Project On

"Evaluation of anti-diabetic and anti-inflammatory activity of selective phytoconstituents from *Hyptis suaveolens (L.) Poit*: an *in silico* molecular docking and ADMET study"

A dissertation submitted to the Department of Pharmacy, Daffodil International University, partially fulfills the needs for the Bachelor of Pharmacy (B. Pharm.) degree

Submitted To

Department of Pharmacy
Faculty of Allied Health Science
Daffodil International University

Submitted By

Mohammed Murshedul Islam
ID: 193-29-1589
Batch: 22-B
Department of Pharmacy
Faculty of Allied Health Science
Daffodil International University

Date of Submission: December, 2023

Approval

This project submitted to the Department of Pharmacy, Faculty of Allied Health Science, Daffodil International University, Has been accepted as satisfactory for the partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (B. Pharm) and approved as to its style and contents.

BOARD OF EXAMINERS

Professor Dr. Muniruddin Ahamed
Professor & Head
Department of Pharmacy
Faculty of Allied Health Sciences
Daffodil International University

Internal Examiner-1

Internal Examiner-2

External Examiner

©Daffodil International University

Acknowledgement

I am very grateful to Allah for granting me the capacity to complete this project on time. I would love to express my sincere gratitude to everyone who helped me to complete this project and for shining a success light on this essential resource. I would especially want to mention my wonderful supervisor, **Mr. Subrato Kumar Barman** sir.

A project is never created by one person. It is more than just a compilation of people's thoughts, evaluations, contributions, and labor. The guidance and assistance of several people are crucial to a project's development and final outcome, thus I consider myself quite lucky to have had this throughout the whole project completion process.

My sincere gratitude goes out to my project supervisor, **Mr. Subrato Kumar Barman**, lecturer, Department of Pharmacy. He showed a great deal of interest in my work and helped me every step of the way, providing me with all the information I needed to create a functional system and successfully finish the project. I am grateful to **Prof. Dr. Muniruddin Ahmed**, The Head, Department of Pharmacy, DIU, for giving me the opportunity to work on the research and for all of his guidance and support, which allowed me to complete the project before the deadline.

I am grateful that the pharmacy department's teaching team provided me with continuous support, guidance, and encouragement. This allowed us to successfully finish our project work. Also, I would like to extend our sincere esteems to all staff in for their timely support.

Finally, I would like to thank all of my friends and well-wishers for their unfailing support and my parents for their financial and spiritual assistance.

Mohammed Murshedul Islam
The Author,

©Daffodil International University

Dedication

DEDICATED TO

ALMIGHTY, FAMILY, FRIENDS, AND ALL OF MY RESPECTED TEACHERS WHO HAVE ALWAYS SUPPORTED AND ENCOURAGED ME

Declaration

I hereby certify that I independently completed this project report under the supervision of Mr. Subrato Kumar Barman, Lecturer, Department of Pharmacy, Faculty of Allied Health Sciences, Daffodil International University, in order to fulfill the requirements for the Bachelor of Pharmacy (B. Pharm) degree. By signing this, I certify that I am the only author of this work. I further certify that I have never turned in this project or any of its components to another university for credit toward a bachelor's or other degree.

Supervised By:

Mr. Subrato Kumar Barman
Lecturer
Department of Pharmacy
Faculty of Allied Health Sciences
Daffodil International University

Submitted By:

Mohammed Murshedul Islam
ID: 193-29- 1589
Department of Pharmacy
Faculty of Allied Health Sciences
Daffodil International University

Abstract

The use of medicinal plants to treat DM and Inflammation is popular, as herbal drugs are generally regarded as free of toxic effects. Moreover**,** the limitations of oral anti-diabetic and anti-inflammatory drugs urge to find new drugs for treatment of DM and inflammation. Therefore, the search for more effective and safer herbal hypoglycemic agents, anti-inflammatory agents and developing new anti-diabetic drugs, anti-inflammatory drugs with improved clinical profiles simultaneously have become an area of active research. Numerous animal studies have shown positive anti-inflammatory and antioxidant capabilities of several physiologically active compounds present in plant extracts. It is yet unclear how these substances, which are found in phytochemical extracts, interact molecularly with the target proteins or enzymes that provide antioxidant and anti-inflammatory properties.

The current effort attempts to identify and evaluate putative biological targets as proteins or enzymes involved in these targeted studies using molecular docking as a computational approach. To extract a variety of phytochemicals from Hyptis suaveolens (L.) Poit, the curated database IMPPAT: Indian Medicinal Plants, Photochemistry and Therapeutics has been used. These phytochemicals are further evaluated by molecular docking against two proteins (4EMA and 1PXX) associated with antioxidant and antidiabetic properties. Beta-sitosterol, ovatodiolide, neoabietinol, apigenin, dehydroabietinol, cyclooxygenase-2 (COX-2) (PDB: 1PXX), and peroxisome proliferator-activated receptor gamma (PPAR-γ) (PDB ID: 4EMA) are a few phytochemicals that have shown encouraging binding affinities towards target proteins.

To determine the pharmacokinetic and pharmacodynamic properties of these compounds as possible therapeutic agents against inflammatory and antidiabetic disorders, employing ADMET prediction and in silico docking studies of specific phytoconstituents. Molecular docking was also utilized to confirm these. Based on their binding affinities and docking scores, many phytochemicals (Beta-Sitosterol, Ovatodiolide, Neoabietinol, Apigenin, Dehydroabietinol, and Spathulenol) were demonstrated to be able to bind protein targets (4EMA, 1PXX). More in vitro research is needed to fully understand the target-based anti-inflammatory and anti-diabetic effects of these newly identified phytochemicals.

Keywords: *Anti-diabetic activity, ADMET study, Inflammation, Diclofenac, Hyptis Suaveolens,Discovery Studio, AutoDockVina, Rosiglitazone, Diabetes Mellitus, Insulin resistance, , in silico molecular docking, Anti-inflammatory agent, COX-2,* PPAR-γ**,** NSAIDs

Table of Contents

List of Tables

List of Figures

©Daffodil International University

List of Abbreviation

NSAID = Non-steroidal anti-inflammatory drugs
IMPPAT=Indian Medicinal Plants, Phytochemistry And Therapeutics
PGI2 = Prostacyclin
Pre-SAP = Prevention of Spontaneous Adenomatous Polyps
RA = Rheumatoid arthritis
RCT = Randomized controlled trials
ROS = Reactive oxygen species
RR = Relative risk
RF = Rheumatoid factor
TARGET = Therapeutic Arthritis Research and Gastrointestinal Event Trial
TXA2 = Thromboxane A2
VICTOR = Vioxx In Colorectal cancer Therapy: definition of Optimal Regimen
VIGOR = Vioxx gastrointestinal outcomes research
BNH = Bangladesh National Herbarium
CADD = Computer-aided drug design
CHF = Congestive heart failure
DM = Diabetes mellitus
EA = Enzyme activity
FDCC = Food, Drug, Chemical, and Cosmetics Testing & Research Laboratory
GDM = Gestational diabetes
icddr,b = International Center for Diarrheal Disease Research, Bangladesh
IGT = Impaired glucose tolerance
mg/kg = Milligram per kilogram
PDB = Protein Data Bank
PPAR = Peroxisome proliferator-activator receptor
SARs = Structure–activity relationships
SEM = Standard error of mean
TCM = Traditional Chinese medicine
TKM = Traditional Korean medicine
TM = Traditional medicine
TZDs = Thiazolidinediones
WHO = World Health Organization
µg/ml = Microgram per milliliter

Chapter 1: Introduction

1.1 Diabetes

The World Health Organization (WHO) defines diabetes as a chronic disorder caused by either insufficient insulin synthesis by the pancreas or inadequate body use of the generated insulin. Insulin is one hormone that regulates blood sugar. Uncontrolled diabetes often leads to hyperglycemia, or high blood sugar, which damages several physiological systems over time, including blood vessels and neurons.

Countries with middle-class and lower-class populations have seen a faster increase in the prevalence of diabetes. Diabetes is the main cause of lower limb amputations, heart attacks, strokes, renal failure, and blindness. [1]

1.1.1 Types of Diabetes Mellitus

The medical conditions that are used to diagnose diabetes mellitus are currently categorized into four groups:

Type 1, *insulin-dependent diabetes, juvenile onset diabetes mellitus:*

Type 1 diabetes is characterized by selective beta cell (B cell) death and severe or total insulin deficiency.

Idiopathic (type 1B) and immunological (type 1A) forms of type 1 diabetes are distinguished by the absence of β cell antibodies and the presence of antibodies that kill β cells in the blood. This kind is less common and has less hereditary predisposition.

Type 2, *non–insulin-dependent diabetes, maturity onset diabetes mellitus:*
A high degree of genetic predisposition, tissue resistance to the action of insulin, no loss or a moderate reduction in β cell mass, low, normal, or even high insulin in circulation, no detectable anti-β-cell antibody, and usually a late onset (past middle age) are the characteristics of type 2 diabetes. More than 90% of instances of diabetes are type 2 in nature.

Type 3, *other*:

"Type 3" refers to several other unique causes of elevated blood sugar, including pharmaceutical therapy, non-pancreatic diseases, pancreatitis, and pancreatectomy.

Type 4, *gestational diabetes mellitus*:

Gestational diabetes (GDM) is the term used to describe any abnormalities in glucose levels that are noticed for the first time during pregnancy. Gestational diabetes is diagnosed in 7% of pregnancies in the United States. The placenta and placental hormones cause insulin resistance to develop during pregnancy, peaking in the final trimester. [2, 3]

©Daffodil International University

1.2 Diabetes status

1.2.1 Worldwide diabetes status

According to predictions from the International Diabetes Federation (IDF), 537 million people globally, aged 20 to 79, would have diabetes in 2021. By 2030, 643 million individuals globally are expected to have diabetes, and by 2045, 783 million. Three of every four adults with diabetes live in low- and middle-income countries. [4]

1.2.2 Diabetes status in Bangladesh

According to a 2019 World Health Organization report, diabetes mellitus is responsible for 20.6% of deaths in Bangladesh among females. [5]

According to 2016 WHO estimate states that diabetes affected 12.88 million people in Bangladesh, or 8% of the country's total population, and that it was the reason behind 3% of all fatalities in the nation, regardless of age [6]. Additionally, the frequency of DM among Bangladeshis has increased over time [7].

Table 1.1: Diabetes estimates (20-79 y) in Bangladesh [8]

Age (20-79 y)	Year		
	2000	2011	2021
People with diabetes, in 1,000s	1,759.7	8,405.6	13,136.3
Age-adjusted comparative prevalence of diabetes, %	---	10.5%	14.2%
Proportion of people with undiagnosed diabetes, %			43.5%

©Daffodil International University

Table 1.2: Impaired glucose tolerance (IGT) estimates in Bangladesh (20-79 y) [8]

Age	**Year**	
	2011	**2021**
People with IGT, in 1,000s	2,141.1	5,011.4
Age-adjusted comparative prevalence of IGT, %	2.8	5.5

Table 1.3: Mortality Attributable to Diabetes (20-79y) in Bangladesh [8]

Age	**Year**	
	2011	**2021**
Deaths attributable to diabetes	144,443.0	75,617.0
Proportion of diabetes-related deaths in people under 60 y, %	-----	4.2%

©Daffodil International University

1.3 Drugs available for treatment of Diabetes

The survival rate of a patient with type 1 diabetes Exogenous insulin, however most type 2 diabetics do not require exogenous insulin to survive; rather, many require exogenous replenishment of their endogenous secretion to attain optimal health. Seven distinct types of oral antidiabetic medications are currently available for the treatment of type 2 diabetes in the United States: Biguanides, thiazolidinediones, α-glucosidase inhibitors, bile acid binding sequestrant, amylin analog, meglitinides, and D-phenylalanine derivatives are among the drugs that have been shown to cause insulin secretagogues. Because they have been around the longest, biguanides and sulfonylureas are the most often used traditional therapies for type 2 diabetes. Meglitinides and D-phenylalanine derivatives are novel classes of fast-acting insulin secretagogues that can be used as an alternative to short-acting sulfonylureas. Insulin secretagogues cause beta cells to emit more insulin. Biguanides prevent the liver from producing glucose. The thiazolidinediones reduce insulin resistance.

Incretin-based medications control post-meal hyperglycemia excursions by increasing insulin release and decreasing glucagon secretion. The amylin analog also reduces post-meal glucose levels and appetite. Alpha-glucosidase inhibitors reduce the digestion and absorption of starches and disaccharides. Although the exact method by which bile acid sequestrant lowers blood sugar levels is unknown, it is believed to be related to a decrease in the quantity of glucose that the liver produces. [2]

1.4 Limitations of current antidiabetic drugs [9]

The major limitations of current anti-diabetic drugs are following:

- Hypoglycemia, weight gain, lipodystrophy, and infrequently, exogenous insulin.
- Sulfonylureas: hypoglycemia and weight gain
- Glitinides: hypoglycemia
- Biguanides: lactic acidosis (in rare cases), gastrointestinal problems; not recommended in cases of alcoholism, hypoxic/acidotic conditions, congestive heart failure (CHF), or compromised renal or hepatic function.
- Alpha-glucosidase inhibitors should not be utilized in patients with compromised renal or hepatic function or intestinal abnormalities resulting from gastrointestinal complaints.

- Agonists of the glucagon-like polypeptide-1 (GLP-1) receptor have been linked to anorexia, mild weight loss, nausea, headaches, vomiting, and pancreatitis.
- Dipeptidyl peptidase-4 (DPP-4) is inhibited by the following conditions: upper respiratory infections, pancreatitis, headaches, rhinitis, and allergic reactions.
- Amylin analog: anorexia, headache, nausea, and low blood sugar
- Acid sequestrant bubbling: flatulence, dyspepsia, constipation
- Thiazolidinediones:
    - Pioglitazone: contraindicated in women with bone fractures, CHF, hepatic disease, bladder cancer, fluid retention, edema, anemia, weight gain, and retinal edema.
    - Women who shouldn't have CHF but use rosiglitazone have the risk of developing hepatic illness, fluid retention, anemia, weight gain, and bone fractures.

1.5 Inflammation:

The term "inflammatory response" describes the complex biochemical response of the body to pathogens, injured cells, or irritants. [10] It may be understood as a defensive reaction involving immune cells, blood vessels, and molecular mediators that aims to remove the primary cause of cell damage, removing shock from wounded tissues and necrotic cells, and starting the healing process.
The main signs of inflammation include heat from stimulation, pain from substances that excite nerve ends, such as bradykinin and histamine, oedema from fluid accumulation, redness from increased blood flow to the area, and loss of function from insensitivity of the nerve endings. An acute or chronic inflammation might result from the duration of the stimulus. The body's initial reaction to damaging stimuli is acute inflammation, which often lasts for three months or more. Local immune cells such mast cells, dendritic cells, histiocytes, kupffer cells, and macrophages are responsible for acute inflammation in tissue injury. Due to the short lifespan of most mediators, this process terminates when the stimulus is removed or they sustain damage. [10]

Prolonged acute inflammation caused by stimuli, non-degradable microbes, persistent foreign substances, and the immune system can progress into chronic inflammation, which can last up to six months. Fibroblasts and mononuclear cells (monocytes, macrophages, lymphocytes, and plasma cells) are its defining characteristics. Hydrolytic enzymes, growth factors, reactive oxygen species (ROS), and cytokines (IFN-Y) are the primary mediators, and it begins slowly. Fibrosis, necrosis, and tissue destruction are the final results. More research is necessary to determine the molecules that may be able to treat this condition since inflammatory processes are connected to a number of ailments, including cancer, rheumatoid arthritis, asthma, type 2 diabetes, chronic inflammatory bowel diseases, and neurological problems. [10, 11]

1.5.1 Causes of inflammation [13, 14, 15]

A great many things can lead to inflammations. The most common ones are as follows:

• Pathogens, or microorganisms, including bacteria, fungi, and viruses

• Cuts or injuries from foreign objects (like a thorn in your finger) that are on the outside.

• Chemical or radiation effects

Medical conditions and inflammatory disorders are sometimes designated with a "-it is" suffix. As an example:

• Cystitis: an inflammation of the bladder; • Bronchitis: an inflammation of the bronchi

• Otitis media, an infection of the middle ear

• Dermatitis: a skin inflammatory disease

1.5.2 Inflammation can cause chronic disease too [13, 14, 15]

Inflammation can also be the cause of chronic illnesses like rheumatoid arthritis, which causes multiple joints throughout the body to be constantly inflamed, and psoriasis, a skin disorder that lasts a lifetime, also ulcerative colitis.

1.7 Anti-inflammatory Drugs:

NSAIDs

The "cyclo-oxygenase" (COX) enzyme family is the target of all the medications in the broad class of nonsteroidal anti-inflammatory drugs, or NSIADs. All of the drugs in the large class of NSIADs are intended to inhibit the "cyclo-oxygenase" (COX) enzyme family. Since many tissues contain COX, inhibiting it lowers the quantity of "prostaglandins" in those tissues. Arachidonic acid is metabolized by the COX enzymes to produce prostaglandins, which are lipid autacoids. In the body, they have a wide range of functions, including causing inflammation, vasodilatation, and preservation of local homeostasis. The many COX enzymes also play important physiological roles. For example, studies have demonstrated that COX-1 protects the kidneys and stomach epithelial cells, whereas COX-2 is associated with labor and ovulation.

COX-2 becomes more active when inflammation first appears, although COX-1 is more important for the regular operation of organs like the stomach. Because most non-selective NSAIDs block both COX-1 and COX-2, taking them for extended periods of time can have unfavorable effects. NSAIDs that specifically block COX-2 are referred to as "COX-2 selective inhibitors" because they reduce the risk of certain side effects associated with NSAID use. Still, there aren't many NSAIDs with this quality. [11]

Analgesic Drugs

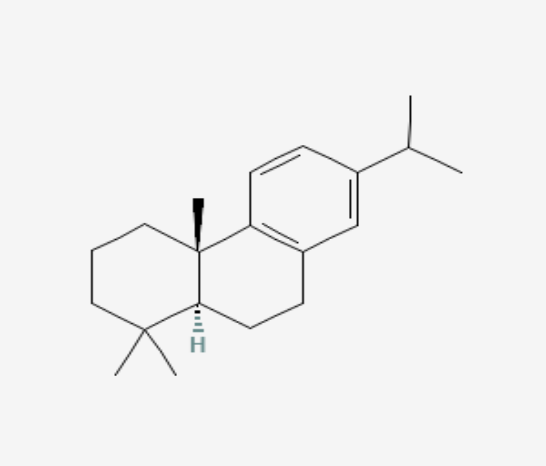
Additionally, since discomfort is typically accompanied by inflammation, medication administration is essential. The opioids are without a doubt the most effective analgesics. Any medication that interacts with the opioid receptors in the nervous system is considered an opioid. These receptors are used by endorphins, which are naturally occurring chemicals that share chemical similarities with opioids used to treat pain. Opioids come in a variety of forms, however due to specific limitations, their use in patient care is restricted. [11]

Corticosteroids

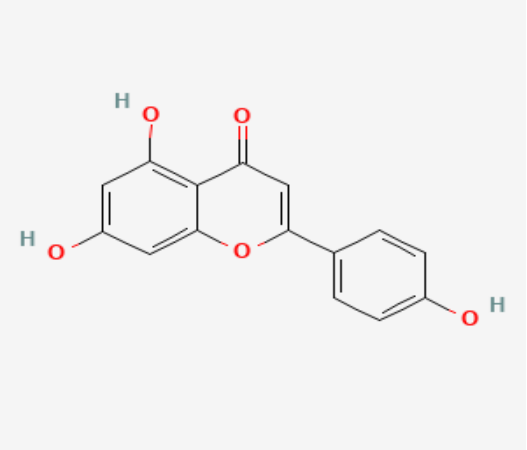
Mineralocorticoids and glucocorticoids are the two types of corticosteroids that are now recognized. Glucocorticoids are mostly used as anti-inflammatory medications. Regardless of the underlying cause of the inflammation, glucocorticoids are effective anti-inflammatory medications because they inhibit the production of lipocortin-1, sometimes referred to as annexin-1. Lipocortin-1 inhibits phospholipase A2, preventing the formation of leukotrienes and prostaglandins and releasing less arachidonic acid as a result. In patients with adrenal insufficiency, low dosages of glucocorticoids may also be administered. Inhaled glucocorticoids are the second-line treatment for asthma; significantly larger dosages of oral or inhaled glucocorticoids are used to diminish a variety of autoimmune, inflammatory, and allergy illnesses. [15, 16]

©Daffodil International University

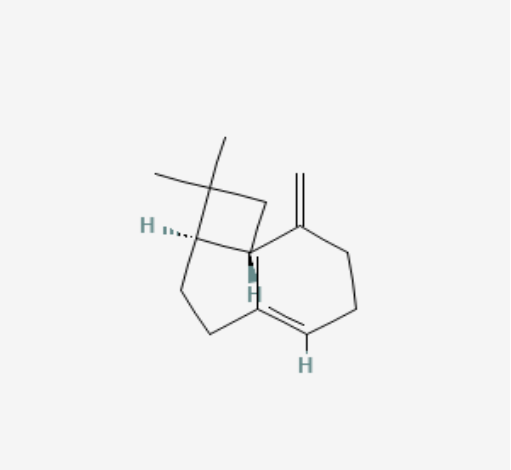
Figure 1.1: Anti-inflammatory constituents' [78]



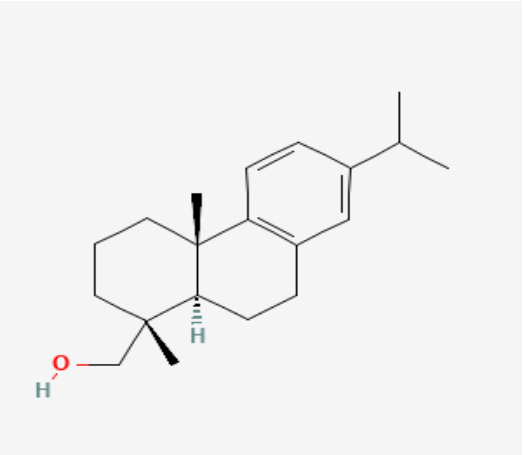
Abietatriene (PubChem CID: 6432211)



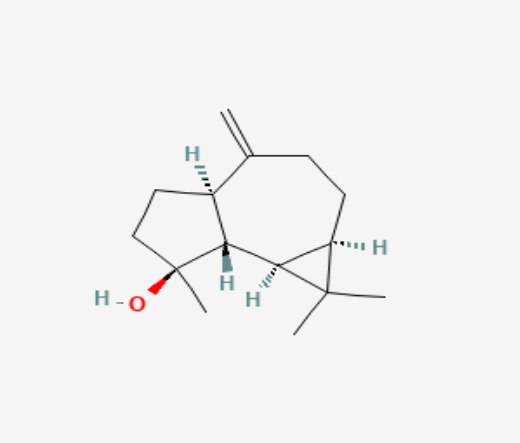
Apigenin (PubChem CID: 5280443)



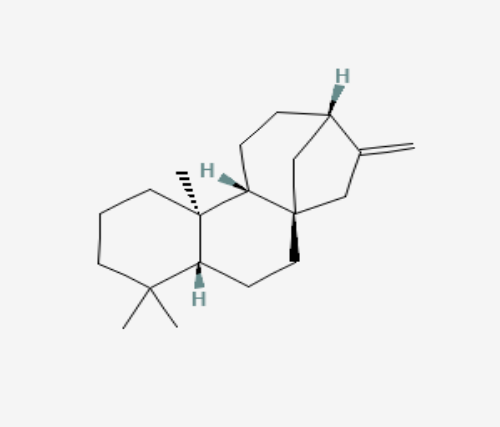
Caryophyllene (PubChem CID: 5281515)



Dehydroabietinol (PubChem CID: 15586718)



Spathulenol (PubChem CID: 92231)



Phyllocladene (PubChem CID: 44559813)

©Daffodil International University

1.8 Side effects of anti-inflammatory drugs

1.9 Gastro-intestinal Side-Effects of Non-steroidal Anti-inflammatory Drugs

Non-steroidal anti-inflammatory drugs, or NSAIDs, cause major injury to the gastrointestinal (GI) tract. Topical irritants that cause damage to the epithelial barrier and cyclo-oxygenase (COX), of which the mucosa mostly produces the COX-1 isoform, are the main causes of stomach injury. Nitric oxide (NO) donors and synthetic prostanoids, two mucosal-protecting agents, as well as antisecretory medications, aid in reducing this harm. Drugs designed to reduce topical irritation or those containing protective agents, as NSAIDs containing NO and CINODs (cyclo-oxygenase-inhibiting NO-donating drugs), reduce mucosal damage. In addition to inhibiting COX, NSAIDs appear to induce harm to the small intestine by the translocation of native bacteria, stimulation of NO synthase, and generation of the cytotoxic chemical peroxynitrite. Coxibs, or COX-2 selective drugs, have proven to be successful in treating NSAID-induced gastrointestinal damage thus far. These compounds do not alter the naturally protective prostanoids that COX-1 produces in the gut; instead, they block the generation of prostanoids in areas of inflammation. The clinical outcomes of employing the second generation of coxibs and the more current NO NSAIDs are still pending. [17]

©Daffodil International University

1.10 Cardiovascular Adverse Effects of Anti-Inflammatory Drugs [18, 19, 20, 21, 22, 23, 24, 25, 26]
Aspirin, glucocorticoids (GCs), and cyclooxygenase-2 (COX-2)-selective inhibitors—often referred to as coxibs—are examples of anti-inflammatory medications. Non-selective non-steroidal anti-inflammatory medications (NSAIDs) are NSAIDs. These are often recommended medications for a variety of musculoskeletal disorders, including inflammatory rheumatic illnesses and osteoarthritis. Among the anti-inflammatory drugs that do not pose a threat to the cardiovascular system is aspirin. Widespread concerns have led to a reevaluation of the benefit/risk ratio of NSAIDs, especially coxibs, because it has been demonstrated that they increase the risk of cardiovascular disease. There is inconsistent evidence about the potential for GCs to cause cardiovascular problems.

Table 1.4: The relationship between NSAIDs and coxibs and cardiovascular events.

Drug	Design	Treatment	Outcome	Reference
Rofecoxib	RCT risk of MI	Naproxen	↑ risk of MI	VIGOR trial Bombardier et al. 2000 [6]
	RCT	Placebo	↑ CV events (MI+strokes) RR 1.92 (1.19-3.11)	APPROVE study Baron et al. 2006 [14]
	Case-control study	Celecoxib Naproxen	↑ risk of MI OR 2.72 (1.24-5.95) OR 3.39 (1.37-8.40)	Kimmel et al. 2005 [10]
Celecoxib	RCT	Placebo	↑CV events Risk ratio 2.6 (1.1-6.1) for 200mgX2/d Risk ratio 3.4 (1.5-7.9) for 400mgX2/d	APC trial Bertagnolli et al. 2006 [20]
	Pooled analysis of RCTs	Placebo	↑ CV risk HR 1.1 (0.6-2.0) for 400 mg/d HR 1.8 (1.1-3.1) for 200mgX2/d HR 3.1 (1.5-6.1) for400mgX2/d	Cross Trial Safety Analysis -Solomon et al. 2008 [25]
Valdecoxib/ Parecoxib	RCT	Placebo	↑ CV events Risk ratio 3.7 (1.0-13.5) for combination Risk ratio 2.0 (0.5-8.1) for valdecoxib alone	Nussmeier et al. 2005 [29]
	RCT	Placebo	CV events	Ott et al. 2003 [28]
NSAIDs	Case-crossover analysis		↑ CV death Ibuprofen: HR 1.50 (1.36-1.67) Diclofenac: HR 2.40 (2.09-2.80)	Gislason et al. 2006 [24]
	RCT	Placebo	↑ deaths, strokes, MI, HF HR 1.63 (1.04-2.55)	Naproxen ADAPT study [42]

©Daffodil International University

1.11 Nature as source of medicine

The master chemist, nature, created an almost infinite diversity of molecular things. It offers an unlimited source of novel chemotypes and pharmacophores, as well as other helpful bioactive compounds for drug creation and scaffolding for amplification into potent treatments for a variety of sickness indications. Natural goods have been the cornerstone of traditional medical treatments throughout for millennia, playing a key role in both history and society.

Initially, a wide range of materials, including marine life, terrestrial plants, terrestrial microorganisms, and terrestrial vertebrates and invertebrates, were used to make all medicines and therapeutic compounds. Plants accounted for the majority of these remedies. Although herbal medicine formulations including bioactive natural products have been used for hundreds or even thousands of years, it wasn't until the 19th century that these ingredients were utilized as distinct and defined chemicals in modern medication discovery and development. It is common knowledge that natural substances played a crucial role in the development of modern medications, especially those with antibacterial and anticancer properties. Despite the growing popularity of synthetic products because of their low production costs, time effectiveness, ease of quality control, strict regulations, and immediate effects, over 80% of the world's population still lives in developing countries and relies on natural products because they have been shown to be safe and effective.

However, it wasn't until it was discovered that bacteria were the root cause of many infectious diseases that there was a genuine push for the development of therapeutic medicines, both natural and synthetic. Significant progress in synthetic organic chemistry and biochemistry coincided with the discoveries in medical microbiology, propelling the field of therapeutic drugs even farther. [27, 28]

1.12 Traditional medicines

Since the dawn of time, people have used natural products—plants, animals, microorganisms, and sea organisms—in medicine to both prevent and treat ailments.
Fossil evidence suggests that people have been using plants as medicine for at least 60,000 years. It must have been extremely difficult for prehistoric humans to employ natural materials as treatments. When hunting for food, it's highly likely that early humans regularly consumed poisonous plants. This might have caused toxic reactions including vomiting, diarrhea, comas, or even death. However, this was also how early humans discovered edible materials and homeopathic cures. Subsequently, people created churches, learned how to brew wine, created fire, advanced technology, and got skilled at creating new narcotics. [29, 30]

Traditional medicine (TM), the oldest medical specialty in the world, is used to treat and prevent both physical and mental illnesses. In the past, several societies have developed a variety of useful medicinal approaches to treat a broad spectrum of ailments, some of which are lethal. Today, TM is still widely used in many nations. Other titles for it include complementary and alternative medicine and ethnic medicine. [30, 31]

©Daffodil International University

1.12.1 Definition of traditional medicine

Traditional medicine is defined by the World Health Organization as all knowledge, skills, and methods derived from the beliefs, practices, and knowledge innate to many cultural contexts. These techniques—which may or may not be explicable—are employed to prevent, identify, treat, or lessen bodily and mental disorders in addition to preserving general health.
In traditional medicine, treatments including minerals, animal parts, or herbal cures are categorized as pharmaceutical therapy. Non-medication treatments include therapies including acupuncture, manual therapy, spiritual therapy, and others that are predominantly provided without the use of medication.
Natural goods and conventional medical treatments are quite significant. In certain parts of the world, traditional medical practices including Ayurveda, Unani, Traditional Chinese medicine, Kampo, and Traditional Korean medicine have been practiced and developed into well-organized, controlled medical systems. [30, 31]

1.12.2 Ayurveda [30]

Ayurveda originated in India, a growing nation; its roots are in the pre-Vedic periods (4000 BC–1500 BC). Characteristics of the Theory or Use. Ayurveda uses natural ingredients to eradicate the root cause of sickness in order to restore harmony.

Living a healthy lifestyle is the aim of Ayurveda in order to avoid imbalance and unnecessary suffering. Several Ayurvedic treatments blend several herbs in a certain ratio to reduce toxicity and maximize therapeutic effect.

Current Role or Status

Over 400,000 Ayurvedic practitioners are registered. The practice, standards, and research and development of Ayurveda are governed by an official organization under the Indian government.

Modern Research:

The knowledge of Ayurvedic medicine's pharmacologically active constituents and their medicinal usefulness has been growing.

1.12.3 Unani medicine [30]

Origin and Developing Nation

India: The 2500-year-old Greco-Arabic medicine is the ancestor of unani medicine, which developed during the Arab era.

Characteristics of Theory or Application

It treats the individual as a whole, mind, body, and soul. Unani contends that the human body is a one, harmonious whole consisting of four basic parts, each with a unique emperament. Temperament is a reflection of an individual's natural personality and physical characteristics. Temperamental variations make the human body susceptible to a wide spectrum of illnesses.

Current Role or Status

Unani has recently received formal approval in India as a way to treat people's medical needs. Unani is an accepted alternative medical system by the WHO. Unani is one of the most important traditional medicinal systems.

Modern Research: Several bioactive components of mangrove plants, used in Unani medicine, have been isolated.

1.12.4 Traditional Chinese medicine (TCM) [30]

China is a long-standing, ancient country that is now under development. These are some of its traits:

The theories of Wuxing and Yinyang form the basis of TCM. A TCM formula is made up of many drugs that complement each other in a beneficial way. A traditional formula consists of four parts: the assistant, servant, minister, and monarch, according to their places in the formula.

Current Role or Status:

Both Western and TCM medications are available at every stage of the healthcare system and are paid for by both public and private insurance. Most traditional hospitals offer TCM services to inpatients as well as outpatients. TCM is becoming more and more popular, accepted, and well-known on a global scale.

Modern Research:

The study of TCM pharmacology has come a long way. In recent decades, several TCM medicinal compounds and compound-based medicines have been discovered. Many efforts have been made to understand the molecular mechanisms of TCM.

1.13 Contribution of plants in modern drugs

Most people still favor herbal medicines over conventional ones, despite the fact that medicinal plants are essential to health care and a major supply of raw materials for both conventional and traditional medicine formulations. Their increased focus was influenced by their effectiveness, cultural preferences, and the growing cost of modern treatments. [32, 33]

There are several ways that plants have helped create contemporary synthetic medications and treatments; a few of these are outlined below [32]:

1. When the structures of physiologically active chemical compounds are found in plant sources, scientists are often motivated to design better or similar semi-synthetic molecules.
2. Chemicals derived from plants with established biological functions are frequently structurally modified to create synthetic drugs with therapeutic efficacies comparable to or greater than those of the natural products.
3. For use as efficient medicines, chemists commonly synthesize a wide range of derivatives and analogues of plant components with better or equivalent pharmacological effects and therapeutic properties.

The WHO estimates that 80% of people on the planet still receive their medical care through conventional means. Humans have been using plants as medicine for 60,000 years. Between 35,000 and 70,000 different plant species have been investigated for possible medicinal use.
Between 1959 and 1980, 25% of all prescriptions written in community pharmacies in the US contained plant extracts or active ingredients. Only around 11% of the 252 essential and basic medications that the WHO is currently researching are derived from flowering plants. In the world, over 25% of prescription drugs come from plants. A 2016 evaluation by the Bangladesh Foreign Trade Institute (BFTI) found that 75% of our people get their basic medical care from herbal treatments. [30, 35, 36, 37]

1.14 Drugs development process from medicinal plants

Since drug creation is a difficult, time-consuming, expensive, and labor-intensive process, comprehensive phytochemical analysis, pharmacological screening, and clinical testing are required in order to manufacture pharmaceuticals from medicinal plants. The process of turning plants into medications involves several processes. Which include [32]:
1. Selecting and accurately recognizing the right medicinal herb.
2. Extracting using the right solvent or solvents.
3. Determining the biological activity present in the crude extract and establishing a bioassay system that enables the separation of active fractions from inactive ones.
4. The finest chromatographic methods should be used to fractionate crude extract, separating the active fractions and physiologically assessing each fraction.
5. Chromatography and/or other suitable techniques to separate the active compounds; repeated chromatography and toxicity testing are then used to purify the separated substances.
6. The chemical structures of the pure compounds are established using diverse physicochemical approaches, and their biological activity is ascertained using a variety of pharmacological and toxicological investigations.
7. manufacturing of the medication within the authorized dose ranges. The following methods might be used to enhance medications made from therapeutic plants:
I. Improvement of the potency and effectiveness of the recently developed drugs by structural changes to the biologically active ingredients.
II. Synthesizing and altering the substances to affect certain biological processes in humans or other organisms.

1.15 Overview of *Hyptis suaveolens (L) Poit*

Hyptis suaveolens (L.)Poit

Synonyms: *Mesosphaerum suaveolens* (L.), chia, pignut, or chan

Common names: *Bilati tulas(Bengali), Horehound, Pignut, Wild spikenard, Gross Baume, Hyptis à odeur(French), Alfavaca- brava, jangli tulsi(Hindi), bhustrena, darp tulas, jungli tulas(Marathi), sirna tulasi(Telugu), Ganga tulasi(Oriya), bhustrena(Sanskrit) [41]*

In India, Hyptis suaveolens is a highly prevalent plant.. Gathered by the side of the road, the plant may be found growing beside waste sites, riverbanks, and coastal places. Indians used to refer to it as "Chan/Wilayati tulsi". [38]

It is stated that tea prepared from the roots of H. suaveolens may cure women of their "diseases" and cleanse the blood.In many regions, it has been utilized as a medicinal tea. [39]
Plants generate unique secondary metabolites, which gives them pharmacognostic qualities. There are many distinct active metabolites found in different stages of a plant's life cycle, but only around 300 species are employed globally in the food, pharmaceutical, cosmetic, and perfume industries. Out of all the plant species on Earth, little over 300 are employed globally in the food, cosmetics, pharmaceutical, and fragrance industries. Plant foods contain components that have been researched for their biological effects, such as their anti-oxidant, anti-mutagenic, antibacterial, and anti-carcinogenic qualities: flavonoids, saponins, tannins, phenolics, terpenoid, alkaloid, and glycosides.

According to Walker, plants' medicinal properties could be able to counteract the harmful effects of microbes. The buildup of photochemicals, some of which have insecticidal, antibacterial, antifungal, and other qualities, near the locations of plant infection [40].Semiarid and subtropical climates are ideal for the plant's growth.

Figure 1.2: *Hyptis suaveolens (L) Poit*

©Daffodil International University

Leaves

In contrast, the length of the elliptical leaves varies from 2.5 to 10 cm. Amazing color is often seen in leaves, especially along the edgesLeaves are used as a carminative, cutaneous parasite stimulant, and [45]

Flower

Hyptis suaveolens has additional flowers with a tall stem and a hairy calyx. The blossom measures around 4 mm in length.

Bloom The blossom has a dark, glandular hue. Blooms: The wide, two-lobed top lip is complemented by dark, beautiful lines at the base of the mauve, two-lipped corolla. Four stamens make up the flower, and a lot of pollinators fertilize it to create a lot of seed.[42]

Stem

The gland patches on the satiny, four-angled stems of the factories resemble long hairs.

Seed

The seeds are mucilaginous and flat. The lengths of fruit, or nutlets, vary from 1.2 to 1.5 mm [42]. Dimorphic in nature, the seeds have a chine purr that aids in dispersing them and a somewhat recessed end. Seed size (wuff91973) reflects this dimorphic, and there is evidence of an inverse link between light need and seed size. This allows seeds to grow in a temperature range of 10 to 40 degrees Celsius, with 25 to 30 degrees Celsius being the best range for growth (Felippe et al., 1983). It also gives seeds good germination conditions throughout a temperature range. [42]

Figure 1.3: *Hyptis suaveolens (L) Poit* seed

©Daffodil International University

1.15.1 Ethnomedicinal use of *Hyptis suaveolens (L.) Poit*

Hyptis Sueveolens is used as a mycotoxic exertion having anti-tumorigenic (Mudgalet al., 1997) and anti-cancerous (Pearzada, 1997) characteristics against the fungus Candida albicia. [42]

Splint extracts are said to be effective in treating hemorrhoids, abscesses, and pimples in hyptis literature. The factory is regarded as a goad, lactogogue, anodynese, and carminative in India. While infusions are used to treat uterine infections, splint juice is used to reduce cramps and stomach discomfort. [43] You may consume the factory-produced shot coverings or use them as seasoning. What makes the factory effective as an insectifuge is its distinct sweet aroma, which is similar to that of mint or thyme. As implied by its English name, backcountry tea, H. suaveolens is employed as a suitable tea infusion cover in West Africa. Its qualities include anti-parasitic, carminative, lactogenic, sudorific (increases or produces sweat), and anticatarrhal. Crude splint extract is also used to relieve constipation and stomachaches.

Smothers composed of dried leaves keep insects out of stored grains and discourage mosquitoes. Leaves and outgrowths are thought to have antispasmodic qualities and are utilized in antirheumatic medications. [44]

1.15.2 Phytoconstituents of *Hyptis Suaveolens (L) Poit*

Table 1.5: Phytoconstituents of *Hyptis Suaveolens (L) Poit* [77]

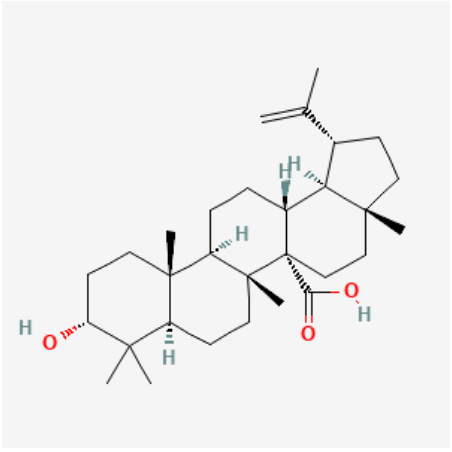
Sl. No.	Plant Part	Class	Name of Compound	PubChem CID
1	Leaves	sesquiterpenoid	Suaveolol	11975519
2	Leaves, stem, and flowers	abietane diterpenoid	Dehydroabietinol	15586718
3	Leaves (essential oil)	monoterpene	Sabinene	18818
4	Leaves (essential oil)	cyclic ether and monoterpenoid	Eucalyptol/1,8-Cineole	2758
9	Leaves and stem	phytosterols	β-Sitosterol	222284
10	Leaves	trihydroxyflavone	Apigenin	5280443
11	Leaves	antioxidant flavonoid	Catechin	9064
12	Flowers	sesquiterpene lactones	Ovatodiolide	38347030
13	aerial part	monoterpene	Myrcene	31253
14	aerial part	terpenoid	beta-Bisabolene	10104370
15	aerial part	phenole	Eugenol	3314
16	aerial part	monoterpene	p-Cymene	7463
17	aerial part	terpenes	Humulene	5281520
18	aerial part	terpineol	4-Carvomenthenol	11230
19	flower	monoterpene	beta-Pinene	14896
20	flower	terpenoid	Spathulenol	92231
21	leaf	monoterpene ketone	Camphor	2537
22	leaf	monoterpene	alpha-Phellandrene	7460
23	leaf	sesquiterpene	Bicyclogermacrene	13894537
24	leaf	terpene	gamma-Terpinene	7461
25	leaf	terpene	Terpinolene	11463
26	leaf	terpene	trans-Sabinene hydrate	12315151
31	leaf	monoterpene	Limonene	22311
32	leaf	terpenes	alpha-Copaene	19725

©Daffodil International University

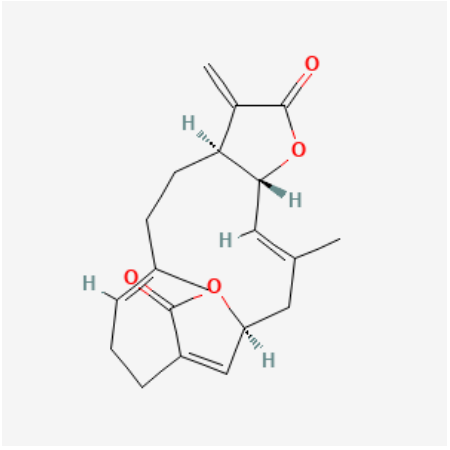
33	leaf	monoterpene	p-Mentha-1,3,8-triene	176983
34	flower	terpenes	(1R)-2-methyl-5-propan-2-ylbicyclo[3.1.0]hex-2-ene / alpha-thujene	637518
35	aerial part	alcohol	2-(4-Methylphenyl)propan-2-ol	14529
36	aerial part	terpenoid	Viridiflorene	10910653
37	roots	triterpene	3β-hydroxylup-20(29)-en-27-oic acid	445927722
38	aerial part	alcohol	4-Isopropylbenzyl alcohol	325
39	aerial part	Terpine	alpha-Terpinene	7462
40	flower	monoterpenoid	Linalool	6549
41	flower	monoterpenoid	Citronellol	8842
42	flower	sesquiterpenoid	(-)-alpha-Cadinol	10398656
43	flower	diterpene alcohol	Phytol	5280435
44	flower	monoterpenoid	Fenchone	14525
45	flower	alcohol	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-, trans-	122484
46	leaf	alcohol	1-Octen-3-OL	18827
47	leaf	monoterpene	Tricyclene	79035
48	leaf	diterpene	Abieta-7,13-diene	443470
49	leaf	sesquiterpene	Rimuene	12314971
50	leaf	diterpenoid	Neoabietinol	443476

©Daffodil International University

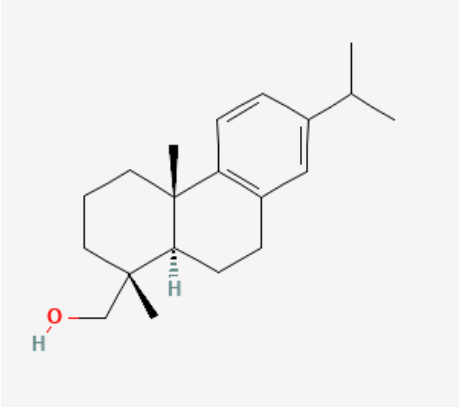
Figure 1.4: Chemical Structure



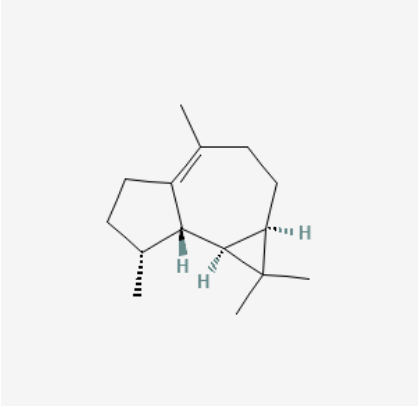
3β-hydroxylup-20(29)-en-27-oic acid **PubChem:** 445927722



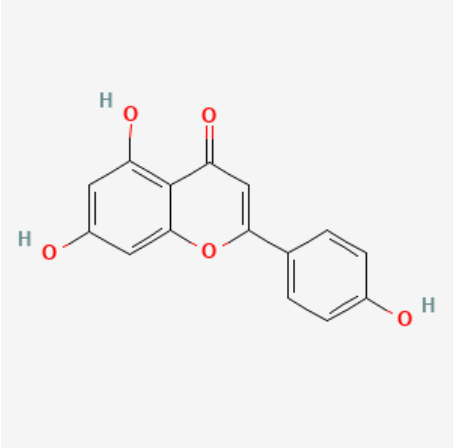
Ovatodiolide **PubChem CID:** 38347030



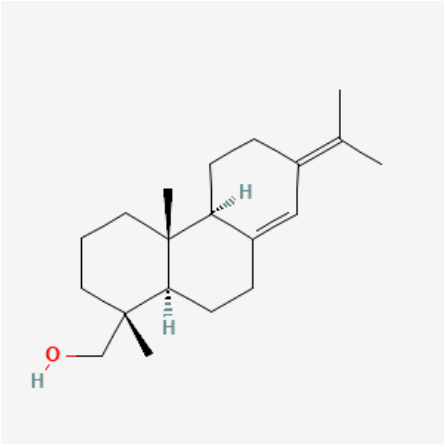
Dehydroabietinol **PubChem:** 15586718



Viridiflorene **PubChem:** 10910653

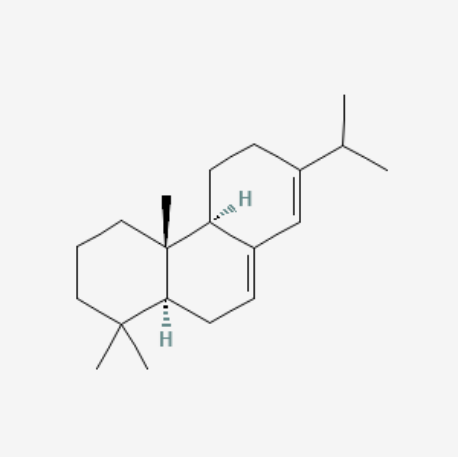


Apigenin **PubChem:** 5280443

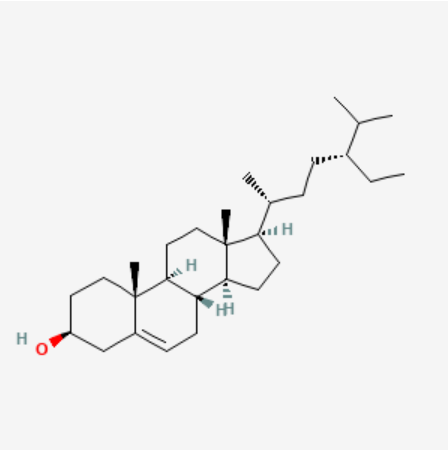


Neoabietinol **PubChem:** 443476

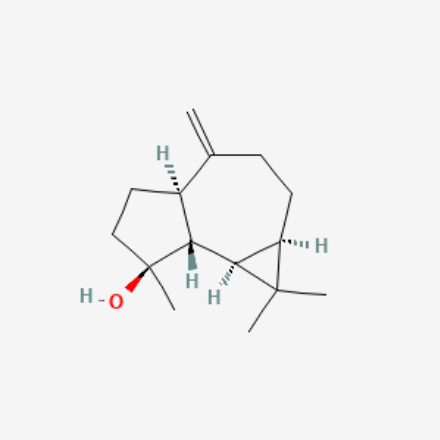
©Daffodil International University



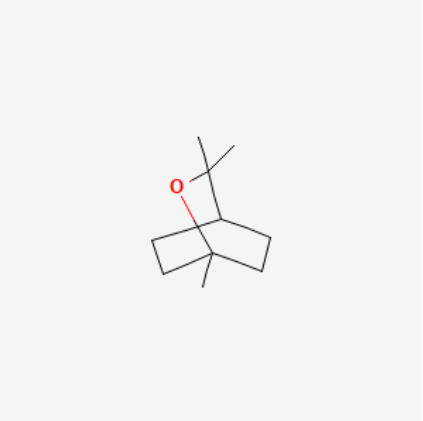
Abieta-7,13-diene **PubChem CID:**443470



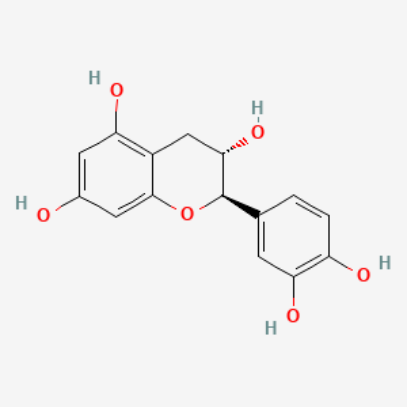
β-Sitosterol **PubChem:** 222284



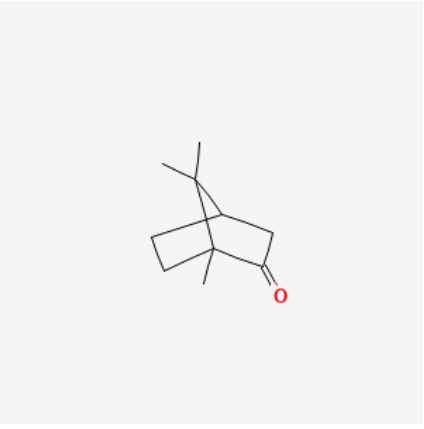
Spathulenol **PubChem CID:** 92231



Eucalyptol **PubChem CID:** 2758



Catechin **PubChem CID:** 9064



Camphor **PubChem CID:** 2537

©Daffodil International University

1.16 Major ligands for Anti-inflammatory activity

Table 1.6: Major ligands for Anti-inflammatory activity [78]

Sl. No.	PubChem CID	Ligands
1	3033	Diclofenac
2	222284	Beta-Sitosterol
3	10398656	alpha-cadinol
4	19725	Alpha-copaene
5	5280443	Apigenin
6	10104370	Beta-bisabolene
7	13894537	Bicyclogermacrene
8	2537	Camphor
9	8842	Citronellol
10	15586718	Dehydroabietinol
11	3314	Eugenol
12	5281520	Humulene
13	443476	Neoabietinol
14	38347030	Ovatodiolide
15	5280435	Phytol
16	12314971	Rimuene
17	18818	Sabinen
18	11975519	Suaveolol
19	11463	Terpinolene
20	79035	Tricyclene
21	18827	1-Octen-3-OL
22	11230	4-Carvomenthenol
23	443470	Abieta-7,13-diene
24	14525	Fenchone
25	7461	gamma-Terpinene
26	6549	Linalool
27	31253	Myrcene
28	92231	Spathulenol
29	7460	alpha-PHELLANDRENE
30	14896	beta-Pinene
31	637518	alpha-Thujene
32	7463	4-Isopropyltoluene
33	5281515	β-Caryophyllene
34	6432211	Abietatriene
35	44559813	Phyllocladene

©Daffodil International University

1.17 Major ligands for Anti-diabetic activity

Table 1.7: Major ligands for Anti-diabetic activity [78]

1	77999	rosiglitazone
2	18827	1-Octen-3-OL
3	11230	4-Carvomenthenol
4	7463	4-Isopropyltoluene
5	443470	Abieta-7,13-diene
6	10398656	Alpha-cadinol
7	19725	Alpha-copaene
8	7460	alpha-PHELLANDRENE
9	637518	alpha-Thujene
10	5280443	Apigenin
11	222284	Beta Sitosterol
12	10104370	Beta-bisabolene
13	14896	beta-Pinene
14	13894537	Bicyclogermacrene
15	2537	Camphor
16	8842	Citronellol
17	15586718	Dehydroabietinol
18	3314	Eugenol
19	14525	Eugenol
20	7461	gamma-Terpinene
21	5281520	Humulene
22	6549	Linalool
23	31253	Myrcene
24	443476	Neoabietinol
25	38347030	Ovatodiolide
26	5280435	Phytol
27	12314971	Rimuene
28	18818	Sabinen
29	92231	Spathulenol
30	11975519	Suaveolol
31	11463	Terpinolene
32	79035	Tricyclene
33	7463	4-Isopropyltoluene

©Daffodil International University

1.18 Modern drug discovery, design, and development [46]

In recent years, there has been a significant shift in medicinal chemistry. We now know a great deal more about the cellular and molecular functions of the body thanks to the rapid advancement of the biological sciences.

As a result, most research projects in both the academic and pharmaceutical domains now begin with the discovery of a physiological target that is appropriate and the development of a medication to interact with it. Understanding the target's makeup, function, and potential drug interactions is essential to using this method. The stages of drug design, development, and discovery often go like this:

Drug discovery: finding a lead

- Choosing a disease
- Choosing a drug target
- Identifying a bioassay
- Finding a _lead compound‘
- Isolation and purification of the lead compound if necessary
- Determination of the structure of the lead compound if necessary

Drug design:

- Identifying structure–activity relationships (SARs)
- Identifying the pharmacophore
- Improving target interactions (pharmacodynamics)
- Improving pharmacokinetic properties

Drug development:

- Patenting the drug
- Carrying out preclinical trials (drug metabolism, toxicology, formulation and stability tests, pharmacology studies, etc.)
- Design a manufacturing process (chemical and process development)
- Carrying out clinical trials
- Registering and marketing the drug
- Making money

1.18.1 *In silico* molecular docking: Role in drug development

As the world develops, so do the diseases, which now cause more suffering than in the past owing to their increased severity and prevalence. For this reason, the discovery and development of pharmaceuticals is an essential subject in the life sciences.
Drug development is the process of evaluating a medication against a target that has been selected or found through drug discovery. However, all of this work fits within the category of modern drug development approaches.
The conventional approach to medicine research has been rather wide, risky, and wasteful (the cost of generating a new treatment is presently over US $800 million and rising at a pace of 7.4% annually). Ninety percent of the drugs that are discovered fail the rigorous trials and testing.
The present drug development pipeline makes extensive use of bioinformatics tools and techniques in a number of domains, including interaction, target identification44, toxicity testing45, etc. [47, 48, 49, 50]

All biological processes are fundamentally based on interactions between biomolecules. The upkeep of intricate networks of metabolic and regulatory interactions—which collectively comprise the activities of living things—requires these linkages. The primary scientific methods for comprehending these processes and identifying compounds that might be used as bioactive agents to alter and regulate them are experimental research and computer simulation and analysis. On the one hand, knowledge analysis of molecular interactions necessitates a basic comprehension of the molecules' three-dimensional shapes. However, building a global model of the activities within a live creature requires understanding of the specific molecular relationships. The amount of bio-molecular structural data is growing exponentially, which allows computer models for bio-molecular docking to be calibrated against a growing dataset.

Computational approaches are becoming a vital component of many drug development strategies, from hit detection to lead optimization and beyond. In these sorts of efforts, techniques such as ligand- or structure-based virtual screening are often used. The critical method of "docking" small molecules to protein binding sites was discovered in the early 1980s and is currently a burgeoning area of research. Molecular docking is an optimization problem where the objective is to discover the best-fit orientation of a ligand that binds to a target protein in order to predict the structure of the intermolecular complex that will form between two or more molecules. Because of its applications in medicine, the protein-ligand interaction is the most fascinating example. A little chemical called a ligand interacts with protein binding sites. Mutual conformations can lead to binding in a number of ways. These are referred to as binding modalities.

In the last several years, the process of finding new pharmaceuticals has changed from being a trial-and-error exercise to a complex process that makes use of a variety of computer-based tactics. Structure-based design uses the structures of known target proteins to identify new compounds that may have therapeutic uses.

©Daffodil International University

The two main categories of approaches are de novo design and docking. The former method produces new ligands that are tailored to the protein target, while the latter is used to assess if compounds that are already on the market have sufficient steric and chemical complementarities to the protein that is supplied.

Often employed in hit identification and lead optimization, molecular docking refers to computer techniques that "dock" tiny molecules into the structures of macromolecular targets and "score" their potential complementarity to binding sites. [51, 52, 53]

1.18.2 Molecular docking for structure based drug design

Docking is a crucial part of the rational design of drugs. Docking is a typical approach in virtual screening or lead optimization for drug screening and design.

Alongside the incredible advances in physics, chemistry, biochemistry, computers, information technology, and biochemistry, docking has evolved greatly as a technique and a powerful tool in drug screening, protein–protein interactions, and nanomaterial behavior since its start in the 1960s. In the field of computer-aided drug design (CADD), the use of technology to affix tiny chemicals onto macromolecules, particularly protein targets, is growing in popularity. In modern CADD, structure-based drug design is a critical component, and most big pharma companies have this section. Several commercial drugs are directly created using the CADD method.

Three eminent computer scientists were awarded the Chemistry Nobel Prize in 2013, drawing attention to the fact that docking approaches are unquestionably crucial scientific advancements for comprehending chemical molecules.

Viral lead discovery for synthetic drugs is facilitated by protein-ligand molecular docking and in silico analysis. Comprehending the three-dimensional structures of proteins facilitates the development of drugs that exhibit high binding affinity and specificity to the receptor sites of target proteins. This informatics-based approach can produce pharmaceuticals with higher efficacy, fewer side effects, and reduced toxicity in a far shorter amount of time and money than the traditional trial-and-error approach. [53, 54, 55]

1.18. 3 Binding interactions in molecular docking

It is challenging to simulate how a medication interacts with its receptor. The intermolecular interaction is mediated by a variety of forces, such as hydrophobic, dispersion, van der wall, hydrogen bonding, and electrostatic. Although hydrophobic interactions appear to be the main force behind binding, the degree of specificity in the binding is often controlled by hydrogen bonds and electrostatic interactions. The intermolecular interactions in the ligand-protein complex are difficult to model due to their several degrees of freedom. Moreover, it is yet unclear how the solvent affects the binding relationship. By choosing the lowest energy route, the method of docking a ligand to a binding site seeks to replicate the natural interaction between a ligand and its receptor.

In order to use computational methodologies for structure-based design, a few assumptions need to be met. Despite the difficulties of docking conformationally flexible ligands with receptors generally, there are straightforward ways for docking rigid ligands with rigid receptors and flexible ligands with rigid receptors. [53]

1.18.4 Application of molecular docking in drug design

The binding relationship between an enzyme protein and a small molecule ligand has the potential to either activate or inhibit the enzyme. Antagonism or agonism may arise from ligand binding if the protein is a receptor. Since most medications are tiny organic compounds, docking is most frequently utilized in the field of drug creation [53]:

- Hit identification: In order to efficiently search through massive databases of possible medications in silico and find molecules that have the best likelihood of binding to a target protein, docking and a scoring function are used.
- Lead optimization: Docking, also known as the binding mode or pose, can be used to anticipate the relative orientation and location of a ligand's binding to a protein. Therefore, this knowledge might be used to create stronger and more accurate analogs.
- Bioremediation: Protein ligand docking may also be utilized to anticipate contaminants that enzymes will be able to break down.

1.9 Study objectives

The use of medicinal plants to treat DM and Inflammation is popular, as herbal drugs are generally regarded as free of toxic effects. Moreover**,** the limitations of oral anti-diabetic and anti-inflammatory drugs urge to find new drugs for treatment of DM and inflammation. Therefore, the search for more effective and safer herbal hypoglycemic agents, anti-inflammatory agents and developing new anti-diabetic drugs, anti-inflammatory drugs with improved clinical profiles simultaneously have become an area of active research. [56]

So, this research study entitled **"Evaluation of anti-diabetic and anti-inflammatory activity of selective phytoconstituents from *Hyptis suaveolens (L.) Poit*: an *in silico* molecular docking and ADMET study"** was carried out to attain the following specific research objectives:

- Screening hypoglycemic activity and anti-inflammatory activity of *Hyptis Suaveolens* phytoconstituents.
- To predict the leads from modeled ligands for discovering and developing new potential oral anti-diabetic and anti-inflammatory drug through *in silico* molecular docking studies

1.19.1 Data collection

All the relevant data has been collected from two types of sources:

- Primary sources: Research database like Google Scholar, PubChem, IMPPAT, PubMed, Elicit.org
- Secondary sources: various publications like research articles, review papers, books,and websites.

1.19.2 Plant selection:

Several in-vivo/in-vitro studies have been done on Hyptis suaveolens (L.) Poit. However, in silico study not done yet. Therefore I decided to do work on this plant.

Table 1.8: In-vivo/in-vitro study on *Hyptis suaveolens (L.) Poit*

Plant part	Pharmacological acitvity (in vitro/in vivo)	In silico activity? (yes /no)	Chemical constituents	Reference
leaves	Cytotoxic activity (Prostate cancer)	No	Silver nanoparticles	79
leaves	against MCF-7 cell lines	No	chlorophore extracts	80
leaves	against MCF-7 cell lines	No	butanolic extracts	81
leaves	Anti-parasitic	No	diterpene (dehydroabietinol)	82
leaves	Anti-Plasmodium falciparum D10	No	13α-epi-dioxiabiet-8(14)-en-18-ol	83
leaves	potent antioxidant activity	No	methanolic extract	84
seeds	antioxidant action	No		85
aerial parts	neuroprotective potential	No	methanolic extract	86
leaves	anti-inflammatory potential	No	ethanolic extract	87
leaves	gastroprotective effect	No	suaveolol	88
leaves	antiulcer potential	No	ethanolic extract	89
leaves	antidiarrheal potential	No	ethanolic extract	90
leaves	antidiabetic activity	No	ethanolic extract	91
aerial parts	Hepatoprotective Effect	No	methanolic extract	92
aerial parts	To stop the growth of plasmodium falciparum strains that are resistant to and sensitive to chloroquine	No	Dehydroabietinol	93
aerial parts	antioxidant activity	No	methanolic extract	94
aerial parts	neuroprotective activity	No	methanolic extract	94
seed	antihyperuricemic	No	4,5-dicaffeoylquinic acid	95
seed	antihyperuricemic	No	methyl 3,5-dicaffeoylquinate	95
aerial parts	larvicidal activity	No	1,8-Cineole/Eucalyptol	96
leaves	Anti-inflammatory activity	No	suaveolol and methyl suaveolate	97

©Daffodil International University

1.20 Research protocol:

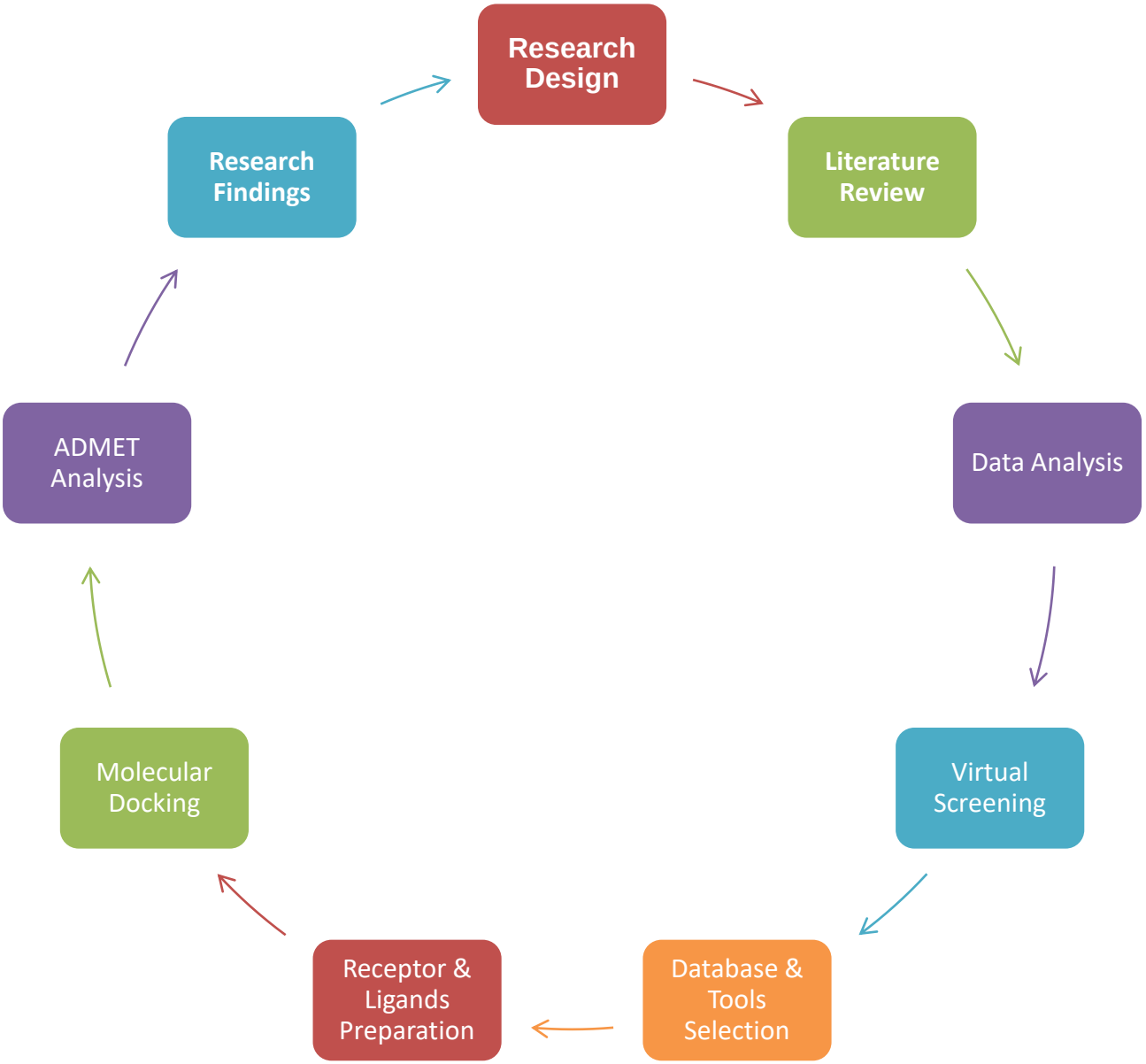


Figure 1.5: Research Protocol

Chapter 2: Literature Review

2.1 Biological activities of *Hyptis suaveolens (L.)*

2.1.1 *Antimicrobial Acitiviy*

Strong antibacterial activity against pathogenic Gram-positive and Gram-negative bacteria, such as Salmonella typhi, Pseudomonas aeruginosa, Lactobacillus planetarium, Escherichia coli, Vibrio vulnificus, Enterococcus fecalis, and Streptococcus fecalis, is demonstrated by the flavonoids and phenolic composites found in the essential oil painting of *H. suaveolens*. [57, 58] Strong antifungal effect against A. spergillus spp. (A. flavus, A. parasiticus, A. niger, A. ochraceus, A. fumigates), Saccharomyces cerevisiae, Mucor spp., Fusarium moniliforme, etc., is demonstrated by essential oil painting from H. suaveolens. [59]

By boosting hydroxyproline content, collagen deposit, and dry weight of granulation tissue, H. suaveolens has demonstrated the potential to heal fissures. Additionally, it showed that granuloma cells with antioxidant enzymes added might enhance the effort put into fracture healing and release innovative scavenging activity. [60]

Gram-negative bacteria (P. aerogenoides and E. coli) are less susceptible to lipophilic essential oil painting than Gram-positive bacteria because of their hydrophilic outer membrane, which shields them from the paint. [61].

2.1.2 *Anti-cancer Acitivity*

A condition known as cancer arises when cells in a certain bodily area multiply out of control. Terpenoids, such as sabinene, βcaryophyllene, trans-caryophyllene, Spatulenol, βspathulenol, β-elemene, γ-elemene, Rimuene, αhumulene, Eucalyptol, 1-8-cineole, etc., are the primary constituents of H. suaveolens' essential oil painting. The cancer cell line MCF-7, derived from mortal bone, has anti-cancer properties due to these chemicals. [63] By inhibiting proteasome activity, ursolic acid and similar triterpenoids cause cancer-causing enzymes to be targeted and malignant cell lines to enter a cell cycle arrest.[62] Thus, ursolic acid and its derivatives have potential applications as cancer-prevention remedies. [64] *H. suaveolens* essential oil painting was assessed in vitro against the deadly bone cancer cell line MCF-7 using the MTT test, which gauges the essential oil painting's cell survival [65].

2.1.3 *Anti-inflammatory Action*

Prevention of Seditious Activities Hyptis suaveoleans has implicit topical anti-inflammatory properties, more so than indomethacin. [66] Pentacyclic triterpenoid ursolic acid possesses potent and enduring anti-inflammatory properties. [67] H. suaveolens possesses anti-seditious properties that are comparable to those of traditional anti-inflammatory drugs by scavenging free revolutionaries. A number of authors mentioned taking ibuprofen. [68, 69, 70]

2.1.4 *Anti-diabetic Acitivity*

Anti-diabetic drugs are used to lower the blood's abnormally high glucose, or sugar, level. Strong hypoglycemic medication pentacyclic triterpenoid ursolic acid promotes vesicular insulin transit and stashing. Additionally, it increases intracellular calcium accumulation, which promotes insulin absorption via the glucose transporter protein (GLUT4), which is located on the tube membrane. [70].

2.1.5 *Antioxidant Acitivity*

Strong radical scavenging characteristics are exhibited by a number of logical types, including DPPH (,2-diphenyl-1-picrylhydrazyl) and ABTS (,2′ azino-bis-(3- ethylbenzothiazoline- 6- sulfonic acid), which are found in *Hyptis suaveolens.* [71] Natural polyphenolic composites with different hydroxyl groups can scavenge different ROS species by altering the enzyme effort required to generate them and by limiting their conformation. This gives them antioxidative characteristics. [72].

Polyphenols exhibit their antioxidant action through the singlet-electron transfer (SET) and hydrogen-snippet transfer (chapeau) media. In the former case, the structure of the radical cation results from a single electron transfer, while in the later one, a fragment of hydrogen is transferred by the phenolic functional group to liberate revolutionaries. [73]

Similar to flavonoids, polyphenols can interact with non-polar composites in the membrane lipid to stop lipid oxidation and protect the membrane's structure and functionality. [74]

Chapter 3: Molecular Docking & ADMET Study

3.1 *In-silico* molecular docking

3.1.1 Receptor preparation

3D Structure in PDB format of Human peroxisome proliferator-activated receptor gamma in complex with rosiglitazone (PDB: 4EMA) and cyclooxegenase-2 (PDB: 1PXX) was extracted From RCSB PDB (https://www.rcsb.org/). Chain B, water, and heteroatoms were removed using Discovery Studio 4.5 Client. Then it was saved in PDB format. Then Check and correct missing residue atoms using Swiss-PdbViewer and save as .pdb format. And then it was opened with AutoDockTools-1.5.7 and polar Hydrogen, Kollman Charges was added. It was saved in .pdbqt format using Grid-macromolecule functions.

3.1.2 Ligand preparation

3.1.2.1 Rosiglitazone, Abieta-7,13-diene, Beta-Sitosterol, Neoabietinol, And Ovatodiolide preparation for anti-diabetic activity

Human peroxisome proliferator-activated receptor gamma in complex with rosiglitazone (PDB: 4EMA) was opened with Disocovery Studio 4.5 Client. Then chain A, B and Water molecules were removed, remaining only rosiglitazone. It was saved in .pdb format. The energy of the rosiglitazone was mininmized by using Chimera 1.13.1. After energy minimization it was saved in .pdb format. Then it was opened with AutoDockTools-1.5.7 which automatically merges nonpolar hydrogen and leaves polar hydrogen. Torsion was chosen as default with focusing particulary on amide bond, which was kept non-rotatable. Then it was saved in .pdbdqt format.

Abieta-7,13-diene, Beta-Sitosterol, Neoabietinol, And Ovatodiolide were downloaded from PubChem in SDF format. Their energy mininmized by using Chimera 1.13.1 and saved in .pdb format. These were opened with AutoDockTools-1.5.7 which which automatically merges nonpolar hydrogen and leaves polar hydrogen. Torsion was chosen as default with focusing particulary on amide bond, which was kept non-rotatable. Then it was saved in .pdbdqt format.

3.1.2.2 Diclofenac, Apigenin, Dehydroabietinol, Neoabietinol, Abieta-7,13-diene, Spathulenol, Abietatriene preparation for Anti-inflammatory activity:

Crystal structure of Diclofenac (PDB: 1PXX) bound to the cyclooxygenase active site of Cox-2. And all the ligands preparation procedures done similarly as mentioned on ligands preparation for anti-diabetic activity. Diclofenac was opened with Disocovery Studio 4.5 Client. Then chain A, B and Water molecules were removed, remaining only rosiglitazone. It was saved in .pdb format. The energy of the rosiglitazone was mininmized by using Chimera 1.13.1. After energy minimization it was saved in .pdb format. Then it was opened with AutoDockTools-1.5.7 which automatically merges nonpolar hydrogen and leaves polar hydrogen. Torsion was chosen as default with focusing particulary on amide bond, which was kept non-rotatable. Then it was saved in .pdbdqt format. And all others procedures done as previously explained.

©Daffodil International University

3.2 Docking

(A) "Rosiglitazone, Abieta-7, 13-diene, Beta-Sitosterol, Neoabietinol, And Ovatodiolide"

(B) "Diclofenac, Apigenin, Dehydroabietinol, Neoabietinol, Abieta-7,13-diene, Spathulenol, Abietatriene.

3.2.1 Grid box set up

(A) For docking purpose co-crystalized rosiglitazone's binding site at Human peroxisome proliferator-activated receptor gamma (PDB ID: 4EMA) was used for target binding site. Using AutoDockTools-1.5.7 grid box was set up at center_x = 15.915, center_y = 8.115, center_z = 37.546.896 with size_x = 26, size_y = 25, size_z = 23 and adjusting spacing 1 Å for enclosing SER289, TYR473 of chain A.

(B) For docking purpose co-crystalized Crystal structure of Diclofenac bound to the cyclooxygenase-2 (PDB: 1PXX) for target binding site. Using AutoDockTools-1.5.7 grid box was set up at center_x = 25.465, center_y = 24.471 , center_z = 13.293 with size_x = 25, size_y = 25, size_z = 25 and adjusting spacing 1 Å for enclosing TYR385, SER530 of chain A.

©Daffodil International University

3.2.2 Preparation of configuration file.txt

(A) A configuration file in .txt format was made where all information for docking was providedin following way for an example considering rosiglitazone as ligand.

receptor = ProteinH.pdbqt
ligand = BRL.pdbqt

size_x = 26
size_y = 25
size_z = 23

center_x = 15.915
center_y = 8.115
center_z = 46.896

out = BRL_out.pdbqt
log = BRL.txt

energy_range = 3
num_modes = 9
exhaustiveness = 8

(B) A configuration file in .txt format was made where all information for docking was providedin following way for an example considering diclofenac as ligand.

receptor = ProteinM.pdbqt
ligand = DIF.pdbqt

size_x = 25
size_y = 25
size_z = 25

center_x = 25.465
center_y = 24.471
center_z = 13.293

out = DIF_out.pdbqt
log = DIF.txt

energy_range = 3
num_modes = 9
exhaustiveness = 8

©Daffodil International University

3.2.3 Running docking

Prior to execution, every ligand file—receptor.pdbqt, ligand.pdbqt, and configuration.txt—was kept in a single folder. The docking procedure was carried out using AutoDock Vina 1.1.2 66. There was a command to create a log.txt file and a configuration.txt file supplied.

©Daffodil International University

3.5 Drug-likeness analysis & ADMET Study

3.5.1 Drug-likeness analysis

After being chosen, compounds were subjected to further screening using Lipinski's rule of five (Ro5) [75]. Lipinski's screening was performed using the Molinspiration server (http://www.molinspiration.com/cgi-bin/properties), SwissADME (http://www.swissadme.ch/), and the computation of ligand physicochemical parameters. Drug scores were calculated using the OSIRIS Property Explorer (http://www.organic-chemistry.org/prog/peo). [76]

3.5.2 Absorption, distribution, metabolism, elimination and toxicity (ADMET) studies

The molecular structures of both ligands were submitted to the ADMET-SAR server (http://lmmd.ecust.edu.cn:8000) in order to evaluate the druglikeness of both ligands as well as different pharmacokinetic and pharmacodynamic parameters, such as bloodbrain barrier penetration, human intestinal absorption, Caco-2 permeability, cytochrome P450 solubility, cytochrome P (CYP) inhibitory promiscuity, renal organic cation transportation, inhibition of human ether-a-go-go related genes, rat acute toxicity, fish toxicity, Tetrahymena pyriformis toxicity, and Ames toxicity. [76]

Table 3.1: Comparison of Drug-likeness properties of rosiglitazone, Beta-Sitosterol, Neoabietinol, And Ovatodiolide

Drug-likeness properties	**Rosiglitazone**	**Beta-Sitosterol**	**Neoabietinol**	**Ovatodiolide**
Molecular weight	**357.43 g/mol**	**414.71 g/mol**	**288.47 g/mol**	**328.40 g/mol**
LogP -	**2.41**	**4.79**	**3.65**	**2.73**
LogS -	**-3.91**	**-7.90**	**-4.83**	**-3.38**
H-bond acceptors	**4**	**1**	**1**	**4**
H-bond donors	**1**	**1**	**1**	**0**
Rotatable bonds	**7**	**6**	**1**	**0**
Heavy atoms	**25**	**30**	**21**	**24**
Hydrogen atoms				
TPSA	**96.83 Å²**	**20.23 Å²**	**20.23 Å²**	**52.60 Å²**
RO5 violation	**0 violation**	**Yes; 1 violation: MLOGP>4.15**	**Yes; 1 violation: MLOGP>4.15**	**0 violation**
Refractivity	**101.63**	**133.23**	**91.61**	**92.59**
Drug-Likeness score				
Drug score				

©Daffodil International University

Table 3.2 : ADMET predicted feature of rosiglitazone, Beta-Sitosterol, Neoabietinol, And Ovatodiolide

Property	Rosiglitazone	Beta-Sitosterol	Neoabietinol	Ovatodiolide
Blood-brain barrier	**BBB+**	**BBB+**	**BBB+**	**BBB+**
Human intestinal absorption	**HIA+**	**HIA+**	**HIA+**	**HIA+**
Caco-2 permeability	**Caco2+**	**Caco2+**	**Caco2+**	**Caco2+**
P-glycoprotein substrate	**Substrate**	**Substrate**	**Substrate**	**Non-substrate**
Renal organic cation transporter	**Non-inhibitor**	**Non-inhibitor**	**Non-inhibitor**	**Non-inhibitor**
CYP450 2C9 substrate	**Non-substrate**	**Non-substrate**	**Non-substrate**	**Non-substrate**
CYP450 2D6 substrate	**Non-substrate**	**Non-substrate**	**Non-substrate**	**Non-substrate**
CYP450 3A4 substrate	**Substrate**	**Substrate**	**Substrate**	**Substrate**
CYP450 1A2 inhibitor	**Inhibitor**	**Non-inhibitor**	**Non-inhibitor**	**Inhibitor**
CYP450 2C9 inhibitor	**Inhibitor**	**Non-inhibitor**	**Inhibitor**	**Non-inhibitor**
CYP450 2D6 inhibitor	**Non-inhibitor**	**Non-inhibitor**	**Non-inhibitor**	**Non-inhibitor**
CYP450 2C19 inhibitor	**Inhibitor**	**Non-inhibitor**	**Inhibitor**	**Non-inhibitor**
CYP450 3A4 inhibitor	**Inhibitor**	**Non-inhibitor**	**Non-inhibitor**	**Non-inhibitor**
CYP inhibitory promiscuity	**High CYP Inhibitory Promiscuity**	**Low CYP Inhibitory Promiscuity**	**Low CYP Inhibitory Promiscuity**	**Low CYP Inhibitory Promiscuity**
AMES toxicity	**Non AMES toxic**	**Non AMES toxic**	**Non AMES toxic**	**Non AMES toxic**
Carcinogens	**Non-carcinogens**	**Non-carcinogens**	**Non-carcinogens**	**Non-carcinogens**
Fish ioxicity	**High FHMT**	**High FHMT**	**High FHMT**	**High FHMT**
Tetrahymena pyriformis toxicity	**High TPT**	**High TPT**	**High TPT**	**High TPT**
Honey bee toxicity	**Low HBT**	**High HBT**	**High HBT**	**High HBT**
Biodegradation	**Not ready biodegradable**	**Not ready biodegradable**	**Not ready biodegradable**	**Not ready biodegradable**
Acute oral toxicity	**III**	**I**	**IV**	**III**
Carcinogenicity (three-class)	**Non-required**	**Non-required**	**Non-required**	**Non-required**

Table 3.3 : comparison of Drug-likeness properties of Diclofenac, Apigenin, Spathulenol.

Drug-likeness properties	**Diclofenac**	**Apigenin**	**Spathulenol**
Molecular weight	**296.15 g/mol**	**270.24 g/mol**	**220.35 g/mol**
LogP -	**1.98**	**1.89**	**2.88**
LogS -	**-4.65**	**-3.94**	**-3.17**
H-bond acceptors	**2**	**5**	**1**
H-bond donors	**2**	**3**	**1**
Rotatable bonds	**4**	**1**	**0**
Heavy atoms	**19**	**20**	**16**
Hydrogen atoms			
TPSA	**49.33 Å²**	**90.90 Å²**	**20.23 Å²**
RO5 violation	**0 violation**	**0 violation**	**0 violation**
Refractivity	**77.55**	**73.99**	**68.34**
Drug-Likeness score			
Drug score			

Table 3.4: ADMET predicted feature of Diclofenac, Apigenin, Spathulenol.

Property	Diclofenac	Apigenin	Spathulenol
Blood-brain barrier	**BBB+**	**BBB+**	**BBB+**
Human intestinal absorption	**HIA+**	**HIA+**	**HIA+**
Caco-2 permeability	**Caco2+**	**Caco2+**	**Caco2+**
P-glycoprotein substrate	**Non-substrate**	**Non-substrate**	**Substrate**
Renal organic cation transporter	**Non-inhibitor**	**Non-inhibitor**	**Non-inhibitor**
CYP450 2C9 substrate	**Non-substrate**	**Non-substrate**	**Non-substrate**
CYP450 2D6 substrate	**Non-substrate**	**Non-substrate**	**Non-substrate**
CYP450 3A4 substrate	**Non-substrate**	**Non-substrate**	**Substrate**
CYP450 1A2 inhibitor	**Inhibitor**	**Inhibitor**	**Non-inhibitor**
CYP450 2C9 inhibitor	**Inhibitor**	**Inhibitor**	**Non-inhibitor**
CYP450 2D6 inhibitor	**Non-inhibitor**	**Non-inhibitor**	**Non-inhibitor**
CYP450 2C19 inhibitor	**Non-inhibitor**	**Inhibitor**	**Non-inhibitor**
CYP450 3A4 inhibitor	**Non-inhibitor**	**Inhibitor**	**Non-inhibitor**
CYP inhibitory promiscuity	**Low CYP Inhibitory Promiscuity**	**High CYP Inhibitory Promiscuity**	**Low CYP Inhibitory Promiscuity**
AMES toxicity	**Non AMES toxic**	**Non AMES toxic**	**Non AMES toxic**
Carcinogens	**Non-carcinogens**	**Non-carcinogens**	**Non-carcinogens**
Fish ioxicity	**High FHMT**	**High FHMT**	**High FHMT**
Tetrahymena pyriformis toxicity	**High TPT**	**High TPT**	**High TPT**
Honey bee toxicity	**Low HBT**	**High HBT**	**High HBT**
Biodegradation	**Not ready biodegradable**	**Not ready biodegradable**	**Not ready biodegradable**
Acute oral toxicity	**II**	**III**	**III**
Carcinogenicity (three-class)	**Non-required**	**Non-required**	**Non-required**

©Daffodil International University

Chapter 4: Result and Discussion

4.1 Molecular docking study

4.1.1 Model 1 ligands

Table 4.1: Binding Affinity, Hydrophobic Interactions, Electrostatic/Other Interactions, Hydrogen Bonds and Hydrogen Bond Distance between **4EMA** and rosiglitazone, Abieta-7, 13-diene, Beta-Sitosterol, Neoabietinol, And Ovatodiolide

Ligand	Binding Affinity (Kcal/mol)	Hydrophobic Interactions	Electrostatic /Other Interactions	Hydrogen Bonds	Hydrogen Bond Distance (Å)
Rosiglitazone	-8.4	LEU330, ARG288, ILE326, ILE341, GLY284, MET348, ILE281, VAL339, LEU353, PHE363, TYR327, HIS449, PHE282, GLN286, LEU465, LEU453, LEU453, LEU469, HIS323	MET 364	TYR 473, SER 289, CYS 285	5.11, 4.50, 4.70
Abieta-7,13-diene	-7.6	GLY284, ARG288, ILE 341, LEU 330, CYS285, MET364, MET348, VAL339, LEU353, SER342, PHE287			
Beta-Sitosterol	-8	GLY258, SER342, ILE341, VAL339, ARG288, TYR327, MET364, LEU330, ILE326, SER289, PHE363, CYS285, GLY284, ALA292, ILE281, MET348, LEU255		GLU259	4.64
Ovatodio-lide	-7.3	PHE287, SER342, ILE341, ARG288, MET348, ILE281, GLY284, ARG280		CYS285	4.27
Neoabieti-nol	-7.1	GLY258, SER342, CYS285, ARG288, LEU255, MET348, ILE 341, ARG280, GLY284, ILE281		GLU259	5.06

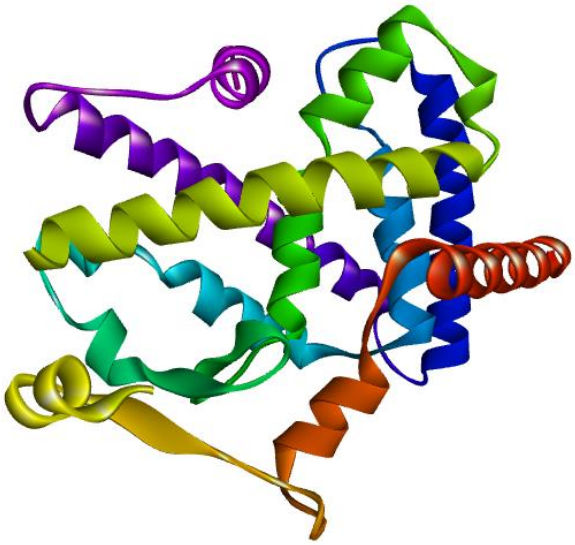


Figure 4.1: Prepared receptor (PDB ID: 4EMA)

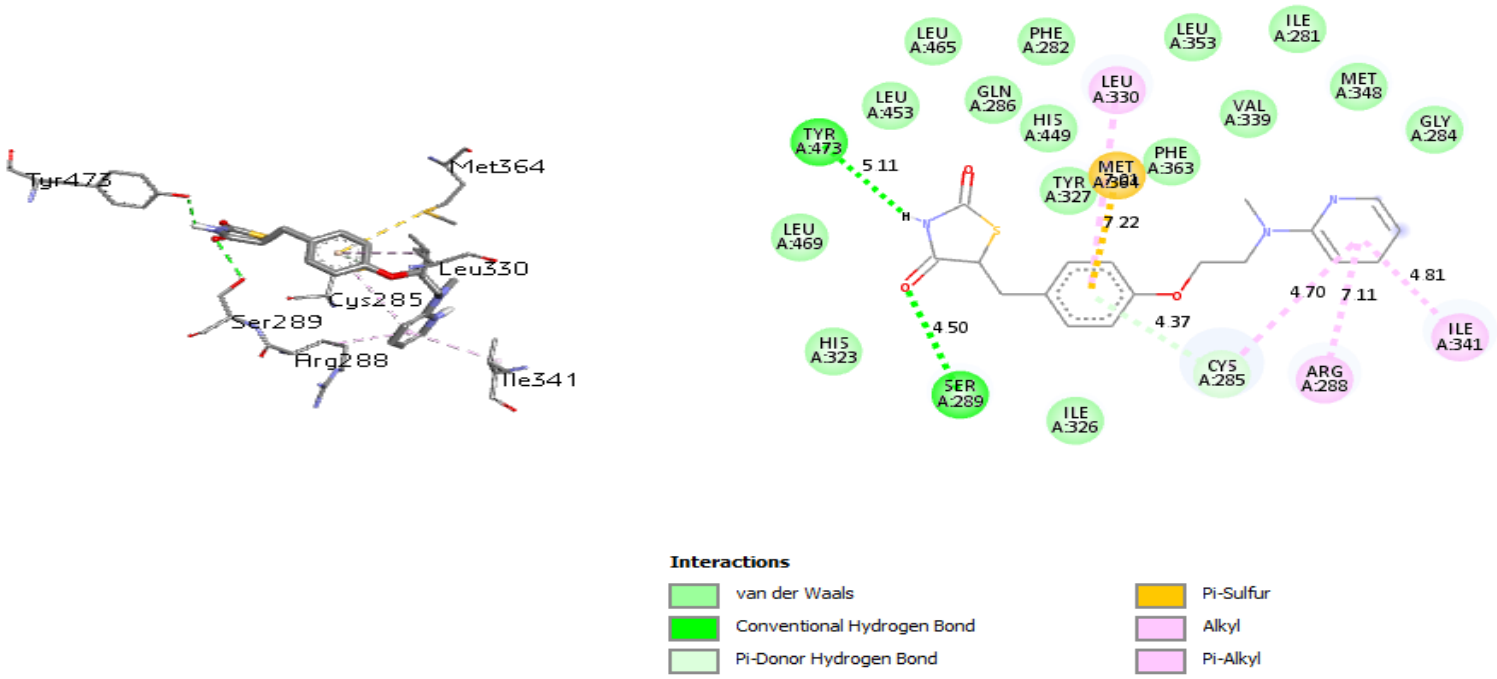


Figure 4.2: 3D and 2D structure of receptor-rosiglitazone interactions (-8.4 kcal/mol)

All the ligands which showed higher docking scores (that is low energy values) than or equivalent to standard rosiglitazone (-8.4 Kcal/mol), their interactions with the receptor (4EMA) are shown in the following figures.

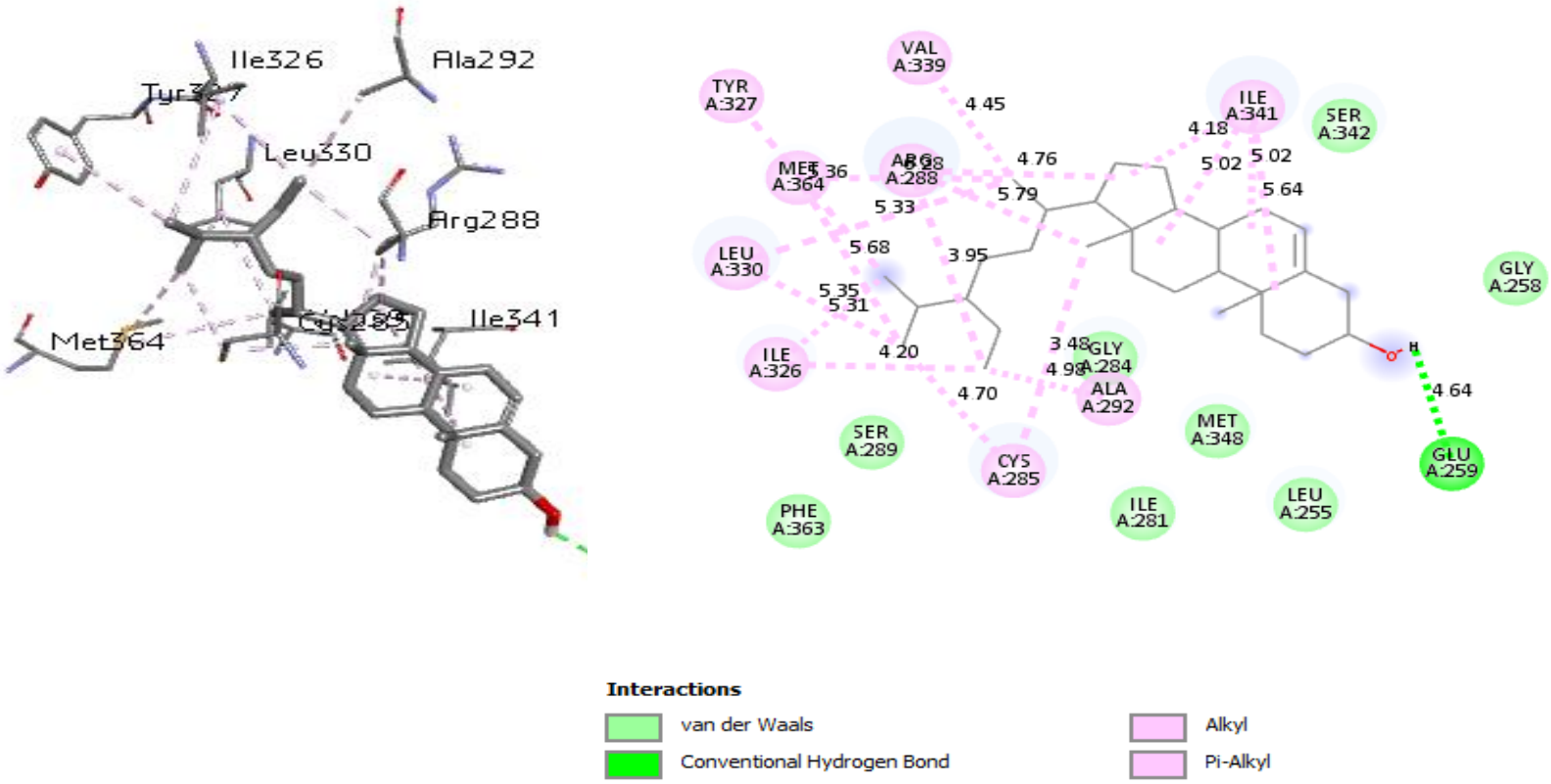


Figure 4.3: 3D and 2D structure of receptor- Beta-Sitosterol interactios (-8 kcal/mol)

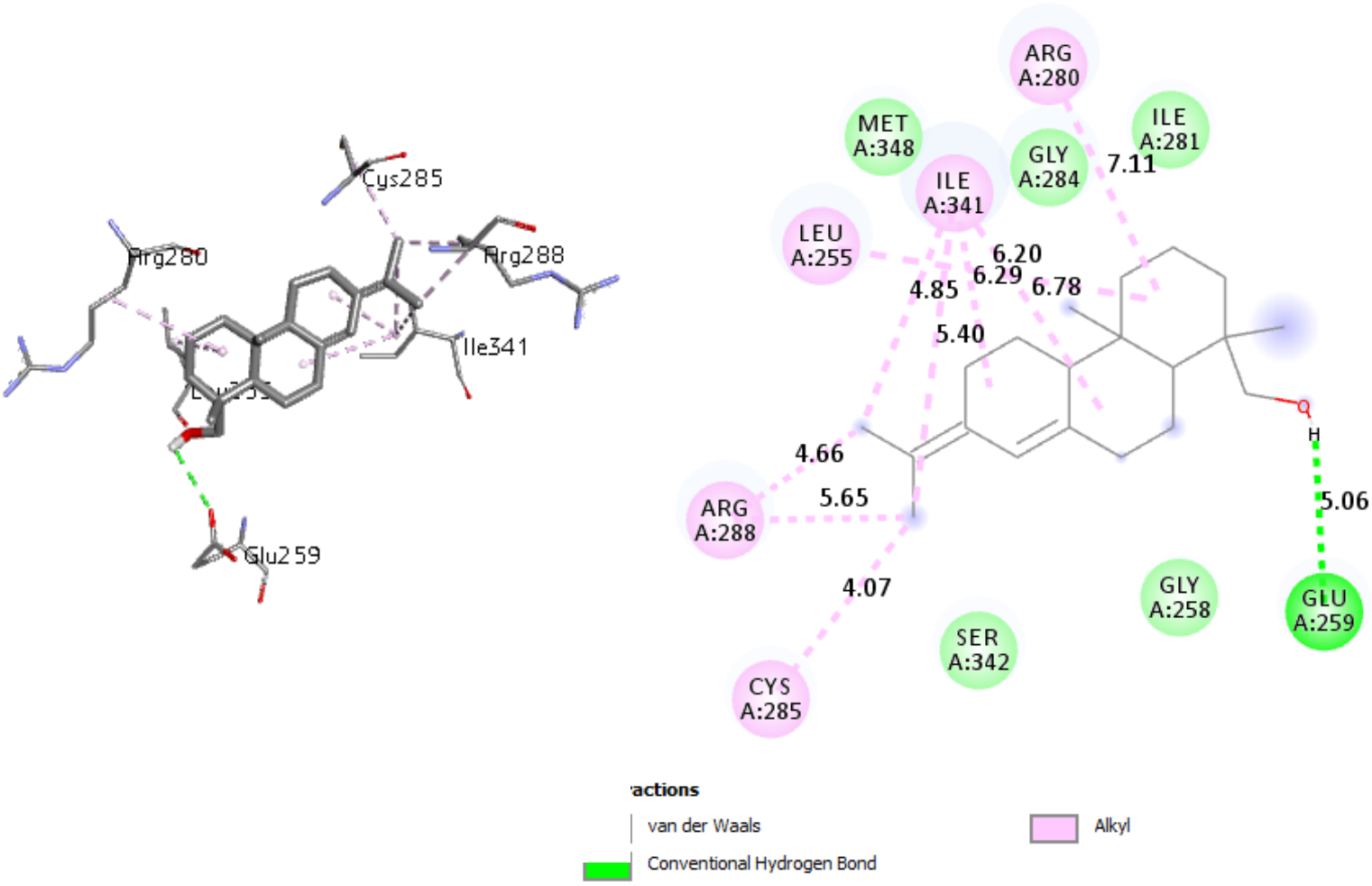


Figure 4.4: 3D and 2D structure of receptor- Neoabietinol interactions (-7.1 kcal/mol)

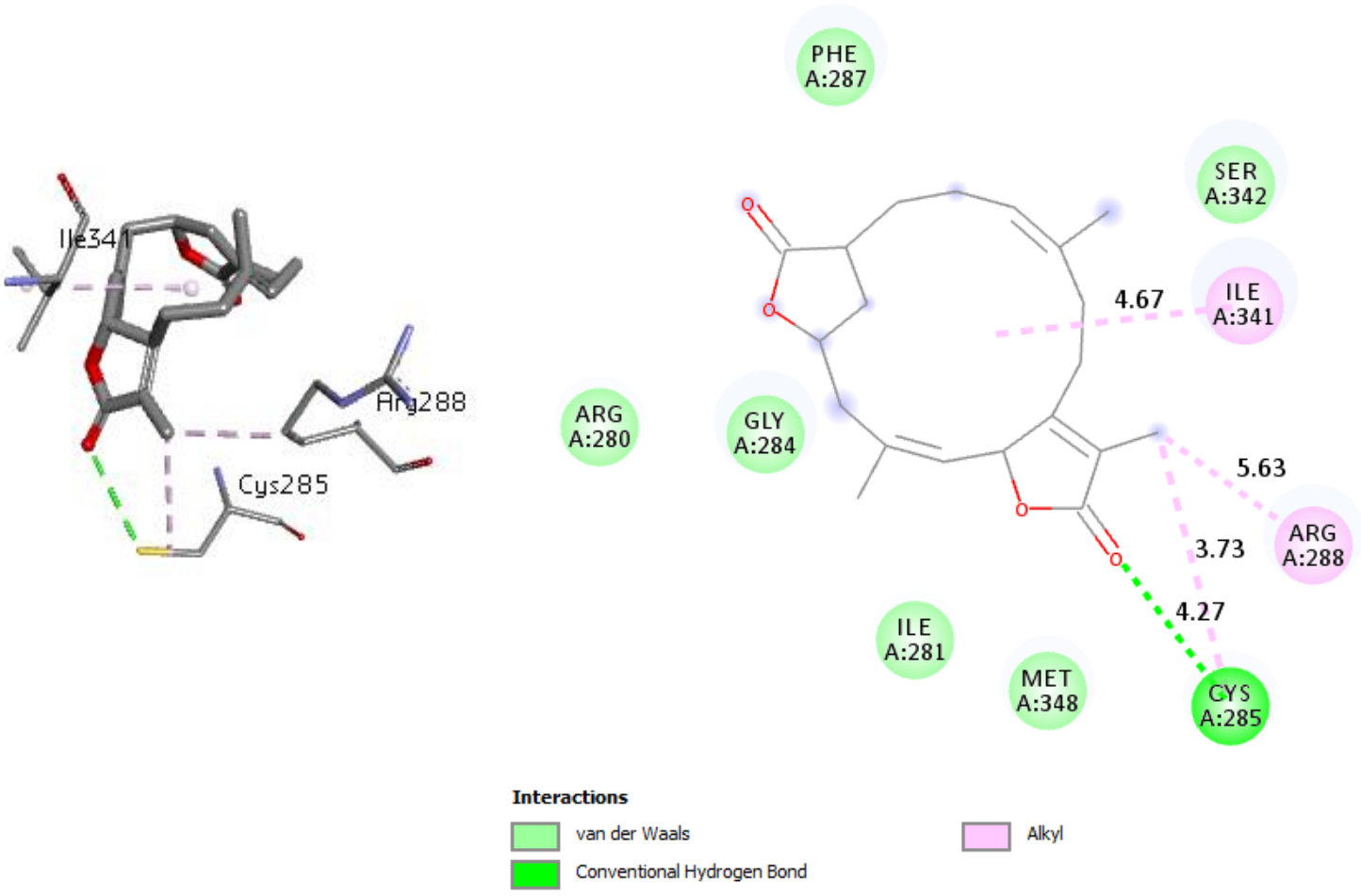


Figure 4.5: 3D and 2D structure of receptor-Ovatodiolide interactions (-7.3 kcal/mol)

4.1. 2 Model 2 ligands

Table 4.2: Binding Affinity, Hydrophobic Interactions, Electrostatic/Other Interactions, Hydrogen Bonds and Hydrogen Bond Distance between **1PXX** and Diclofenac, Apigenin, Dehydroabietinol, Neoabietinol, Abieta-7,13-diene, Spathulenol, Abietatriene.

Ligand	Binding Affinity (Kcal/mol)	Hydrophobic Interactions	Electrostatic/ Other Interactions	Hydrogen Bonds	Hydrogen Bond Distance (Å)
Diclofenac	-8.4	PHE381, TYR348, PHE205, LEU352, PHE518, SER353, VAL523, TYR355, ALA527, ARG120, VAL349, LEU531, LEU384, GLY526	MET522, TRP387	TYR385, SER530	6.05, 4.64
Apigenin	-8.7	TYR385, ALA202, TYR348, ALA199, LEU390, LEU391, TYR148, THR212, PHE210, HIS386, HIS207	ASN382	GLN203, THR206, HIS388, TRP387	4.53, 4.05, 5.89, 6.19
Dehydroabietinol	-8.7	VAL523, ALA527, ARG120, VAL116, MET113, TYR355, LEU359, LEU531, VAL349,TYR348, SER530, TYR385, LEU352, PHE381, PHE518, TRP387, GLY526, LEU384		MET522	3.63
Neoabietinol	-8.9	VAL349, SER530, TYR348, TYR385,PHE381,PHE518,GLY526, LEU384, MET522, TRP387, LEU352, VAL523, ALA527, MET113, LEU359, LEU531, VAL116, TYR355			
Abieta-7,13-diene,	-9.2	VAL349, ALA527, SER353, LEU359, LEU531, MET113, VAL116, TYR355, ARG120, SER530, VAL523, TYR385, PHE381, MET522, PHE518, LEU384, GLY526, LEU352, TRP387			
Spathulenol	-7	SER353, TYR3555, LEU352, VAL523, GLY526, PHE518, VAL349, SER530, ALA527, LEU531	ARG120	TYR355	6.48

©Daffodil International University

Abietatriene	-9.6	ARG120, TYR355, MET113, TYR348, SER530, TYR385, LEU352, TRP387, PHE381, MET522, GLY526, LEU384, PHE518, VAL523, VAL349, SER353, ALA527, LEU531, LEU359, VAL116			

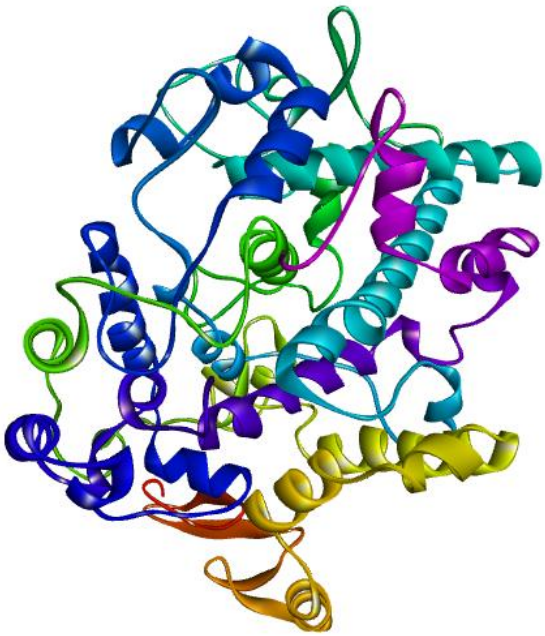


Figure 4.6: Prepared receptor (PDB ID: 1PXX)

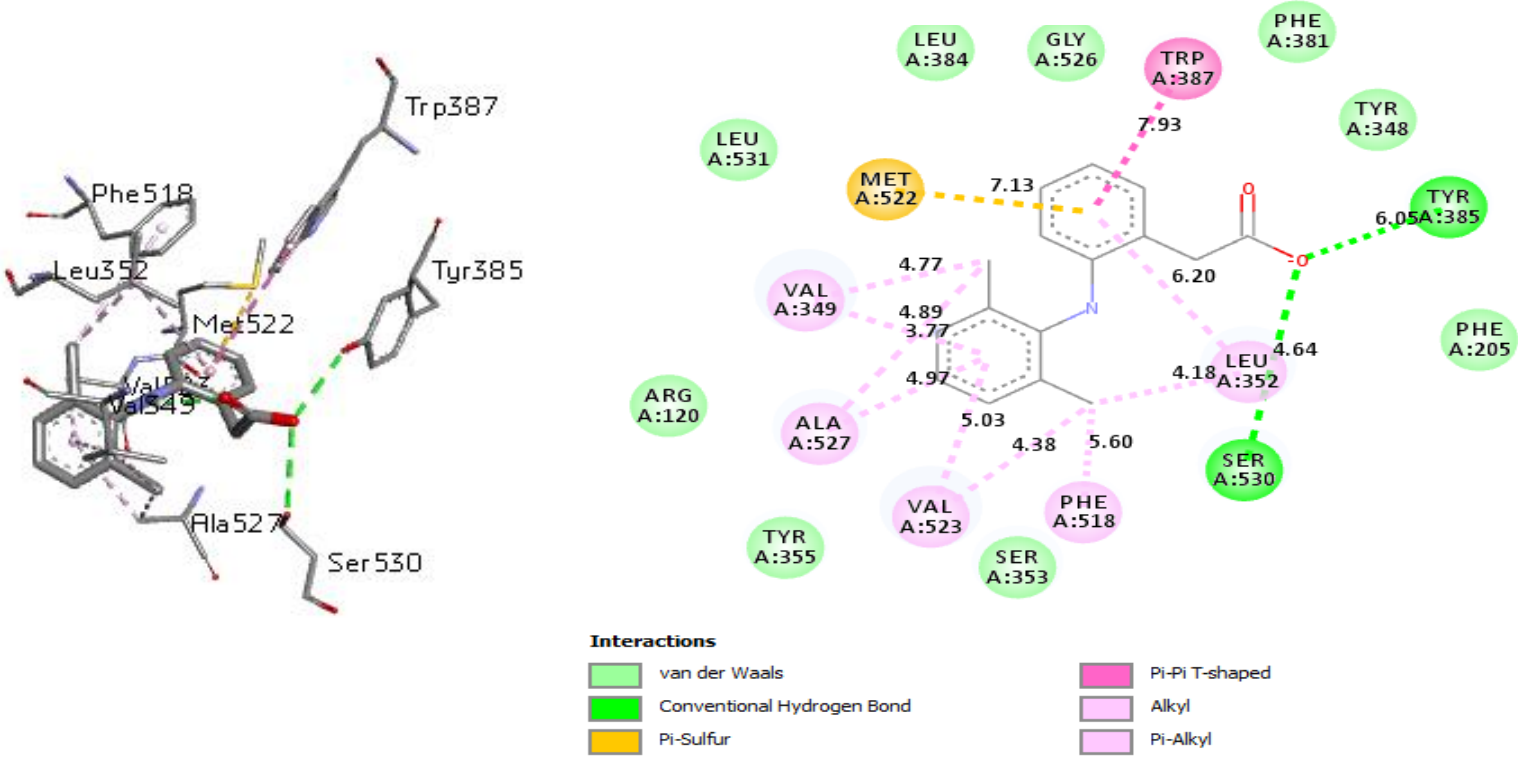


Figure 4.7: 3D and 2D structure of receptor- Diclofenac interactions (-8.4 kcal/mol)

All the ligands which showed higher docking scores (that is low energy values) than or equivalent to standard Diclofenac (-8.4 Kcal/mol), their interactions with the receptor (4EMA) are shown in the following figures.

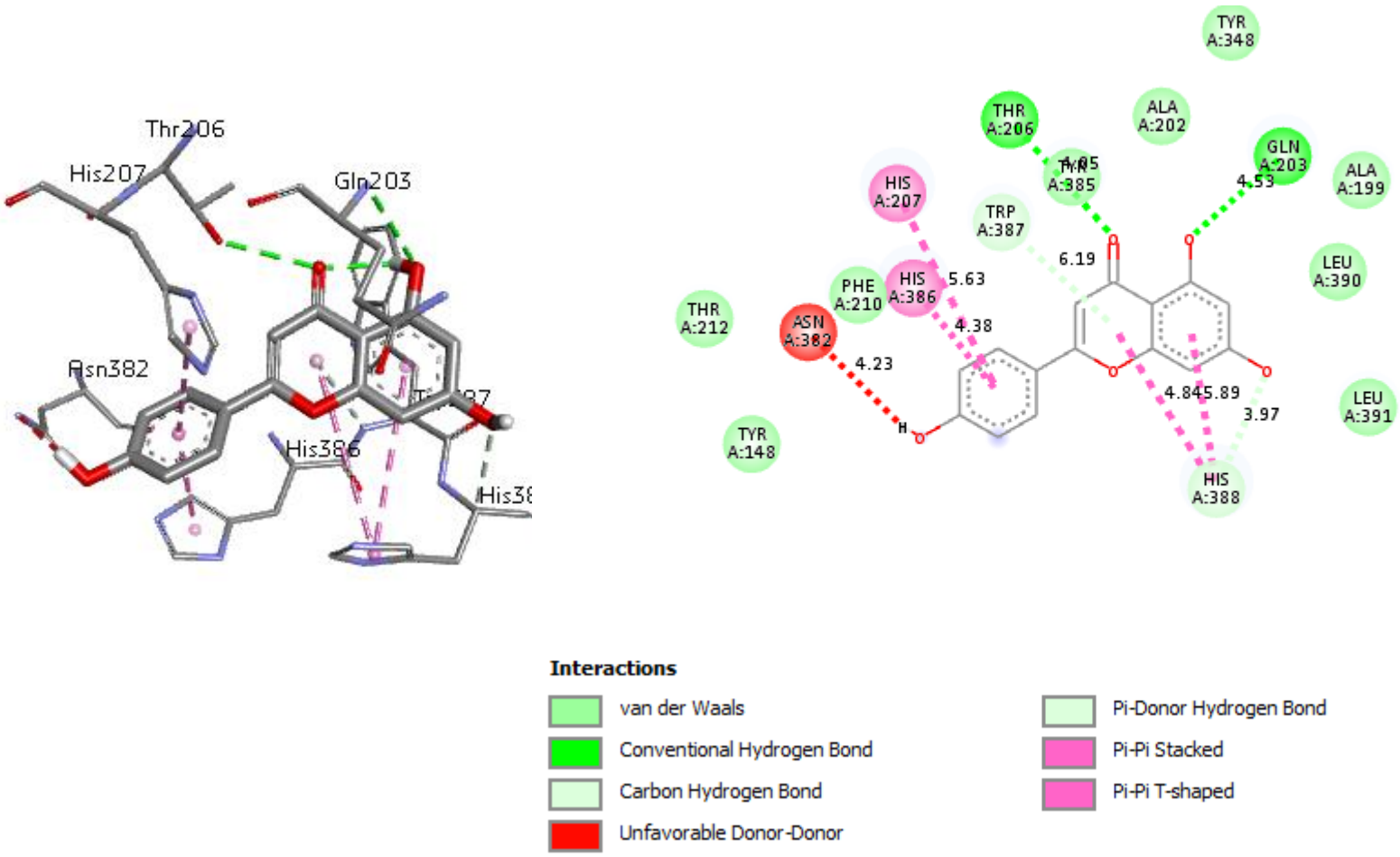


Figure 4.8: 3D and 2D structure of receptor-Apigenin interactions (-8.7 kcal/mol)

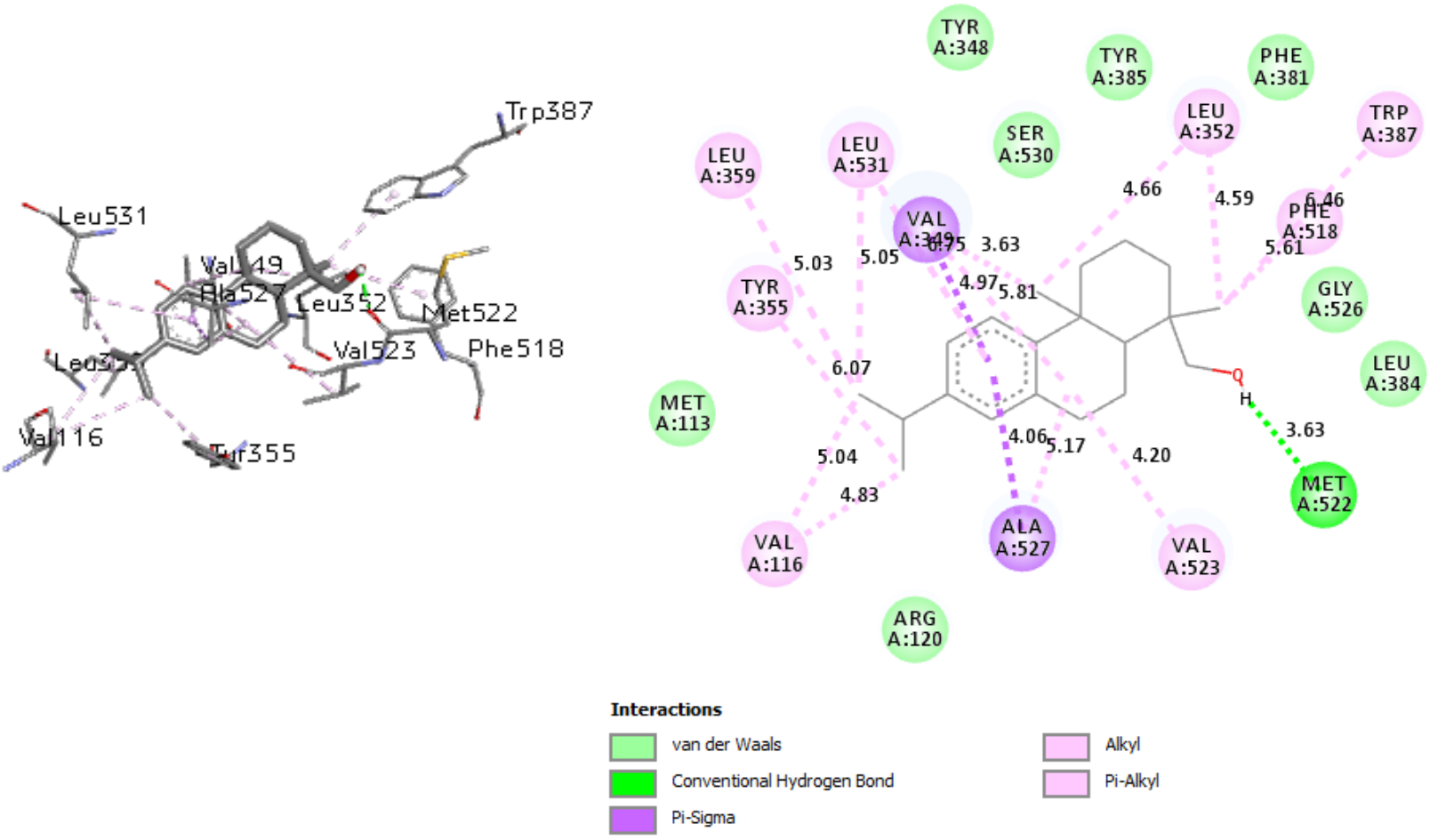


Figure 4.9: 3D and 2D structure of receptor- Dehydroabietinol interactions (-8.7 kcal/mol)

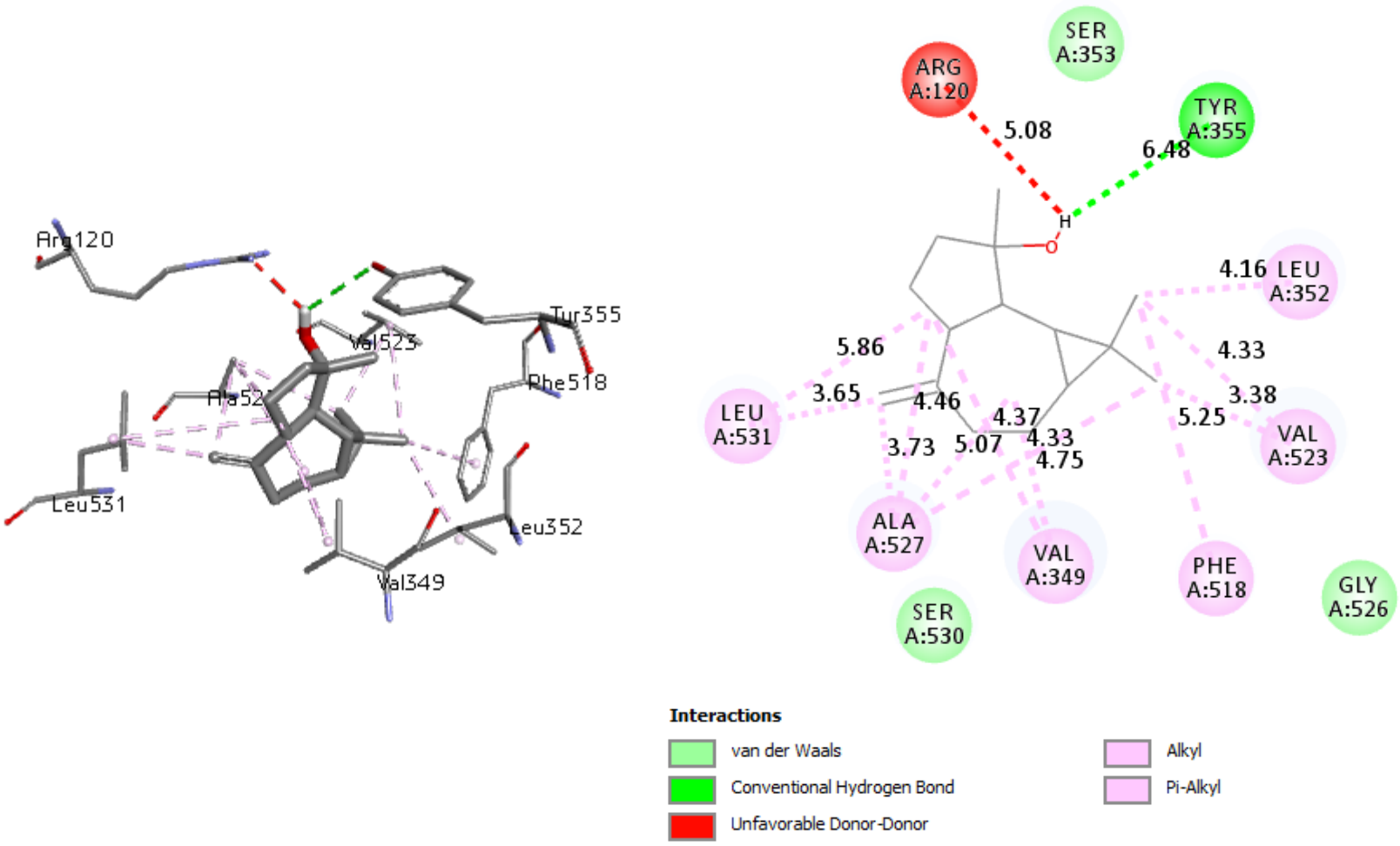


Figure 4.10: 3D and 2D structure of receptor- Spathulenol interactions (-7 kcal/mol)

Chapter 5: Conclusion

5.1 Limitations

The issue of practicality prevented us from synthesizing, characterizing, and testing the biological activity of the proposed modeled ligands in a wet laboratory. Should the opportunity present itself, we would like to investigate this subject more in the future.

5.2 Conclusion:

These days, diabetes and inflammation-related risks to human health are the fastest-growing worldwide threats. Thus, research is now being conducted to find safer and more efficient herbal hypoglycemic medicines as well as to produce novel anti-diabetic medications with better clinical characteristics.

Utilizing IMPPAT, a well managed database, the phytochemical list from Hyptis suaveolens (L.) Poit was acquired. In order to identify the potent biological targets generating the anti-inflammatory and anti-diabetic actions, a computational screening of the small molecule library was conducted.

Almost ten out of thirty docked ligands in silico molecular docking studies had docking scores that were greater than or equal to standard, which might lead to the identification and development of novel oral antidiabetic and anti-inflammatory medicines in the future. *Hyptis suaveolens (L.) Poit* might be a useful natural source for the creation of novel anti-inflammatory and oral hypoglycemic medications. Research on molecular dynamics is required to advance the ligand-receptor complex model.

Strong hydrogen bonding and van der Waals interactions were observed in ligand-receptor complexes, indicating that these phytochemicals are good candidates for use as anti-inflammatory and anti-diabetic medicines and are safe to be developed into a commercial medication. Comparing these molecules to other medications, it was also discovered that they had less adverse effects. For the same reason, molecular docking was also utilized to confirm these. Based on their binding affinities and docking scores, many phytochemicals (Beta-Sitosterol, Ovatodiolide, Neoabietinol, Apigenin, Dehydroabietinol, and Spathulenol) were demonstrated to be able to bind protein targets (4EMA, 1PXX). More in vitro research is needed to fully understand the target-based anti-inflammatory and anti-diabetic effects of these newly identified phytochemicals.

©Daffodil International University

References:

1] World Health Organization. (2018, October 30). Diabetes. World Health Organization. https://www.who.int/news-room/fact-sheets/detail/diabetes

2] Kennedy, M. S. N., & Masharani, U. (2018). Pancreatic hormones & antidiabetic drugs. *Basic & clinical pharmacology*, *13*, 723-47.

3] Tripathi, K. D. (2013). *Essentials of medical pharmacology*. JP Medical Ltd.

4] *IDF diabetes atlas*. IDF Diabetes Atlas. (2021). https://diabetesatlas.org/

5] World Health Organization. (2019). *Bangladesh data*. World Health Organization. https://data.who.int/countries/050

6] World Health Organization. Diabetes country profiles, 2016: Bangladesh. France: WHO (2016).

7] Akter, S., Rahman, M. M., Abe, S. K., & Sultana, P. (2014). Prevalence of diabetes and prediabetes and their risk factors among Bangladeshi adults: a nationwide survey. *Bulletin of the World Health Organization*, *92*, 204-213A.

8] The International Diabetes Federation (IDF). (2021). *Bangladesh diabetes report 2000 - 2045*. diabetes report 2000 - 2045. https://diabetesatlas.org/data/en/country/16/bd.html

9] Lee, M. A., Tan, L., Yang, H., Im, Y.-G., & Im, Y. J. (2017). Structures of PPARΓ complexed with lobeglitazone and pioglitazone reveal key determinants for the recognition of Antidiabetic Drugs. *Scientific Reports*, *7*(1). https://doi.org/10.1038/s41598-017-17082-x

10] R Scrivo; M Vasile; I Bartosiewicz; G Valesini. Aut Rev. 2011, 10(7), 369-374.

11] BG Katzung. Basic and Clinical Pharmacology. (Fifth edition). Prentice Hall, London. 1992

12] Andreae S. Lexikon der Krankheiten und Untersuchungen. Stuttgart: Thieme; 2008.

13] Kaspers DL, Hauser SL, Jameson JL, Fauci AS, Longo DL, Loscalzo J. Harrison's Principles of internal medicine. New York: McGraw-Hill Companies. 19th ed; 2015.

14] Pschyrembel W. Klinisches Wörterbuch. Berlin: De Gruyter; 2017.

15] IH Tarner; Englbrecht M; Schneider M; Vander Heijde DM; Muller-Ladner U. The J Rheu. 2012, 90,17-20.

16] A Haywood; P Good; S Khan; A Leupp; S Jenkins- Marsh; K Rickett; JR Hardy. The Coc Database Sys Rev. 2015, 4, 1-5.

17] Whittle, B. J. (2003). Gastrointestinal effects of nonsteroidal anti-inflammatory drugs. *Fundamental & Clinical Pharmacology*, *17*(3), 301–313. https://doi.org/10.1046/j.1472-8206.2003.00135.x

18] Bombardier, C., Laine, L., Reicin, A., Shapiro, D., Burgos-Vargas, R., Davis, B., Day, R., Ferraz, M. B., Hawkey, C. J., Hochberg, M. C., Kvien, T. K., & Schnitzer, T. J. (2000). Comparison of upper gastrointestinal toxicity of rofecoxib and naproxen in patients with rheumatoid arthritis. *New England Journal of Medicine*, *343*(21), 1520–1528. https://doi.org/10.1056/nejm200011233432103

19] Kimmel, S. E., Berlin, J. A., Reilly, M., Jaskowiak, J., Kishel, L., Chittams, J., & Strom, B. L. (2005). Patients exposed to rofecoxib and celecoxib have different odds of nonfatal myocardial infarction. *Annals of Internal Medicine*, *142*(3), 157. https://doi.org/10.7326/0003-4819-142-3-200502010-00005

20] Baron, J. A., Sandler, R. S., Bresalier, R. S., Quan, H., Riddell, R., Lanas, A., Bolognese, J. A., Oxenius, B., Horgan, K., Loftus, S., & Morton, D. G. (2006). A randomized trial of rofecoxib for the chemoprevention of colorectal adenomas. *Gastroenterology*, *131*(6), 1674–1682. https://doi.org/10.1053/j.gastro.2006.08.079

21] Bertagnolli, M. M., Eagle, C. J., Zauber, A. G., Redston, M., Solomon, S. D., Kim, K., Tang, J., Rosenstein, R. B., Wittes, J., Corle, D., Hess, T. M., Woloj, G. M., Boisserie, F., Anderson, W. F., Viner, J. L., Bagheri, D., Burn, J., Chung, D. C., Dewar, T., … Hawk, E. T. (2006). Celecoxib for the prevention of sporadic colorectal adenomas. *New England Journal of Medicine*, *355*(9), 873–884. https://doi.org/10.1056/nejmoa061355

22] Gislason, G. H., Jacobsen, S., Rasmussen, J. N., Rasmussen, S., Buch, P., Friberg, J., Schramm, T. K., Abildstrom, S. Z., Køber, L., Madsen, M., & Torp-Pedersen, C. (2006). Risk of death or reinfarction associated with the use of selective cyclooxygenase-2 inhibitors and nonselective nonsteroidal antiinflammatory drugs after acute myocardial infarction. *Circulation*, *113*(25), 2906–2913. https://doi.org/10.1161/circulationaha.106.616219

23] Solomon, S. D., Wittes, J., Finn, P. V., Fowler, R., Viner, J., Bertagnolli, M. M., Arber, N., Levin, B., Meinert, C. L., Martin, B., Pater, J. L., Goss, P. E., Lance, P., Obara, S., Chew, E. Y., Kim, J., Arndt, G., & Hawk, E. (2008a). Cardiovascular risk of celecoxib in 6 randomized placebo-controlled trials. *Circulation*, *117*(16), 2104–2113. https://doi.org/10.1161/circulationaha.108.764530

24] Ott, E., Nussmeier, N. A., Duke, P. C., Feneck, R. O., Alston, R. P., Snabes, M. C., Hubbard, R. C., Hsu, P. H., Saidman, L. J., & Mangano, D. T. (2003). Efficacy and safety of the cyclooxygenase 2 inhibitors parecoxib and valdecoxib in patients undergoing coronary artery bypass surgery. *The Journal of Thoracic and Cardiovascular Surgery*, *125*(6), 1481–1492. https://doi.org/10.1016/s0022-5223(03)00125-9

25] Nussmeier, N. A., Whelton, A. A., Brown, M. T., Langford, R. M., Hoeft, A., Parlow, J. L., Boyce, S. W., & Verburg, K. M. (2005). Complications of the COX-2 inhibitors parecoxib and valdecoxib after cardiac surgery. *New England Journal of Medicine*, *352*(11), 1081–1091. https://doi.org/10.1056/nejmoa050330

26] Cardiovascular and cerebrovascular events in the randomized, controlled alzheimer's disease anti-inflammatory prevention trial (ADAPT). (2006). *PLoS Clinical Trials*, *1*(7). https://doi.org/10.1371/journal.pctr.0010033

27] Veeresham, C. (2012). Natural products derived from plants as a source of drugs. *Journal of Advanced Pharmaceutical Technology and Research*, *3*(4), 200-201.

28] Iman Islamieh, D., Afshar, D., Yousefi, M., & Esmaeili, D. (2018). Efflux pump inhibitors derived from natural sources as novel antibacterial agents against Pseudomonas aeruginosa: a review. *International Journal of Medical Reviews*, *5*(3), 94-105.

29] Fabricant, D. S., & Farnsworth, N. R. (2001). The value of plants used in traditional medicine for drug discovery. *Environmental health perspectives*, *109*(suppl 1), 69-75.

30] Yuan, H., Ma, Q., Ye, L., & Piao, G. (2016). The traditional medicine and modern medicine from natural products. *Molecules*, *21*(5), 559.

31] World Health Organization. (2002). *404*. World Health Organization. http://whqlibdoc.who.int/hq/2002/WHO_EDM_TRM_2002.1.PDF.

32] Ghani A. *Medicinal Plants of Bangladesh with Chemical Constituents and Uses*. 2nd ed. Dhaka, Bangladesh: Asiatic Society of Bangladesh; 2003

33] Heinrich, M. (2000). Ethnobotany and its role in drug development. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, *14*(7), 479-488.

34] Tabuti, J. R., Lye, K. A., & Dhillion, S. S. (2003). Traditional herbal drugs of Bulamogi, Uganda: plants, use and administration. *Journal of ethnopharmacology*, *88*(1), 19-44.

35] Farnsworth, N. R., Akerele, O., & Bingel, A. S. (1987). Medicinal plants in therapy. *Journal of Ethnopharmacology*, *19*(3), 336.

36] Rates, S. M. K. (2001). Plants as source of drugs. *Toxicon*, *39*(5), 603-613.

37] Bangladesh Foreign Trade Institute (BFTI). (2016, June). A Study on Sector-based Need Assessment of Business Promotion Council-Herbal Products and Medicinal Plants. 2016.http://www.bfti.org.bd/pdf/Herbal%20products%20and%20medicinal%20plants%20BPC%20research.pdf published June, 2016.

38] Wood, J. R., Bedolla Garcia, B. Y., Pink, L., & King, C. (2022). 1028. SALVIA HISPANICA: Lamiaceae. *Curtis's Botanical Magazine*, *39*(2), 359-378.

39] Mozhiyarasi, P., & Anuradha, R. (2018). A study on antioxidant activity of Hyptis suaveolens (L) poit. *Int J Curr Microbiol Appl Sci*, *7*(06), 376-382.

40] Dhamale, P., Pardhe, A. T., Manikpuriya, S., Sanap, G., Khadekar, K., & Jangle, K. (2023). FORMULATION AND EVALUATION OF HERBAL CREAM CONTAINING EXTRACTS OF HYPTIS SUAVEOLENS AND MURRAYA KOENIGII.

41] Gayathri, Y., Babu, A. N., Lakshmi, J. N., Kiranmai, K., & Kavya, G. (2021). Review on phyto-pharmacological and medicinal uses of Hyptis suaveolens (L) Poit. *Lipids*, *4*(3.00), 2-00.

42] Raizada, P. (2006). Ecological and vegetative characteristics of a potent invader, Hyptis suaveolens Poit. from India. *Lyonia*, *11*(2), 115-120.

43] Pachkore, G. L., & Dhale, D. A. (2011). Pharmacognostic Evaluation of Hyptis Suaveolens (L. Poit) Lamiaceae. *Journal of Ecobiotechnology*, *3*(3).

44] Mandal, S. M., Mondal, K. C., Dey, S., & Pati, B. R. (2007). Antimicrobial Activity of the Leaf Extracts of Hyptis suaveolens (L.) Poit. *Indian Journal of Pharmaceutical Sciences*, *69*(4).

45] Iwu, M. M., Ezeugwu, C. O., Okunji, C. O., Sanson, D. R., & Tempesta, M. S. (1990). Antimicrobial activity and terpenoids of the essential oil of Hyptis suaveolens. *International Journal of Crude Drug Research*, *28*(1), 73-76.

46] Patrick, G. L. (2023). *An introduction to medicinal chemistry*. Oxford university press.

47] Blundell, T. L., Sibanda, B. L., Montalvão, R. W., Brewerton, S., Chelliah, V., Worth, C. L., ... & Burke, D. (2006). Structural biology and bioinformatics in drug design: opportunities and challenges for target identification and lead discovery. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *361*(1467), 413-423.

48] Rawlins, M. D. (2004). Cutting the cost of drug development?. *Nature reviews Drug discovery*, *3*(4), 360-364.

49] Rowland, M., Peck, C., & Tucker, G. (2011). Physiologically-based pharmacokinetics in drug development and regulatory science. *Annual review of pharmacology and toxicology*, *51*, 45-73.

50] Jiang, Z., & Zhou, Y. (2005). Using bioinformatics for drug target identification from the genome. *American Journal of Pharmacogenomics*, *5*, 387-396.

51] Lengauer, T., & Rarey, M. (1996). Computational methods for biomolecular docking. *Current opinion in structural biology*, *6*(3), 402-406.

52] Claußen, H., Buning, C., Rarey, M., & Lengauer, T. (2001). FlexE: efficient molecular docking considering protein structure variations. *Journal of molecular biology*, *308*(2), 377-395.

53] Sharma, N. K., & Jha, K. K. (2010). Molecular docking: an overview. *J Adv Sci Res*, *1*(1), 67-72.

54] Chen, Y. C. (2015). Beware of docking!. *Trends in pharmacological sciences*, *36*(2), 78-95.

55] Xiong, J. (2006). *Essential bioinformatics*. Cambridge University Press.

56] Rao, B. K., Sudarshan, P. R., Rajasekhar, M. D., Nagaraju, N., & Rao, C. A. (2003). Antidiabetic activity of Terminalia pallida fruit in alloxan induced diabetic rats. *Journal of Ethnopharmacology*, *85*(1), 169-172.

57] Pham, H. N. T., Sakaff, J. A., Vuong, Q., Von Bowyer, M. C., & Scarlett, C. J. (2018). Screening of phytochemical content, antioxidants, antimicrobial and cytotoxic activities of Catharanthus roseus (L.) G. Don. Stem extract and its fraction. Biocatal Agric Biotechnol 16: 405–411.

58] Evans, W. C. (2009). *Trease and Evans' pharmacognosy*. Elsevier Health Sciences.

59] Moreira, A. C. P., Lima, E. D. O., Wanderley, P. A., Carmo, E. S., & Souza, E. L. D. (2010). Chemical composition and antifungal activity of Hyptis suaveolens (L.) Poit leaves essential oil against Aspergillus species. *Brazilian Journal of Microbiology*, *41*, 28-33.

60] Shenoy, C., Patil, M. B., & Kumar, R. (2009). Wound healing activity of Hyptis suaveolens (L.) Poit (Lamiaceae). *Int J Pharm Tech Res*, *1*(3), 737-744.

61] Mann, C. M., Cox, S. D., & Markham, J. L. (2000). The outer membrane of Pseudomonas aeruginosa NCTC 6749 contributes to its tolerance to the essential oil of Melaleuca alternifolia (tea tree oil). *Letters in Applied Microbiology*, *30*(4), 294-297.

62] Dai, J., & Mumper, R. J. (2010). Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules*, *15*(10), 7313-7352.

63] Poonkodi, K., Karthika, J., Tamilselvi, V., Anitha, R., & Vasanthamani, S. (2017). Chemical composition of essential oil of Hyptis suaveolens (L.) POIT and its in vitro anticancer activity. *Journal of Pharmacy Research*, *11*(5), 410-413.

64] Neuhouser, M. L. (2004). Flavonoids and cancer prevention: What is the evidence in humans?. *Pharmaceutical biology*, *42*(sup1), 36-45.

65] Poonkodi, K., Karthika, J., Tamilselvi, V., Anitha, R., & Vasanthamani, S. (2017). Chemical composition of essential oil of Hyptis suaveolens (L.) POIT and its in vitro anticancer activity. *Journal of Pharmacy Research*, *11*(5), 410-413.

66] Grassi, P., Reyes, T. S. U., Sosa, S., Tubaro, A., Hofer, O., & Zitterl-Eglseer, K. (2006). Anti-inflammatory activity of two diterpenes of Hyptis suaveolens from El Salvador. *Zeitschrift für Naturforschung C*, *61*(3-4), 165-170.

67] Baricevic, D., Sosa, S., Della Loggia, R., Tubaro, A., Simonovska, B., Krasna, A., & Zupancic, A. (2001). Topical anti-inflammatory activity of Salvia officinalis L. leaves: the relevance of ursolic acid. *Journal of ethnopharmacology*, *75*(2-3), 125-132.

68] Dhamale, P., Manikpuriya, S., Sanap, G., & Pardhe, A. (2023). The Phytochemical and Pharmacological Screening of Hyptis Suaveolens.

69] Uritu, C. M., Mihai, C. T., Stanciu, G. D., Dodi, G., Alexa-Stratulat, T., Luca, A., ... & Tamba, B. I. (2018). Medicinal plants of the family Lamiaceae in pain therapy: A review. *Pain Research and Management*, *2018*.

70] Castro, A. J. G., Frederico, M. J. S., Cazarolli, L. H., Mendes, C. P., Bretanha, L. C., Schmidt, E. C., ... & Silva, F. R. M. B. (2015). The mechanism of action of ursolic acid as insulin secretagogue and insulinomimetic is mediated by cross-talk between calcium and kinases to regulate glucose balance. *Biochimica et Biophysica Acta (BBA)-General Subjects*, *1850*(1), 51-61.

71] Agarwal, K. (2013). Antioxidant activity and Phytochemical analysis of Hyptis suaveolens (L.) Poit. *Journal of Advanced Pharmacy Education & Research Oct-Dec*, *3*(4).

72] Hussain, T., Tan, B., Yin, Y., Blachier, F., Tossou, M. C., & Rahu, N. (2016). Oxidative stress and inflammation: what polyphenols can do for us?. *Oxidative medicine and cellular longevity*, *2016*.

73] Quideau, S., Deffieux, D., Douat-Casassus, C., & Pouységu, L. (2011). Plant polyphenols: chemical properties, biological activities, and synthesis. *Angewandte Chemie International Edition*, *50*(3), 586-621.

74] Oteiza, P. I., Erlejman, A. G., Verstraeten, S. V., Keen, C. L., & Fraga, C. G. (2005). Flavonoid-membrane interactions: a protective role of flavonoids at the membrane surface?. *Clinical and Developmental Immunology*, *12*(1), 19-25.

75] Lipinski, C. A., Lombardo, F., Dominy, B. W., & Feeney, P. J. (1997). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced drug delivery reviews*, *23*(1-3), 3-25.

76] Malik, A., Manan, A., & Mirza, M. U. (2017). Molecular docking and in silico ADMET studies of silibinin and glycyrrhetic acid anti-inflammatory activity. *Tropical Journal of Pharmaceutical Research*, *16*(1), 67-74.

77] (asamal@imsc.res.in), A. S. (n.d.). *Imppat: Imppat: Indian medicinal plants, phytochemistry and therapeutics*. IMPPAT | IMPPAT: Indian Medicinal Plants, Phytochemistry And Therapeutics. https://cb.imsc.res.in/imppat/basicsearch/phytochemical

78] U.S. National Library of Medicine. (n.d.). *PubChem*. National Center for Biotechnology Information. PubChem Compound Database. https://pubchem.ncbi.nlm.nih.gov/

79] Botcha, S., & Prattipati, S. D. (2019). Green synthesis of silver nanoparticles using Hyptis suaveolens (L.) Poit leaf extracts, their characterization and cytotoxicity evaluation against PC-3 and MDA-MB 231 cells. *Biologia*, *74*, 783-793.

80] Lautié, E., Quintero, R., Fliniaux, M. A., & Villarreal, M. L. (2008). Selection methodology with scoring system: Application to Mexican plants producing podophyllotoxin related lignans. *Journal of Ethnopharmacology*, *120*(3), 402-412.

81] De Barros, M. P., Lemos, M., Maistro, E. L., Leite, M. F., Sousa, J. P. B., Bastos, J. K., & de Andrade, S. F. (2008). Evaluation of antiulcer activity of the main phenolic acids found in Brazilian Green Propolis. *Journal of ethnopharmacology*, *120*(3), 372-377.

82] Ziegler, H. L., Jensen, T. H., Christensen, J., Stærk, D., Hägerstrand, H., Sittie, A. A., ... & Jaroszewski, J. W. (2002). Possible artefacts in the in vitro determination of antimalarial activity of natural products that incorporate into lipid bilayer: apparent antiplasmodial activity of dehydroabietinol, a constituent of Hyptis suaveolens. *Planta Medica*, *68*(06), 547-549.

83] Chukwujekwu, J. C., Smith, P., Coombes, P. H., Mulholland, D. A., & Van Staden, J. (2005). Antiplasmodial diterpenoid from the leaves of Hyptis suaveolens. *Journal of ethnopharmacology*, *102*(2), 295-297.

84] Agarwal, K. (2013). Antioxidant activity and Phytochemical analysis of Hyptis suaveolens (L.) Poit. *Journal of Advanced Pharmacy Education & Research Oct-Dec*, *3*(4).

85] Hsu, F. C., Tsai, S. F., & Lee, S. S. (2019). Chemical investigation of Hyptis suaveolens seed, a potential antihyperuricemic nutraceutical, with assistance of HPLC-SPE-NMR. *journal of food and drug analysis*, *27*(4), 897-905.

86] Ghaffari, H., Ghassam, B. J., Chandra Nayaka, S., Ramachandra Kini, K., & Prakash, H. S. (2014). Antioxidant and neuroprotective activities of Hyptis suaveolens (L.) Poit. against oxidative stress-induced neurotoxicity. *Cellular and Molecular Neurobiology*, *34*, 323-331.

87] Shenoy, R., & Shirwaikar, A. (1970, January 1). *Anti inflammatory and free radical scavenging studies of Hyptis suaveolens (Labiatae)*. Manipal Academy of Higher Education, Manipal, India. https://researcher.manipal.edu/en/publications/anti-inflammatory-and-free-radical-scavenging-studies-of-hyptis-s

88] Vera-Arzave, C., Antonio, L. C., Arrieta, J., Cruz-Hernández, G., Velázquez-Méndez, A. M., Reyes-Ramírez, A., & Sánchez-Mendoza, M. E. (2012). Gastroprotection of suaveolol, isolated from Hyptis suaveolens, against ethanol-induced gastric lesions in Wistar rats: role of prostaglandins, nitric oxide and sulfhydryls. *Molecules*, *17*(8), 8917-8927.

89] Jesus, N. Z. T., Falcão, H. S., Lima, G. R. M., Caldas Filho, M. R. D., Sales, I. R. P., Gomes, I. F., ... & Batista, L. M. (2013). Hyptis suaveolens (L.) Poit (Lamiaceae), a medicinal plant protects the stomach against several gastric ulcer models. *Journal of ethnopharmacology*, *150*(3), 982-988.

90] Shaikat, M. Z. H., Hossain, M. T., & Azam, G. (2012). Phytochemical screening and antidiarrhoeal activity of Hyptis suaveolens. *International Journal of Applied Research in Natural Products*, *5*(2), 1-4.

91] Mishra, S. B., Verma, A., Mukerjee, A., & Vijayakumar, M. (2011). Anti-hyperglycemic activity of leaves extract of Hyptis suaveolens L. Poit in streptozotocin induced diabetic rats. *Asian Pacific Journal of Tropical Medicine*, *4*(9), 689-693.

92] Ghaffari, H., Ghassam, B. J., & Prakash, H. S. (2012). Hepatoprotective and cytoprotective properties of Hyptis suaveolens against oxidative stress–induced damage by CCl4 and H2O2. *Asian Pacific journal of tropical medicine*, *5*(11), 868-874.

93] Ziegler, H. L., Jensen, T. H., Christensen, J., Stærk, D., Hägerstrand, H., Sittie, A. A., ... & Jaroszewski, J. W. (2002). Possible artefacts in the in vitro determination of antimalarial activity of natural products that incorporate into lipid bilayer: apparent antiplasmodial activity of dehydroabietinol, a constituent of Hyptis suaveolens. *Planta Medica*, *68*(06), 547-549.

94] Ghaffari, H., Ghassam, B. J., Chandra Nayaka, S., Ramachandra Kini, K., & Prakash, H. S. (2014). Antioxidant and neuroprotective activities of Hyptis suaveolens (L.) Poit. against oxidative stress-induced neurotoxicity. Cellular and Molecular Neurobiology, 34, 323-331.

95] Hsu, F. C., Tsai, S. F., & Lee, S. S. (2019). Chemical investigation of Hyptis suaveolens seed, a potential antihyperuricemic nutraceutical, with assistance of HPLC-SPE-NMR. *journal of food and drug analysis*, *27*(4), 897-905.

96] Luz, T. R. S. A., Leite, J. A. C., de Mesquita, L. S. S., Bezerra, S. A., Silveira, D. P. B., de Mesquita, J. W. C., ... & Coutinho, D. F. (2020). Seasonal variation in the chemical composition and biological activity of the essential oil of Mesosphaerum suaveolens (L.) Kuntze. *Industrial crops and products*, *153*, 112600.

97] Luz, T. R. S. A., Leite, J. A. C., de Mesquita, L. S. S., Bezerra, S. A., Silveira, D. P. B., de Mesquita, J. W. C., ... & Coutinho, D. F. (2020). Seasonal variation in the chemical composition and biological activity of the essential oil of Mesosphaerum suaveolens (L.) Kuntze. *Industrial crops and products*, *153*, 112600.