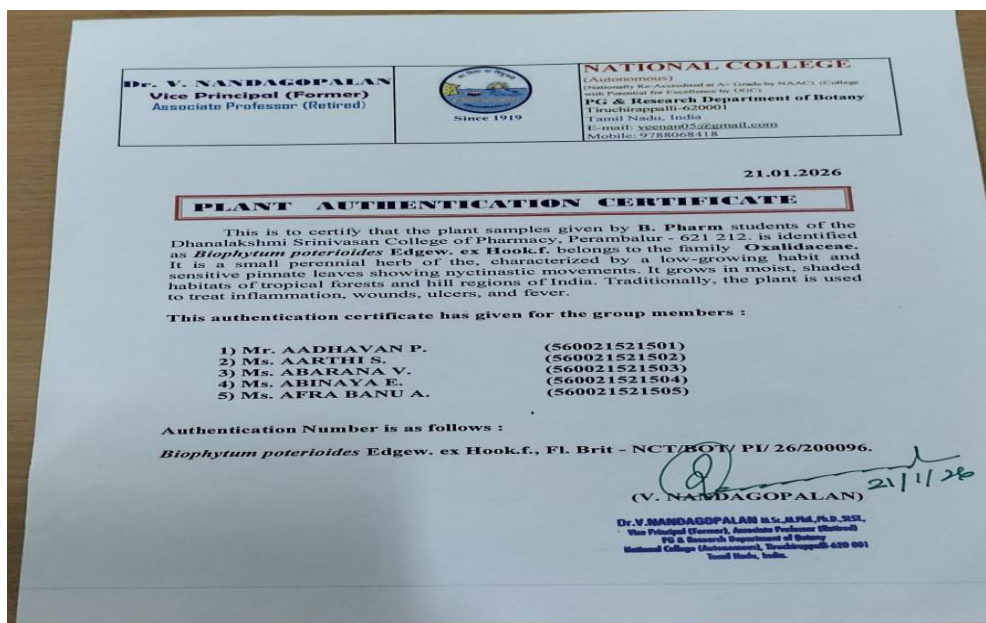
Human skin contains natural antioxidants such as glutathione peroxidase, catalase, superoxide dismutase, and small molecules like vitamin E, vitamin C, and glutathione. Skin aging is accelerated by oxidative stress caused by UV radiation, pollution, and smoking. Cosmetic antioxidants help restore skin cell balance, enhance protection, and delay aging signs like wrinkles. Commonly used antioxidants in cosmetics include ginkgo biloba extract, resveratrol, α -arbutin, vitamin E, and polysaccharides, making them essential ingredients in modern skincare.

3. Food Industry

Lipid oxidation is a major cause of food spoilage, leading to flavor loss and nutrient degradation. Antioxidants act as preservatives, preventing oxidation during processing and storage. They help maintain nutrient content, color, flavor, texture, and freshness, thereby extending shelf life. Due to their stability and low volatility, antioxidants are widely applied in food preservation and packaging.

4. Health Food Industry

Antioxidants play a major role preventing atherosclerosis, neurodegenerative diseases, and premature aging. Foods rich in natural antioxidants such as broccoli (sulforaphane), carrots (β -carotene), and tomatoes (lycopene) are highly beneficial. However, dietary intake alone is often insufficient, especially for the elderly. As a result, many antioxidant-enriched health food products have emerged, particularly those containing Chinese medicinal herbs, which represent a promising future in the health supplement industry^[30]



Plant Profile:

Vernacular Names

- Tamil- Tintanali, Nilalchurungi
- English- Red little tree plant, Red sensitive plant
- Telugu- Pulicenta
- Malayalam- Mukkutti

- Hindi- Lajalu, Lajri
- Marathi- Jharera, Lajvanti
- Bengali- Jhalai
- Sanskrit- Panktipatra, Jhullipuspa

Classification

- Kingdom- plantae
- Phylum- streptophyta
- Class- Equisetopsida
- Subclass- magnoliidae
- Order- oxalidales
- Family- oxalidaceae
- Genus- biophytum
- Species- *Biophytum poterioides* [31]



FIGURE 3: *Biophytum poterioides*

S.No	Characteristics	Description
1.	Common name	Red little tree plant
2.	Plant type	Annual herb (lives for one year) small, slender plant. Grows upto 15cm long.
3.	Habitat & Distribution	Endemic to Tamilnadu (India) Grows in:

		Waste lands, wet agricultural fields. Found at elevation of 100-400 meters.
4.	Flowering season	August – January
5.	Stem	Slender and delicate Upto 15cm long
6.	Leaves	Leaves are arranged in pairs (4-15 pairs). Leaf axis slightly winged Total leaf length: 2.8 to 9.5cm. Surface: sparsely bristly and glandular hairy. Leaflets: often overlap each other. Size: 3-10 mm long, 4-5mm wide. Last leaflet: obovate(egg-shaped), slightly slanted. Others: oblong. Lateral veins: 10-15 pairs, not very visible.
7.	Flowers	Long-styled flowers. Petals: Inverted lance-shaped. Size: 8-10mm long, 4-5mm wide tip flat. Colour: brick red, flame red or pink. Stamens: <ul style="list-style-type: none"> • Shorter ones: 2mm long, smooth. • Longer ones: 3.5mm long finely velvety. Sepals: lance-shaped, 4-4.5mm long, 6-nerved, glandular hairy outside, smooth inside, longer than the fruit capsule. Style: 0.25-2.8mm long. Stigma: flattened, rounded and slightly split.
8.	Inflorescence	Flower cluster Stalk: 1.5- 5cm long covered with bristly hairs. Bracts: ovate, 2.5-3mm long. Flower stalks: 1.5-2mm long.
9.	Fruits	Size: 4-4.5mm long, 3-3.5mm wide. Fringed with hairs at the top.

[32]

IV. Materials and Methods

PREPARATION OF EXTRACT

The coarse powder (100gm) of leaf of *Biophytum poterioides* was extracted with ethanol 500 ml in a soxhlet apparatus for 24 hrs. As cold extraction was performed using water as a solvent through whatman No. 41 filter paper all the extracts were filtered. The flasks containing the mixture was placed in the water bath for 48hrs and it was stirred at regular intervals. It was then filtered, evaporated and concentrated which provided us the final extract. The extracts were subjected to the further analysis.



Figure 4: Water extract



FIGURE 5: Ethanol extract

FOURIER TRANSFORM INFRARED SPECTROSCOPY ANALYSIS

Using a mortar and pestle, little powdered of both ethanol and water extract of *Biophytum poterioides* were separately mixed with KBr salt and compressed in to a thin pellet. On Thermoscientific transmission, between 4000-400 cm⁻¹. infrared spectra were recorded as KBr pellets

GC-MS ANALYSIS:

GC-MS analysis of the active ethanol and water extract of *Biophytum poterioides* was carried out by using the GC-MS instrument (Model Clarus 680 GC and amp, Perkin Elmer USA), equipped with a capillary column 'Elite-5MS' having dimensions-length-60m. ID-0.25mm and film thickness-0.25°. The instrument was operated in electron impact mode at ionization voltage (70eV), an injection volume of 1µl was employed at an injector temperature of 280°C. The carrier gas was Helium (99.9% purity) at a flow rate of 1ml/min. The oven temperature was initially programmed at 60°C for 3 mins and then increased at the rate of 6°C/min to 300° for a holding time of 10mins. The identification of compounds from the spectral data was based on the available mass spectral records (NIST-2008 library).

IN VITRO ANTIOXIDANT ACTIVITY

Sample preparations

Samples were prepared in 20, 40, 60, 80 and 100µg/ml for *in vitro* antioxidant activity of ethanol and Aqueous extract from leaf of *Biophytum poterioides* plant.

DPPH radical-scavenging activity

DPPH radical-scavenging activity was determined by the method of Shimada, *et al.*, (1992).

Procedure:

Briefly, a 2 ml aliquot of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) in methanol solution (25µg/ml) was added to 0.5 ml sample solution at different concentrations. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min. Then the absorbance was measured at 517nm in a spectrophotometer. Lower absorbance of the reaction mixture indicated higher free-radical scavenging activity.

$$\text{Radical scavenging activity (\%)} = 100 - \left(\frac{A_c - A_s}{A_c} \times 100 \right)$$

Where A_c= control is the absorbance and A_s = sample is the absorbance of reaction mixture (in the presence of sample).

Statistical analysis

Tests were carried out in triplicate for 3 separate experiments. The amount of sample needed to inhibit free radicals concentration by 50%, IC₅₀, was graphically determined by a linear regression method using Ms- Windows based graph pad InStat (version 3) software. Results were expressed as graphically/ mean ± standard deviation.^[33,34]

DOCKING

Docking is a method which involves orientation and a best attempt to find a matching between two molecules it involves binding of one ligand to the active site of protein receptor to form a complex. The binding between the biologically relevant molecule like protein, nucleic acid, carbohydrates lipid play role in signal transduction. Therefore, Docking predicts the site for binding the ligand in order to estimate the activity an activity of small molecules.

Types of docking –

-Rigid docking (lock and key); in this both receptor a ligand is rigid in geometry.

-Flexible docking; in this type of docking the rotation of molecules is performed in every rotation.

Docking can be between

- Protein-ligand.
- Protein-protein.
- Protein-nucleotide.

Types of interactions

- Electrostatic forces.
- Electrodynamics forces.
- Steric forces.
- Solvent related forces.

Key stages in docking

- Target selection a preparation
- ligand re a selection
- Docking-Evaluating docking result

Applications of Molecular Docking

The binding of small molecule ligand an enzyme protein may result in activation an inhibition of enzymes. the main

propose of ligand binding is antagonism or agonism. The main importance of docking technique is in drug design process most drug activity are organic molecule an can be applied for

- Hit identification -docking when combined with scoring function results in potential of drug in silico to identify the molecules that are likable to bind protein of target interest.
- Lead optimization- docking can be used in predicting the orientation in which the ligand would bind the protein this is used in more proper implementation of drug design.

- Bioremediation -it's used in prediction of pollutants that degrade enzymes produced.
- Docking is mainly used in determination of protein - protein docking.
- It is used in determination of side effect when used with another molecule.
- It is used in designing of drugs.
- It is used in study of geometry of particular complex.

Example of docking by binding to active site

- -HIV -1 protease is target receptor
- -Aspartyl is group of active site present for receptor to bind
- -Generation of molecular surface for receptors
- -Generate spheres to fill the active site of receptor. The sphere become potential location for ligand atoms.
- Sphere centers are matched with ligand atoms to determine the orientation for the ligand.
- Find the best ranking or score.^[35]

The ligand used in docking was found in extract of the plant through FTIR

Ligand 1 – Cholestane-3,26-diol-22-oxime

Ligand 2 –R (-)3,7-dimethyl-1,6-octadiene

Ligand 3 – Galactopyranose

The protein was used in docking is anti-diabetic protein

1. 3G5E

2. 1J2E

Protein ligand docking:

Molecular Docking Study Using PyRx: Molecular docking was performed to evaluate the binding interaction between selected ligands and the target protein using PyRx virtual screening software (version 0.8), which integrates AutoDock Vina for docking analysis.

Protein Preparation: The three-dimensional structure of the target protein was retrieved from the Protein Data Bank (PDB) in .pdb format. The protein structure was imported into PyRx, and all water molecules, heteroatoms, and co-crystallized ligands were removed. Polar hydrogen atoms were added, and Kollman charges were added to the protein molecule. The prepared protein was saved in pdbqt format for docking analysis.

Ligand Preparation: Ligand structures were obtained from the PubChem database in .sdf

format. The ligands were imported into PyRx and subjected to energy minimization using the Universal Force Field (UFF) to obtain stable conformations. Gasteiger charges were applied, and ligands were converted into pdbqt format prior to docking.

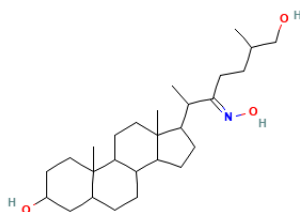
Grid Box Configuration: The docking grid box was set to encompass the active binding site of the target protein. The grid center coordinates (X, Y, Z) and box dimensions were adjusted to ensure complete coverage of the binding pocket

Docking Procedure: Docking simulations were carried out using AutoDock Vina embedded in PyRx. The prepared protein was selected as the macromolecule, and the optimized ligands were docked individually. Docking parameters were kept at default settings, and multiple binding poses were generated for each ligand. Binding affinity values were expressed in kcal/mol.

Analysis of Docking Results: The docked complexes were ranked based on their binding energy scores. The best binding pose for each ligand was selected and analyzed for molecular interactions such as hydrogen bonding, hydrophobic interactions, and π - π interactions. Visualization and interaction analysis were carried out using PyMOL and Discovery Studio Visualizer software.

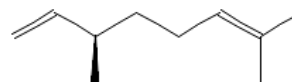
Selective ligands

1. Cholestane-3,26-diol-22-oxime



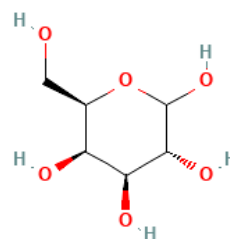
Pubchem ID – 9601628
Molecular Formula – $C_{27}H_{47}NO_3$
Molecular Weight – 433.7g/mol

2. R(-)-3,7-dimethyl-1,6-octadiene



Pubchem ID – 10997105
Molecular Formula – $C_{10}H_{18}$
Molecular Weight – 138.25g/mol

3. Galactopyranose



Pubchem ID – 6036
Molecular Formula – $C_6H_{12}O_6$
Molecular Weight – 180.16g/mol

Selective Protein

1. 3G5E

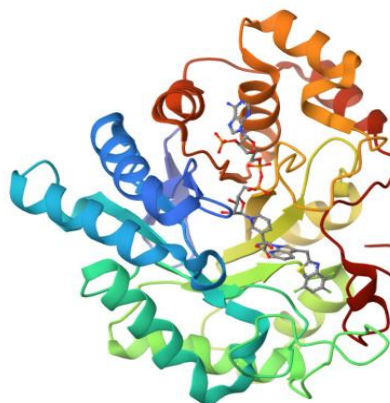


FIGURE 6: 3G5E

2. 1J2E



FIGURE 7: 1J2E

Method for Analysis of Protein–Ligand Docking Results Using Discovery Studio

1. Import of Docked Complex: The docked protein–ligand complex from PyRx (in PDB format) were imported into BIOVIA Discovery Studio Visualizer (DSV) for post-docking analysis. The lowest binding energy pose (most negative binding affinity) for each ligand was selected for detailed interaction studies.

2. Visualization of Docking Pose: The docked complex was visualized in both 3D and 2D interaction modes to examine the orientation of the ligand within the active site of the target protein. The ligand positioning relative to the binding pocket residues was carefully observed to ensure proper accommodation within the active site.

3. Binding Site Analysis: The ligand binding site was analyzed by identifying amino acid residues present within 4–5 Å distance of the ligand. Key active site residues involved in molecular recognition were noted and compared with reported catalytic or functional residues.

4. Protein–Ligand Interaction Analysis: Using the “Analyse Ligand Interactions” tool in Discovery Studio, various intermolecular interactions between

protein and ligand were identified, including:

- Hydrogen bonds
- Hydrophobic interactions (Alkyl, π -Alkyl)
- Van der Waals forces

The type, distance (Å), and participating residues of each interaction were recorded.

5. Binding Energy Correlation: Docking scores (binding affinities in kcal/mol) obtained from PyRx were correlated with interaction profiles observed in Discovery Studio. Ligands showing lower binding energy and higher number of stabilizing interactions were considered to have better binding potential.

6. 2D Interaction Diagram Generation : Two-dimensional interaction diagrams were generated using Discovery Studio to clearly illustrate protein–ligand interactions. These diagrams were used for publication and comparative analysis.

7. Stability and Binding Suitability Assessment: Based on binding energy, interaction type, number of hydrogen bonds, and involvement of key active site residues, the ligand–protein complex stability was assessed. Ligands exhibiting strong interactions with essential residues were identified as potential drug candidates.

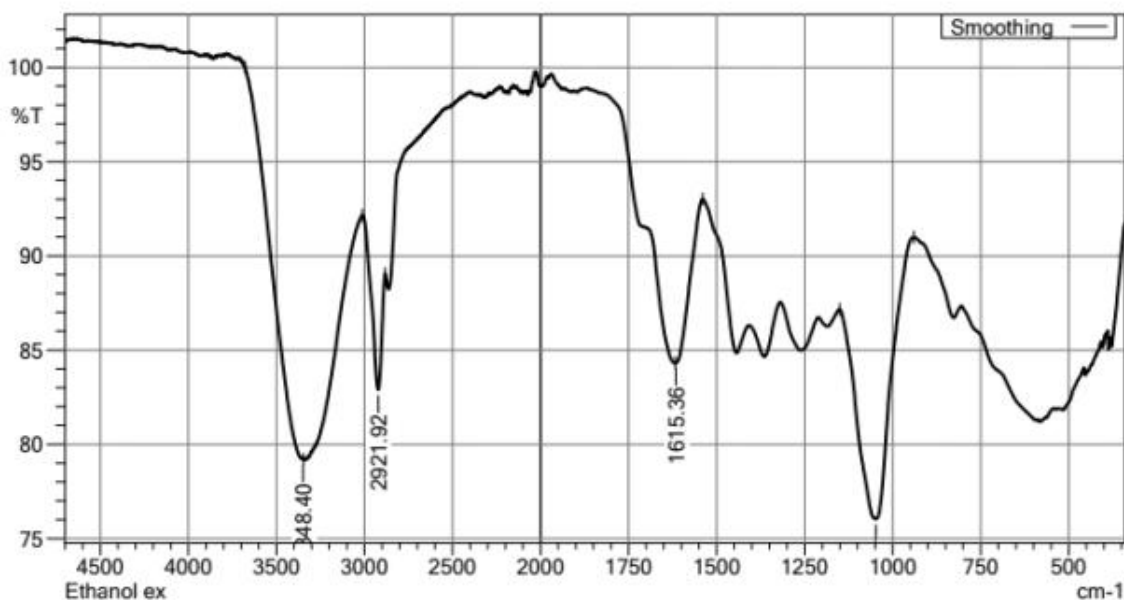
Conclusion of Docking Analysis : The Discovery Studio-based interaction analysis provided detailed insights into molecular binding mechanisms and helped validate the docking results obtained from

PyRx. Ligands demonstrating favourable binding energy and strong interactions were considered promising for further in vitro and in vivo studies. [36]

V. Result & Discussion

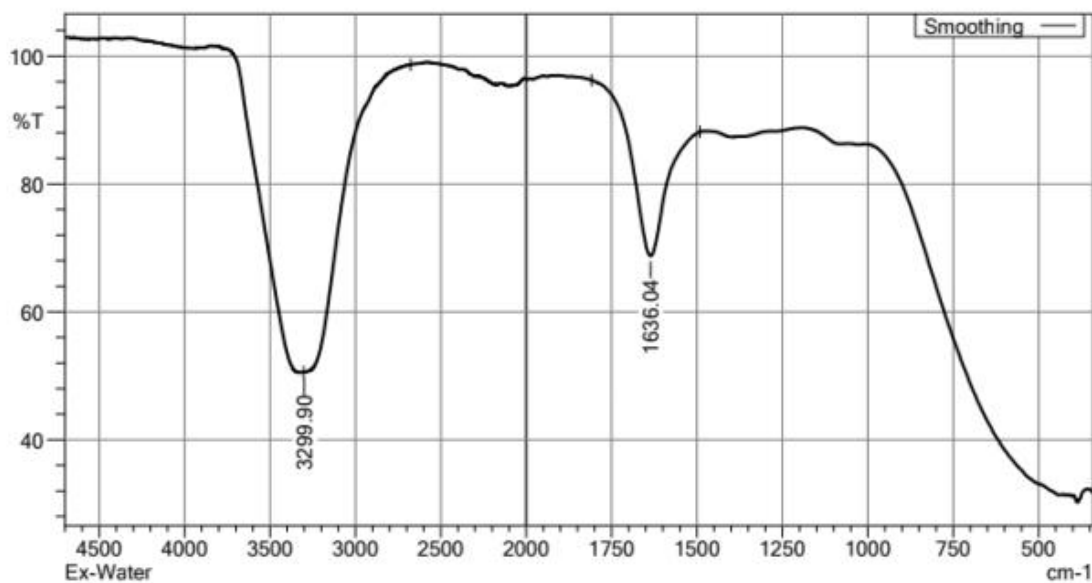
Fourier Transmission Infrared Spectroscopy Ethanol Extract

S.No	Stretching Frequency cm^{-1}	Chemical Bond	Phyto-constituents
1.	877.93	C-H "oop"	Aromatic
2.	1041.96	C-N Stretch	Aliphatic amine
3.	1443.48	C-C Stretch	Aromatic
4.	2915.50	C-H Stretch	Alkane
5.	3332.00	O-H Stretch	Phenolic/ Alcohol group



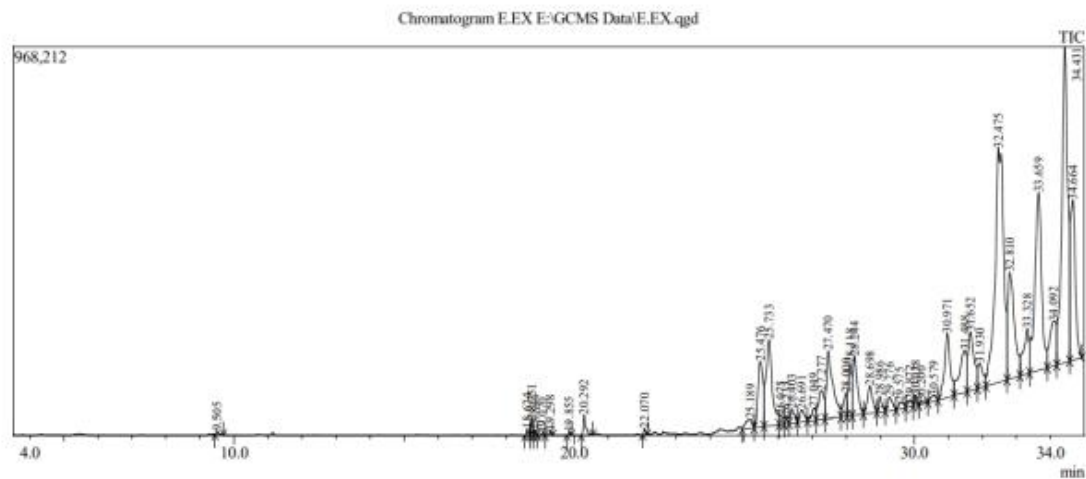
Water Extract

S.No	Stretching Frequency cm^{-1}	Chemical Bond	Phyto-constituent
1.	1636.04	>NH bond	Secondary amines
2.	3299.90	O-H Stretch	Phenolics/ Alcohol group

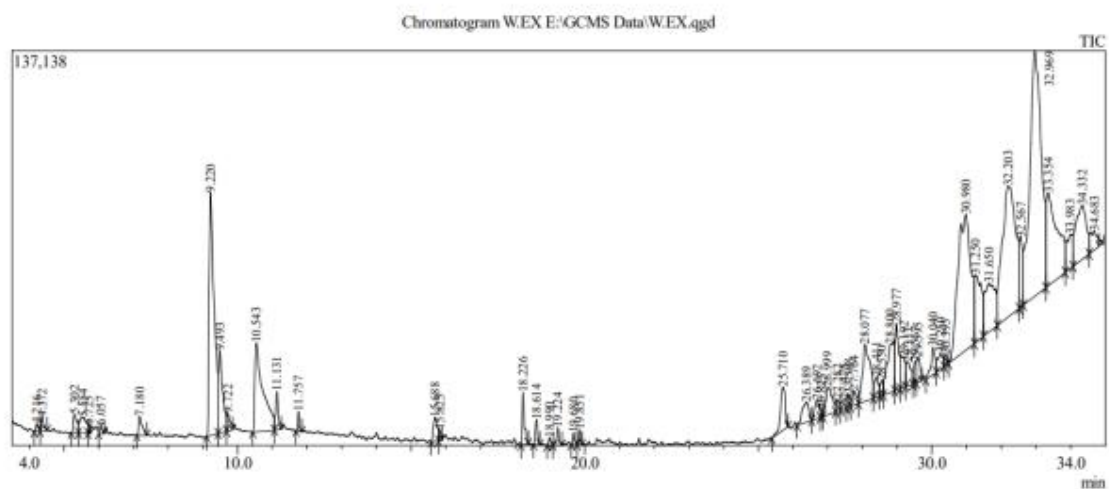


GC-MS Analysis

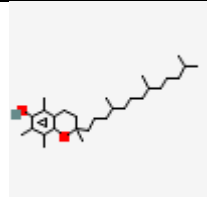
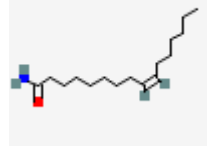
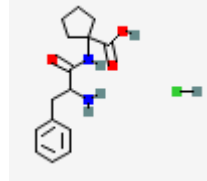
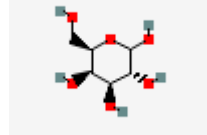
Ethanol Extract




Water Extract

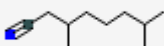
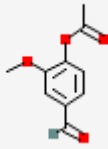


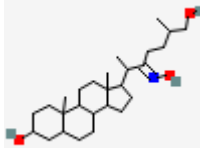


Ethanol extract

S.No	Compound Name	Compound structure
1.	dl-d-Tocopherol	
2.	Palmitoleamide	
3.	1-aminocyclopentane carboxylic acid	
4.	Galactopyranose	

5.	R(-)3,7-dimethyl-1,6-octadiene	
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Water extract

S.No	Compound Name	Compound structure
1.	3,7-dimethyloctanenitrile	
2.	4-o-acetylvannillin	
3.	N-furfuryl-N-methylamine	
4.	Octahydrocyclopenta[c]pyrrole	
5.	Chloestan-3,26-diol-22-oxime	

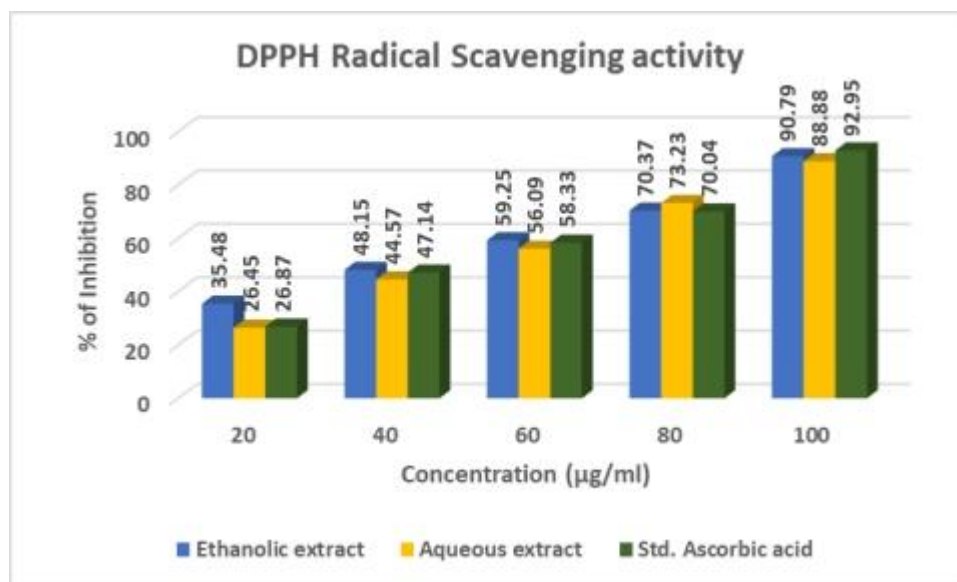
Antioxidant assay

DPPH radical scavenging activity of ethanol and aqueous extract from leaf of *Biophytum poterioides* Edgew at different concentrations

Samples	% of inhibitions					IC ₅₀ value (µg/ml)
	20 (µg/ml)	40 (µg/ml)	60 (µg/ml)	80 (µg/ml)	100 (µg/ml)	
Ethanolic extract	35.48 ± 2.04	48.15 ± 1.69	59.25 ± 1.05	70.37 ± 2.49	90.79 ± 1.5	43.72

Aqueous extract	26.45 ± 2.13	44.57 ± 1.5	56.09 ± 3.12	70.23 ± 1.89	88.88 ± 0.05	49.38
Std. Ascorbic acid	26.87±1.88	47.14±3.29	58.33±0.23	70.04±4.90	92.95±6.51	41.63

Values expressed as Mean ± SD for triplicates



Docking Activity

Ligands

- 1.Cholestane-3,26-diol-22-oxime
- 2.R(-)-3,7-dimethyl-1,6-octadiene
- 3.Galactopyranose

Target Protein

- 1.1J2E (DPP-IV)
2. 3G5E (Human aldose reductase)

Lipinski rule

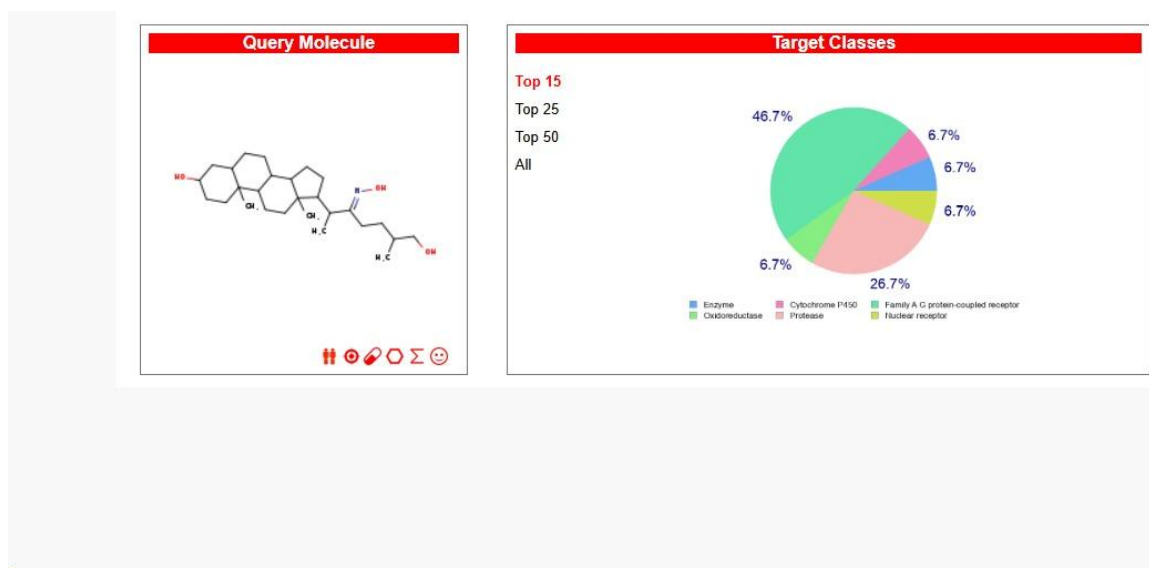
Lipinski's rule of Five (Ro5) is a rule of thumb in drug discovery to predict the oral bioavailability on chemical/physical properties.

- Molecular weight: less than 500 Dalton.
- Lipophilicity: Octanol-Water partition coefficient (log p) less than 5.
- Hydrogen Bond Donor: 5 or fewer (sum of OH and NH group).
- Hydrogen Bond Acceptor: 10 or fewer (sum of N and O atoms).
- No. of violation: 0 or 1

Compound name	Molecular Weight	Lipophilicity	Hydrogen bond acceptor	Hydrogen bond donor	No. of Violation
Chloestan-3,26-diol-22-oxime	433.67 g/mol	4.20	4	3	1

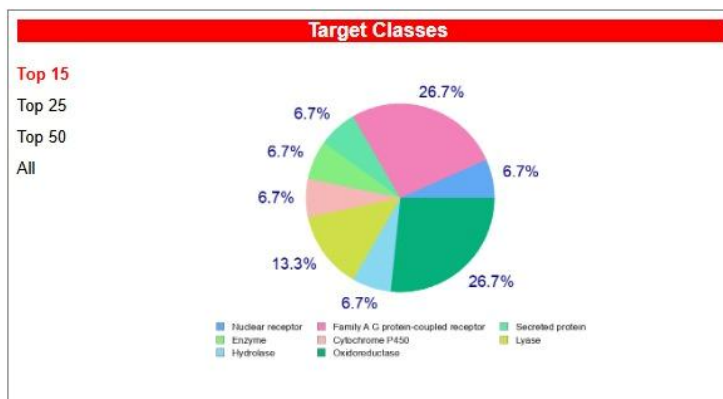
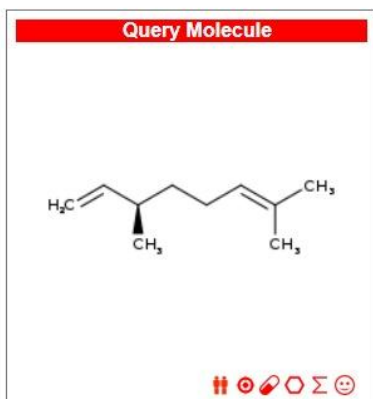
R(-)-3,7-dimethyl-1,6-octadiene	138.25 g/mol	3.02	0	0	0
Galactopyranose	180.16 g/mol	0.73	6	5	0

Cholestan-3,26-diol-22-oxime



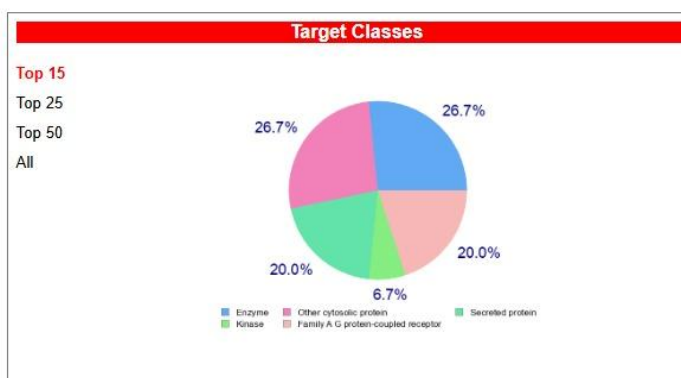
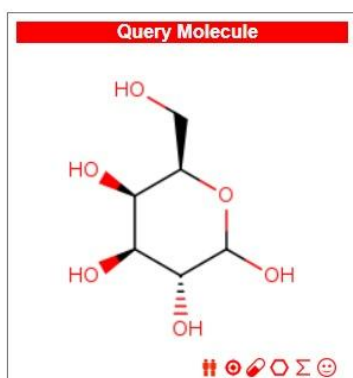
Target	Common name	Uniprot ID	CHEMBL ID	Target Class	Probability*	Known actives (3D/2D)
11-beta-hydroxysteroid dehydrogenase 2	HSD11B2	P80365	CHEMBL3746	Enzyme	<div style="width: 100%;"></div>	8 / 0 ↓
Cytochrome P450 19A1	CYP19A1	P11511	CHEMBL1978	Cytochrome P450	<div style="width: 100%;"></div>	6 / 0 ↓
G-protein coupled receptor 55	GPR55	Q9Y2T6	CHEMBL1075322	Family A G protein-coupled receptor	<div style="width: 100%;"></div>	1 / 0 ↓
Cannabinoid receptor 1	CNR1	P21554	CHEMBL218	Family A G protein-coupled receptor	<div style="width: 100%;"></div>	113 / 0 ↓
N-arachidonyl glycine receptor	GPR18	Q14330	CHEMBL2384898	Family A G protein-coupled receptor	<div style="width: 100%;"></div>	1 / 0 ↓
Cannabinoid receptor 2	CNR2	P34972	CHEMBL253	Family A G protein-coupled receptor	<div style="width: 100%;"></div>	106 / 0 ↓
HMG-CoA reductase (by homology)	HMGCR	P04035	CHEMBL402	Oxidoreductase	<div style="width: 100%;"></div>	85 / 0 ↓
Mu opioid receptor (by homology)	OPRM1	P35372	CHEMBL233	Family A G protein-coupled receptor	<div style="width: 100%;"></div>	155 / 0 ↓
Delta opioid receptor (by homology)	OPRD1	P41143	CHEMBL236	Family A G protein-coupled receptor	<div style="width: 100%;"></div>	111 / 0 ↓
Matrix metalloproteinase 3	MMP3	P08254	CHEMBL283	Protease	<div style="width: 100%;"></div>	25 / 0 ↓
Matrix metalloproteinase 1	MMP1	P03956	CHEMBL332	Protease	<div style="width: 100%;"></div>	32 / 0 ↓
Matrix metalloproteinase 2	MMP2	P08253	CHEMBL333	Protease	<div style="width: 100%;"></div>	28 / 0 ↓

R(-)-3,7-dimethyl-1,6-octadiene



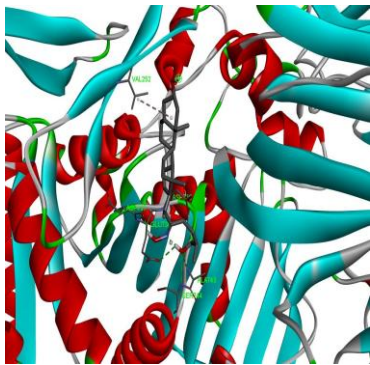
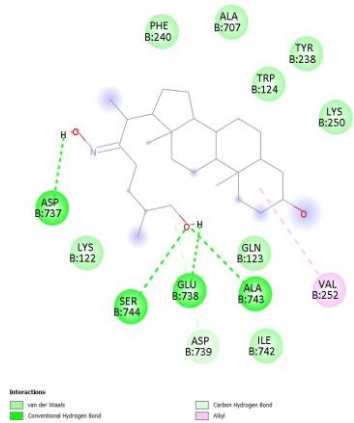
Target	Common name	Uniprot ID	ChEMBL ID	Target Class	Probability*	Known actives (3D/2D)
Peroxisome proliferator-activated receptor alpha	PPARA	Q07869	CHEMBL239	Nuclear receptor	<input type="text"/>	1 / 1
Cannabinoid receptor 2	CNR2	P34972	CHEMBL253	Family A G protein-coupled receptor	<input type="text"/>	1 / 1
Testis-specific androgen-binding protein	SHBG	P04278	CHEMBL3305	Secreted protein	<input type="text"/>	1 / 0
Serotonin 2a (5-HT2a) receptor	HTR2A	P28223	CHEMBL224	Family A G protein-coupled receptor	<input type="text"/>	1 / 0
Monoglyceride lipase	MGLL	Q99685	CHEMBL4191	Enzyme	<input type="text"/>	3 / 0
Cytochrome P450 1A2	CYP1A2	P05177	CHEMBL3356	Cytochrome P450	<input type="text"/>	3 / 0
C-X-C chemokine receptor type 3	CXCR3	P49682	CHEMBL4441	Family A G protein-coupled receptor	<input type="text"/>	2 / 0
Carbonic anhydrase II	CA2	P00918	CHEMBL205	Lyase	<input type="text"/>	1 / 0
Carbonic anhydrase IV	CA4	P22748	CHEMBL3729	Lyase	<input type="text"/>	1 / 0
Butyrylcholinesterase	BCHE	P06276	CHEMBL1914	Hydrolase	<input type="text"/>	1 / 0
Monoamine oxidase A	MAOA	P21397	CHEMBL1951	Oxidoreductase	<input type="text"/>	2 / 0
Cyclooxygenase-1	PTGS1	P23219	CHEMBL221	Oxidoreductase	<input type="text"/>	1 / 0

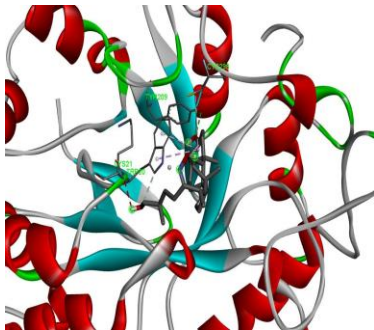
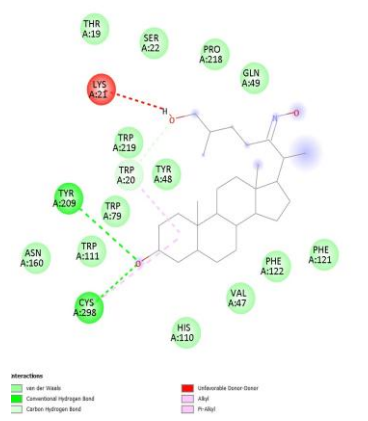
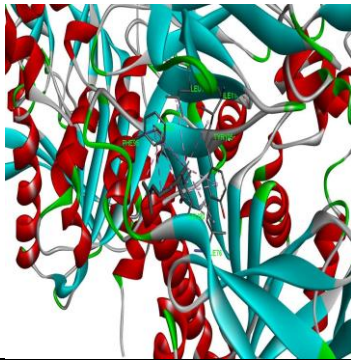
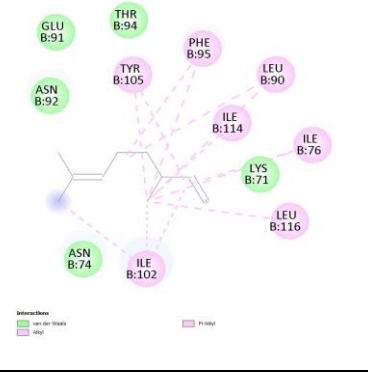
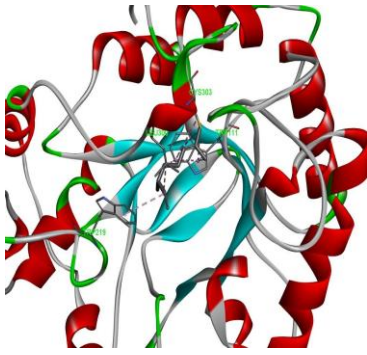
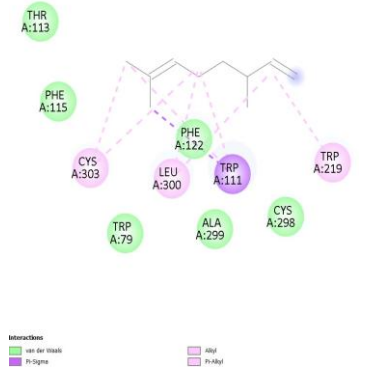
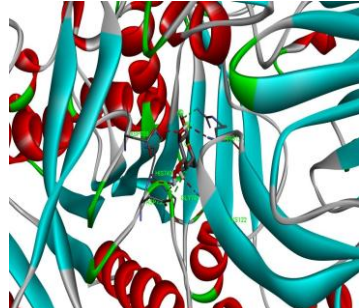
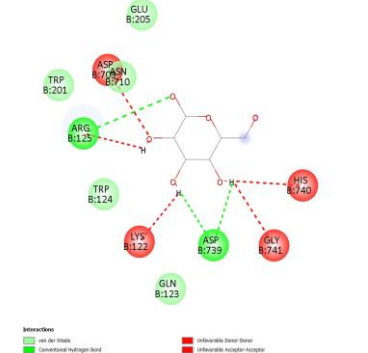
Galactopyranose



Target	Common name	Uniprot ID	ChEMBL ID	Target Class	Probability*	Known actives (3D/2D)
Beta-glucocerebrosidase	GBA	P04062	CHEMBL2179	Enzyme	<div style="width: 100%; height: 10px; background-color: green;"></div>	3 / 4 ↓
Heat shock protein HSP 90-alpha	HSP90AA1	P07900	CHEMBL3880	Other cytosolic protein	<div style="width: 100%; height: 10px; background-color: green;"></div>	0 / 1 ↓
Vascular endothelial growth factor A	VEGFA	P15692	CHEMBL1783	Secreted protein	<div style="width: 100%; height: 10px; background-color: green;"></div>	0 / 2 ↓
Gamma-secretase	PSEN2 PSENEN NCSTN APH1A PSEN1 APH1B	P49810 Q9NZ42 Q92542 Q96BI3 P49768 Q8WW43	CHEMBL2094135	Protease	<div style="width: 100%; height: 10px; background-color: green;"></div>	0 / 10 ↓
Acidic fibroblast growth factor	FGF1	P05230	CHEMBL2120	Secreted protein	<div style="width: 100%; height: 10px; background-color: green;"></div>	0 / 4 ↓
Basic fibroblast growth factor	FGF2	P09038	CHEMBL3107	Secreted protein	<div style="width: 100%; height: 10px; background-color: green;"></div>	0 / 2 ↓
Heparanase	HPSE	Q9Y251	CHEMBL3921	Enzyme	<div style="width: 100%; height: 10px; background-color: green;"></div>	0 / 4 ↓
Cyclin-dependent kinase 1	CDK1	P06493	CHEMBL308	Kinase	<div style="width: 100%; height: 10px; background-color: green;"></div>	0 / 1 ↓
Galectin-4	LGALS4	P56470	CHEMBL1671608	Other cytosolic protein	<div style="width: 100%; height: 10px; background-color: green;"></div>	0 / 2 ↓
Galectin-3	LGALS3	P17931	CHEMBL4531	Other cytosolic protein	<div style="width: 100%; height: 10px; background-color: green;"></div>	0 / 3 ↓
Galectin-8	LGALS8	O00214	CHEMBL5475	Other cytosolic protein	<div style="width: 100%; height: 10px; background-color: green;"></div>	0 / 2 ↓
Brain glycogen phosphorylase	PYGB	P11216	CHEMBL3856	Enzyme	<div style="width: 100%; height: 10px; background-color: green;"></div>	3 / 0 ↓

Ligand	Binding affinity with target protein	
	1J2E	3G5E
Cholestan-3,26-diol-22-oxime	-7.9	-8.1
R(-)-3,7-dimethyl-1,6-octadiene	-5.4	-6.3
Galactopyranose	-6.2	-5.9

S.N O	LIGAND	PROTEIN	SPIRAL STRUCTURE	2D STRUCTURE
1	Chloestane-3,26-diol-22-oxime	1J2E		

		3G5E		
2	R(-)3,7-dimethyl-1,6-octadiene	1J2E		
		3G5E		
3	Galactopyranose	1J2E		



Hydrogen interaction in Docking Compound

Hydrogen interaction		Residue
Cholestan-3,26-diol-22-oxime with 1J2E		
Hydrophilic	Conventional Hydrogen Bond	ASP 737, SER 744, GLU 738, ALA 743
	Carbon-Hydrogen Bond	ASP 739
	Vander Waals Bond	LYS 122, ILE 742, GLN 123, LYS 250, TRP 124, TYR 238, ALA 707, PHE 240
Hydrophobic	Alkyl	VAL 252
Cholestan-3,26-diol-22-oxime with 3G5E		
Hydrophilic	Conventional Hydrogen Bond	TYR 209, CYS 298
	Carbon-Hydrogen Bond	ASN 160, TRP 111, TRP 79, TRP 20, HIS 110, VAL 47, PHE 122, PHE 121
	Vander Waals Bond	TYR 48, TRP 219, THR 19, SER 22, PRO 218, GLN 49
R(-)-3,7-dimethyl-1,6-octadiene with 1J2E		
Hydrophilic	Vander Waals Bond	GLU 91, THR 94, ASN 92, ASN 74, LYS 71
Hydrophobic	Alkyl	ILE 102, LEU 116, ILE 76
	Pi-Alkyl	TYR 105, PHE 95, LEU 90, ILE 114
R(-)-3,7-Dimethyl-1,6-octadiene with 3G5E		
Hydrophilic	Vander Waals Bond	THR 113, PHE 115, PHE 122, TRP 79, ALA 299, CYS 298
Hydrophobic	Pi-Sigma	TRP 111
	Alkyl	CYS 303, LEU 300
	Pi-Alkyl	

		TRP 219
Galactopyranose with 1J2E		
Hydrophilic	Conventional Hydrogen Bond	ARG 125, ASP 739
	Vander Waals Bond	GLU 205, TRP 201, ASN 710, TRP 124, GLN 123
Galactopyranose with 3G5E		
Hydrophilic	Conventional Hydrogen Bond	SER 210, TRP 20, TYR 48
	Vander Waals Bond	CYS 298, TYR 209, PRO 211, SER 214, ASP 216, LYS 262, GLY 18, THR 19, ASP 43, LYS 77, ILE 260

Metformin shows maximum binding affinity with 1J2E and 3G5E was 4.9 and 5.3 respectively. The hydrogen interaction with 1J2E was TYR 238, ASP 709, ALA 707, TRP 124, ASP 739, GLN 123 & LYS 122 and interaction with 3G5E was SER 214, SER 210, GLN 183, SER 159, LYS 177, HIS 110, ILE 260, TYR 209, TYR 48, ASP 43, THR 19, TRP 20, GLY 18 & LYS 262. Metformin shows relatively lower binding affinity for proteins 1J2E and 3G5E in comparison to the affinity exhibited by our selective ligand, indicating a potential difference in interaction dynamics.