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Theme

Therapeutic Potential and Chemical Composition of Basil Essential Oil

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Appreciation

I would like to express my deepest gratitude to everyone who has supported and guided me throughout the process of writing this thesis.

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TABLE OF CONTENT

Table of Figures

Abstract

Basil essential oil, derived from the leaves of the Ocimum basilicum plant, has been widely recognized for its extensive therapeutic properties. This review highlights its primary bioactive compounds, such as linalool, eugenol, and methyl chavicol, which contribute to its pharmacological activities. The oil exhibits potent anti-inflammatory, antimicrobial, antioxidant, and analgesic properties, making it a valuable remedy in traditional and modern medicine. Studies have shown its efficacy in reducing symptoms of stress and anxiety due to its calming effects on the central nervous system. Additionally, basil essential oil demonstrates significant antibacterial and antifungal activities, offering potential in the treatment of infections and skin conditions. Its antioxidant properties help in combating oxidative stress, thus protecting cells from damage. Furthermore, the oil has been found to alleviate pain and inflammation, making it beneficial in managing conditions such as arthritis and muscle spasms. This comprehensive review underscores the therapeutic potential of basil essential oil. Further research is warranted to explore its full therapeutic potential and possible applications in integrative medicine.

يُعرف زيت الريحان العطري، المستخرج من أوراق نباتOcimum basilicum ، بخصائصه العلاجية الواسعة. يستعرض هذا البحث المركبات النشطة بيولوجيًا الرئيسية فيه، مثل اللينالول والأوجينول والميثيل شافيكول، التي تساهم في أنشطته الدوائية. يظهر الزيت خصائص مضادة للالتهابات ومضادة للميكروبات ومضادة للأكسدة ومسكنة للألم، مما يجعله علاجًا قيمًا في الطب التقليدي والحديث. أظهرت الدراسات فعاليته في تقليل أعراض التوتر والقلق بفضل تأثيراته المهدئة على الجهاز العصبي المركزي. بالإضافة إلى ذلك، يُظهر زيت الريحان العطري نشاطًا كبيرًا مضادًا للبكتيريا والفطريات، مما يوفر إمكانيات في علاج الالتهابات وحالات الجلد. تساعد خصائصه المضادة للأكسدة في مكافحة الإجهاد التأكسدي، وبالتالي حماية الخلايا من التلف. علاوة على ذلك، وجد أن الزيت يخفف الألم والالتهابات، مما يجعله مفيدًا في إدارة حالات مثل التهاب المفاصل وتشنجات العضلات. يؤكد هذا البحث الشامل على الإمكانيات العلاجية لزيت الريحان العطري. هناك حاجة إلى مزيد من البحث لاستكشاف إمكانياته العلاجية الكاملة والتطبيقات الممكنة في الطب التكميلي.

Keywords
Linalool, eugenol, methyl chavicol, pharmacological activities, anti-inflammatory, antibacterial, therapeutic potential.

Introduction

Plants play a crucial role in the pharmaceutical industry, serving as a valuable source of medicinal compounds and natural remedies. For centuries, humans have relied on the diverse array of plant species to discover and develop drugs to treat various ailments. Plants produce a wide range of chemical compounds, including alkaloids, flavonoids, terpenoids, and phenolic compounds, which possess therapeutic properties. [43]

Pharmaceutical research extensively explores plant biodiversity to identify bioactive compounds that exhibit potential medicinal effects. Extracts or isolated compounds derived from plants undergo rigorous testing to determine their efficacy, safety, and mechanism of action. Many well-known drugs in modern medicine, such as aspirin derived from willow bark or morphine from the opium poppy, have plant origins. [43]

Furthermore, plants serve as a sustainable and renewable resource for pharmaceutical production. Cultivation, extraction, and processing methods are employed to obtain the desired active compounds in a cost-effective manner. Additionally, advancements in biotechnology and genetic engineering have allowed for the modification and enhancement of plant species to optimize their pharmaceutical potential. [43]

The study of medicinal plants, known as ethno-pharmacology, explores traditional knowledge and practices of various cultures in utilizing plants for medicinal purposes. This interdisciplinary field combines elements of botany, pharmacology, and anthropology to identify, document, and validate traditional plant-based remedies. [43]

Overall, the utilization of plants in the pharmaceutical industry showcases the significant role of nature in providing valuable therapeutic agents. The exploration of plant-based medicine continues to contribute to the development of new drugs, treatments, and healthcare solutions, highlighting the ongoing symbiotic relationship between plants and human well- being. [43]

Chapter I

Introduction to Basil Oil

I.1 Definition & Origins

I.1.1 Definition

Basil, scientifically known as Ocimum basilicum, is a highly valuable crop known for its essential oils and rich content of polyphenols, phenolics, flavonoids, and phenolic acids. [1]

I.1.2 Origins

This annual plant belongs to the mint family and is native to tropical regions. In addition to its culinary uses, basil leaves offer numerous pharmaceutical benefits and are commonly incorporated into dishes such as rice, meat, stews, and soups. Traditional practices have utilized basil for various conditions including kidney problems, as a hemostatic agent during childbirth, for earaches, menstrual irregularities, arthritis, anorexia, and the treatment of colds and malaria. [1]

Basil has demonstrated positive effects against viral, fungal, bacterial, and other infections. Its leaves have been traditionally employed for the treatment of fevers, coughs, flu, asthma, bronchitis, influenza, and diarrhea. Furthermore, basil seed mucilage, also known as basil seed gum, possesses thickening, stabilizing, fat substitute, texturizing, surface-active, and emulsifying properties. Basil's pharmacological potential is extensive and includes anti- cancer, radioprotective, antimicrobial, anti-inflammatory, immunomodulatory, anti-stress, anti-diabetic, anti-pyretic, anti-arthritic, antioxidant, prophylactic, and cardiovascular disease- related activities. [1]

Overall, basil's remarkable composition and diverse pharmacological properties make it a highly valuable plant in various therapeutic applications. [1]

Basil, belonging to the Ocimum genus, encompasses over 30 species of herbs and shrubs with remarkable variability in terms of morphology, flower color, growth habits, chemical composition, leaves, and stems. This genus is native to Asia, central and southern America, and Africa. In ancient Greece, it was hailed as the "herb of kings." Different languages have their own names for basil, such as Basilic in French, Basilikum in German, Albahaca in Spanish, and Reihan in Persian. Ocimum basilicum L., commonly known as sweet basil, is the most recognized species, while other notable members include Ocimum americanum L., Ocimum hispidulum Schum, Ocimum tenuiflorum L., Ocimum sanctum L., and Ocimum gratissimum L. [1]

Basil holds significant importance in various cuisines, including Iranian, Italian, Chinese, and Indian. Its chemical composition, including essential oil levels, varies among species, cultivars, and growing conditions. Factors such as genotype, cropping seasons, and geographical properties can influence the biochemical components of medicinal plants. [1]

The primary volatile components found in basil include linalool, methyl chavicol, eugenol, bergamotene, and methyl cinnamate. [1]

I.2 Basil Oil Characteristics

Basil oil if rich of various compounds, a study was made by a group of reserchers shows that the dominant volatile components of basil are linalool, methyl chavicol, eugenol, anethole, alpha-cadinol, cinnamic acid and cineole [29], the following table (Table I.1) illustrates the major molecules found in basil oil:

Component	%	
	Egyptian	French
Linalool	14.596	-
Eugenol	-	1.053
Estragole	55.373	87.160
Anatole	6.356	-
Alpha-Cadinol	4.720	1.113
Cinnamic Acid	3.458	-
1,8-Cineole	2.712	1.354

In the same study, eugenol found to be > 24% in basil oil.

In this project we are only concerned with only three famous molecules which have therapeutic effects, these three molecules are Linalool, Estragole and Eugenole, the following figure (Figure I.1) represents the 2D shape of them:

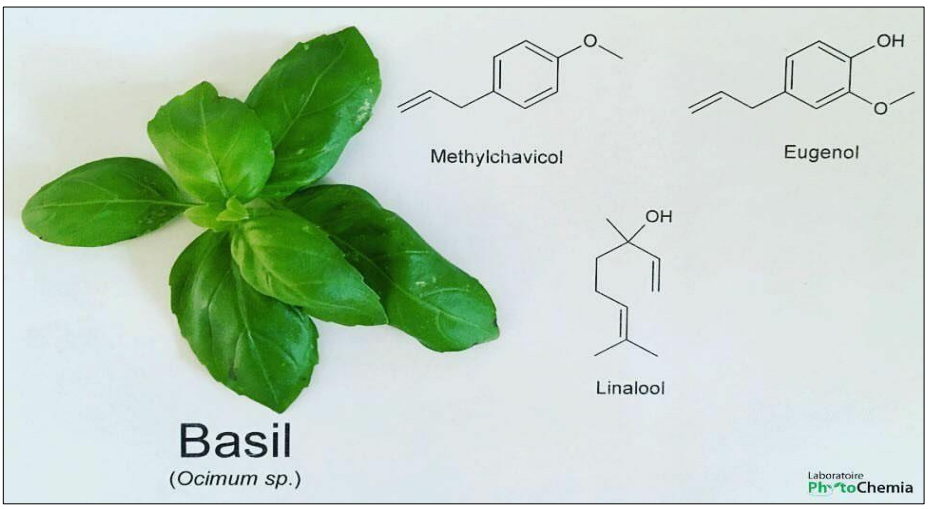


Figure I.1 : Major Basil oil components [Laboratoire Ph to Chemia]

For further understanding of the therapeutic activities of basil oil, we need to study each component individually.

I.1.1 Eugenol (Eugenic acid): Eugenol is a compound that is commonly found in cloves and various other plants [31]. (**Table I.4**) represents properties of eugenol:

IUPAC Name	4-Allyl-2-methoxyphenol
Molecular Formula	$C_{10}H_{12}O_2$
Molecular Weight	164.20 g/mol
Physical Description	Eugenol appears as clear colorless pale yellow or amber-colored liquid. Odor of cloves. Spicy pungent taste. (NTP, 1992)
Boiling Point	489 °F or 253.889 at 760 mmHg (NTP, 1992)
Freezing point	-9.2 to -9.1 °C
Flash Point	>212 °F (>100 °C)
Solubility	In water, 2460 mg/L at 25 °C (Slightly soluble in water; Soluble in ethanol; Soluble in ether, most fixed oils)
Density	1.0652 at 68 °F or 20 °C (NTP, 1992)
Vapour Pressure	0.01 mmHg at 68 °F ; 0.03 mmHg at 77 °F (NTP, 1992)
Viscosity	7.817 centipoise at 20 °C

It is widely used for its aromatic properties and has several applications, including:

I.1.1.1 Flavour and fragrance: Eugenol is known for its pleasant, spicy, and clove-like aroma. It is utilized as a flavouring agent in food and beverages, including baked goods, candies, and chewing gums. Additionally, it is a popular ingredient in perfumes, soaps, and cosmetics due to its appealing scent. [32]

I.1.1.2 Dental applications: Eugenol has been traditionally used in dentistry for its analgesic and antiseptic properties. It is commonly used in dental fillings, cement, and temporary restorative materials. Eugenol's mild numbing effect can help alleviate toothache and gum pain. [31]

I.1.1.3 Antimicrobial properties: Eugenol possesses antimicrobial properties, making it effective against various bacteria, fungi, and viruses. It is often used as an ingredient in mouthwashes, sanitizers, and surface disinfectants.

I.1.1.4 Insect repellent: Eugenol has insect-repelling properties and is used in various formulations to deter pests. It can be found in insect repellent sprays, lotions, and candles. [31]

I.1.1.5 Medicinal uses: Eugenol has been studied for its potential therapeutic benefits, including anti-inflammatory and antioxidant properties. It has been investigated for its role in pain management, cardiovascular health, and gastrointestinal disorders. [31]

It is important to note that while eugenol is generally considered safe when used in appropriate amounts, it can cause skin irritation or allergic reactions in some individuals. [32]

I.1.2 Linalool: Linalool is a naturally occurring compound found in many flowers and spice plants, particularly in lavender, coriander, and citrus fruits. **(Table I.2)** illustrates propreties of

Linalool [10]:

IUAPC Name	3,7-Dimethyl-1,6-octadien-3-ol
Molecular Formula	$C_{10}H_{18}O$, $(CH3)_2C=CH(CH2)_2C(CH3)(OH)CH=CH2$
Molecular Weight	154.25 g/mol
Physical Description	Colorless to pale yellow liquid with an odor of bergamot oil or French lavender with Spicy, citrus taste.
Boiling Point	194.00 to 197.00 °C. at 760.00 mm Hg
Freezing point	below -74 °C
Flash Point	71 °C to 78 °C
Solubility	In water, 1590 mg/L at 25 °C (Soluble in alcohol, ether, fixed oils, propylene glycol; insoluble in glycerine)
Density	0.865 at 15 °C
Vapour Pressure	0.16 [mmHg]
Viscosity	4.465 mPa s at 25 °C (dynamic)

It is widely used for its pleasant aroma and has various applications, including:

I.1.2.1 Fragrance and perfumery: Linalool is valued for its floral and sweet scent. It is a common ingredient in perfumes, colognes, and personal care products such as soaps, lotions, and candles. Linalool's fragrance profile is often described as calming and relaxing. [10]

I.1.2.2 Aromatherapy: Due to its soothing and calming properties, linalool is frequently used in aromatherapy. It is believed to have stress-relieving and anxiety-reducing effects when inhaled or applied topically in diluted form. [10]

I.1.2.3 Flavouring agent: Linalool is used as a flavor enhancer in various food and beverage products. It is added to baked goods, beverages, chewing gums, and candies to provide a subtle citrusy or floral note.[10]

I.1.2.4 Antimicrobial properties: Linalool exhibits antimicrobial activity against certain bacteria and fungi. It is sometimes utilized as a natural preservative in cosmetic and personal care products to inhibit the growth of microorganisms. [10][30]

I.1.2.5 Insect repellent: Linalool has been found to repel certain insects, including mosquitoes, flies, and cockroaches. It is often incorporated into insect repellent products, including sprays, lotions, and candles. [10]

I.1.2.6 Medicinal uses: Linalool has been studied for its potential health benefits. It is believed to have anti-inflammatory, analgesic, and antioxidant properties. Some research suggests that linalool may have potential in pain management, skin conditions, and neuroprotective effects. [10][30]

It's important to note that while linalool is generally regarded as safe, some individuals may have sensitivities or allergic reactions to it.

I.1.3 MethylChavicol (Estragole): Methyl chavicol, also known as Estragole, is a compound commonly found in various plants, including basil, tarragon, and anise. [11] (**Table I.3**) shows the properties of Estragole:

IUPAC Name	1-methoxy-4-prop-2-enylbenzene
Molecular Formula	$C_{10}H_{12}O$
Molecular Weight	148.20 g/mol
Physical Description	Estragole is a Colourless to light yellow liquid with odour of anise. Insoluble in water.
Boiling Point	421 °F (216 °C) at 764 mmHg (NTP, 1992)
Freezing point	N/A
Flash Point	81 °C (178 °F)
Solubility	In water, 178 mg/L at 25 °C (Very soluble in ethanol, chloroform)
Density	0.9645 at 70 °F (NTP, 1992)
Vapour Pressure	0.165 mm Hg at 25 °C (est)
Viscosity	N/A

It has several uses and applications, including:

I.1.3.1 Flavouring agent: Methyl chavicol is known for its sweet, anise-like flavour. It is used as a flavouring component in food and beverages, particularly in products like liqueurs, herbal teas, and confectionery. [11]

I.1.3.2 Aromatic properties: Methyl chavicol possesses a pleasant aroma, often described as sweet, spicy, and reminiscent of licorice. It is used in the production of perfumes, colognes, and other scented products. [11]

I.1.3.3 Traditional medicine: In certain traditional medicine systems, methyl chavicol has been used for its potential medicinal properties. It has been attributed with various benefits, including digestive aid, anti-inflammatory effects, and as a remedy for coughs and respiratory issues. [11]

I.1.3.4 Insecticidal properties: Methyl chavicol has been studied for its insecticidal properties and its potential use as a natural insecticide. It has shown effectiveness against

certain pests and is being explored as an alternative to synthetic insecticides in some cases. [11]

I.1.3.5 Research and studies: Methyl chavicol is the subject of ongoing research and studies to explore its potential health benefits and applications. It is being investigated for its antioxidant, antimicrobial, and anticancer properties. [11]

Some studies have raised concerns about potential toxicity and carcinogenicity in high doses, so it is important to consider the dosage and source of methyl chavicol when using it for any purpose. [12]

Chapter II

Extraction & Isolation

I. Extraction Methods of Basil Oil

There is several extraction methods used to obtain basil oil from the basil plant. The most common methods include:

I.1 Steam Distillation: This is the most traditional and widely used method for extracting essential oils, including basil oil. It involves the use of steam to extract the volatile compounds from the basil leaves. The steam passes through the plant material, carrying the essential oil molecules. The steam is then condensed, and the oil is separated from the water.

I.2 Solvent Extraction: Solvent extraction involves using a solvent, typically ethanol or hexane, to dissolve the essential oil from the basil leaves. The solvent is then evaporated, leaving behind the concentrated basil oil. This method is often used for delicate plant materials that may not withstand the high temperatures of steam distillation.

I.3 Cold Press Extraction: Cold pressing is commonly used for obtaining basil oil from fresh basil leaves. The process involves mechanically pressing the basil leaves to release the essential oil. No heat is applied during this method, which helps preserve the aroma and therapeutic properties of the oil.

I.4 CO_2 Extraction: Carbon dioxide (CO_2) extraction is a method that utilizes pressurized carbon dioxide to extract the essential oil. The CO_2 acts as a solvent at high pressure and temperature, effectively extracting the oil from the basil leaves. This method is known for producing high-quality oils but requires specialized equipment.

Each extraction method has its advantages and may yield slightly different characteristics in the resulting basil oil. Steam distillation is the most commonly used method for basil oil extraction due to its efficiency and ability to retain the aromatic compounds. However, the choice of extraction method may depend on the specific requirements of the manufacturer and the desired qualities of the basil oil.

Let’s discuss each of these methods separately:

I.1 Steam Distillation

I.1.1 Materials & Instruments

- Dried Basil Leaf, Crusher, Distilled water, Boiler, Condenser, collection flask.

I.1.2 Experimental Process

- Assemble the distillation apparatus according to the manufacturer's instructions Ensure that all the connections are tight and secure.
- **Prepare the basil leaves**: Clean the basil leaves thoroughly to remove any dirt or impurities. Roughly chop the leaves to increase the surface area for better extraction.
- **Load the distillation flask**: Place the chopped basil leaves into the distillation flask then adding the distilled water in which the ratio of water to basil leaves will be (3.2: 1 basil leaves).
- **Start Heating**: After placing the distillation set-up, we start heating gradually to the flash point of the basil oil, The flash point of basil oil can vary depending on the specific composition and quality of the oil. However, in general, the flash point of basil oil falls within a range of approximately 57-73 degrees Celsius (135-163 degrees Fahrenheit).
- **Condensation**: As the steam rises from the distillation flask, it enters the condenser, which cools it down and causes it to condense. The condensed liquid, which contains the basil oil, collects in the collection flask.
- **Collect the basil oil**: Once the distillation process is complete, we carefully remove the collection flask containing the basil oil from the condenser. Allow it to cool before sealing it in a dark, airtight container to preserve its quality.

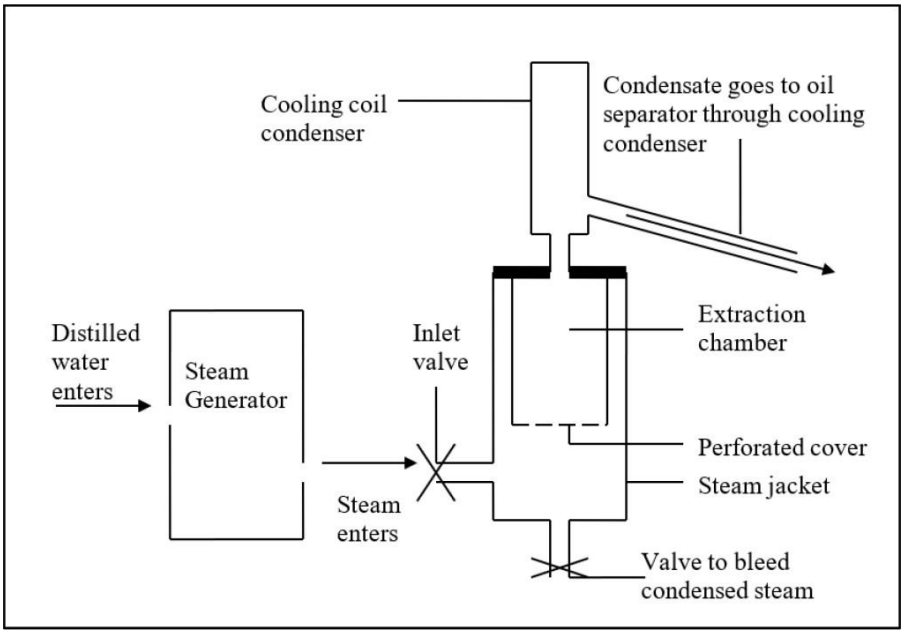


Figure II.1: A schematic flow chart of the Steam Distillation [2]

I.2 Solvent Extraction

An experimental process was made with the collaboration between (the Department of Chemical Engineering, College of Engineering, University of Baghdad, Baghdad, Iraq) and (Department of Chemical and Petrochemical Engineering, College of Engineering, University of Anbar, Anbar, Iraq) in which they used n-hexane and petroleum ether as organic solvents. [3]

I.2.1 Experimental Process

Ocimum basillicum leaves were obtained from middle Baghdad city, Iraq. The solvents used were n-hexane (95 % ALDRICH) and petroleum ether (BDH). [3]

The Basil leaves were dried in an oven at a temperature of 50 °C. Once dried, the leaves were weighed using a digital balance with four decimal points (Sartorius BL 2105). The dried leaves and solvents were then combined in the extraction reaction flask. To control the temperature, the extraction flask (0.5 L) was immersed in a water bath. An electrical mixer was used to thoroughly mix the contents. The solvents used were n-Hexane and petroleum ether, with a ratio of 40 mL solvent to 1 gram of solids, The solubility of oil in hexane was high because of the strong solute-solvent interactions. [3]

The extraction of natural products progresses through the following stages: (1) the solvent penetrates into the solid matrix; (2) the solute dissolves in the solvents; (3) the solute is diffused out of the solid matrix; (4) the extracted solutes are collected. [4]

I.2.2 Simplification of the solvent extraction process

- **Plant Material Preparation**: The first step involves collecting fresh basil leaves and stems. The plant material is usually dried and crushed to increase the surface area for efficient extraction. [3][4]
- **Solvent Selection**: A suitable organic solvent is chosen based on its ability to selectively dissolve the essential oil components while minimizing the extraction of unwanted substances. Common solvents include ethanol, hexane, and supercritical carbon dioxide. [3][4]
- **Extraction**: The plant material is placed in an extraction vessel, and the solvent is added to it. The mixture is then heated or agitated to facilitate the transfer of essential oil components from the plant material to the solvent. This step allows the solvent to

dissolve the essential oils, in our case the temperature is between 40-60°C , the Agitation is between 100-300 rpm.[3]

- **Separation**: After the extraction process, the mixture of solvent and essential oil is obtained. The next step involves separating the solvent from the extracted oil. This can be achieved through various techniques such as filtration, decantation, or centrifugation. [3][4]
- **Solvent Recovery**: To minimize waste and make the process more sustainable, the solvent is often recovered for reuse. Techniques like distillation or evaporation are employed to remove the solvent from the extracted oil, leaving behind pure basil oil. [3][4]
- **Quality Assessment**: The extracted basil oil undergoes quality assessment to ensure it meets the desired standards. This may include analysing its chemical composition, aroma, and other relevant characteristics. [3][4]
- **Storage and Packaging**: Once the basil oil is deemed suitable, it is typically stored in dark airtight containers to preserve its freshness and potency. Proper labelling and packaging are done before the final product is ready for distribution or sale. [3][4]

I.3 Cold Press Extraction

This is the general description of the cold press extraction method for basil oil:

- **Harvesting**: Basil plants are typically harvested when they have reached their peak oil content. The leaves and stems are usually used for extraction. [5]
- **Preparation**: The harvested basil is cleaned to remove any dirt, debris, or impurities. It is important to ensure the plant material is in good condition before proceeding. [5]
- **Grinding**: The cleaned basil is then ground or chopped into smaller pieces to facilitate oil extraction. This step helps break down the plant cells and release the essential oil. [5]
- **Pressing**: The ground basil is placed in a hydraulic press or an oil extraction machine specifically designed for cold-pressing. Pressure is applied to the plant material, and the essential oil is gradually released. [5]
- **Separation**: The extracted oil, along with other liquid components, is collected. To separate the basil oil from any remaining liquid or water, the mixture may go through a separation process like filtration or centrifugation. [5]

- **Storage**: The obtained basil oil is typically stored in dark, airtight containers to protect it from light, heat, and oxidation. Proper storage conditions help maintain the oil's quality and extend its shelf life. [5]

This method doesn't involve the use of high temperatures or chemicals. It is believed to preserve the natural qualities of the basil oil, including its aroma, flavor, and potential therapeutic properties. [5]

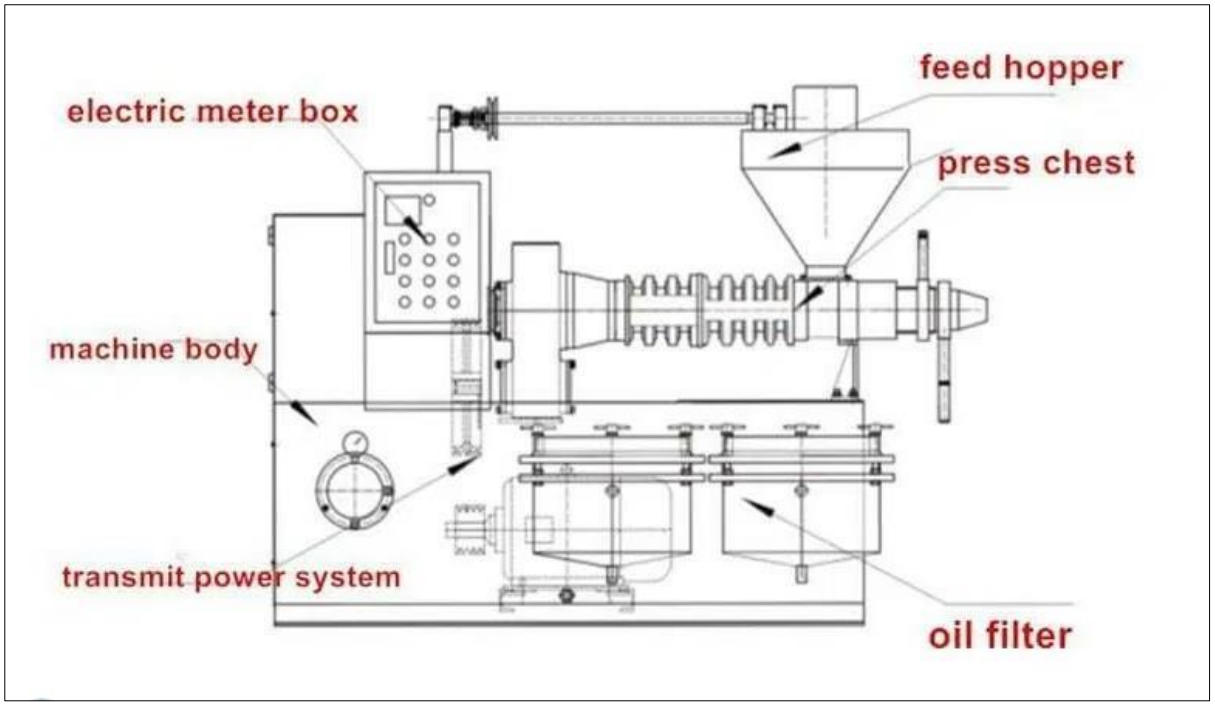


Figure II.2: Cold press Oil Machine (zcoilpress.com)

I.4 CO_2 Extraction

Supercritical carbon dioxide (CO_2) fluid extraction equipment is purposefully engineered to facilitate the extraction of target compounds by employing supercritical CO_2 as the solvent. This extraction technique finds extensive applications across diverse industries including pharmaceuticals, food and beverages, cosmetics, and natural product extraction. The range of available supercritical CO_2 fluid extraction equipment encompasses a variety of sizes and configurations, encompassing both small-scale laboratory units and large-scale industrial systems. The choice of equipment is contingent upon the precise extraction prerequisites, desired production capacity, and financial constraints. [6]

I.4.1 Working Principle

A supercritical fluid (SF or SCF) refers to a substance that exists at a temperature and pressure surpassing its critical point, rendering the separate liquid and gas phases indistinguishable. Such a fluid possesses the ability to dissolve materials akin to a liquid and permeate solids similar to a gas. It exhibits distinctive characteristics, including high density, low viscosity, and a substantial diffusion coefficient, thereby affording commendable solubility and transport properties. Notably, when a supercritical fluid approaches its critical point, its solubility becomes highly sensitive to variations in pressure and temperature. Consequently, by regulating these parameters, operators can conveniently manipulate the fluid's solubility, transport, and reaction capabilities. [6]

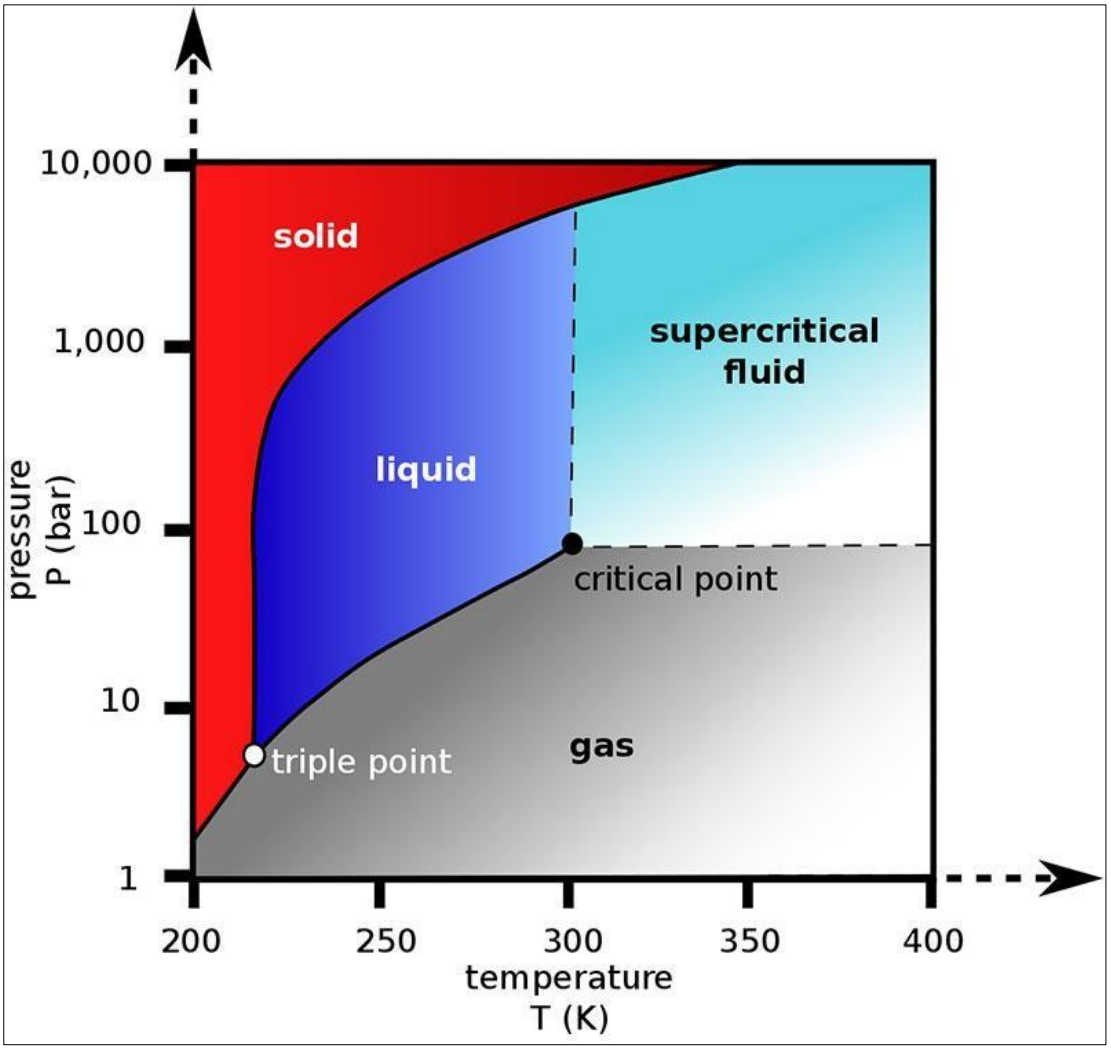


Figure II.3: Phases Diagram shows the range of the super critical state determined by pressure and temperature. [6]

I.4.2 Extraction Flowchart

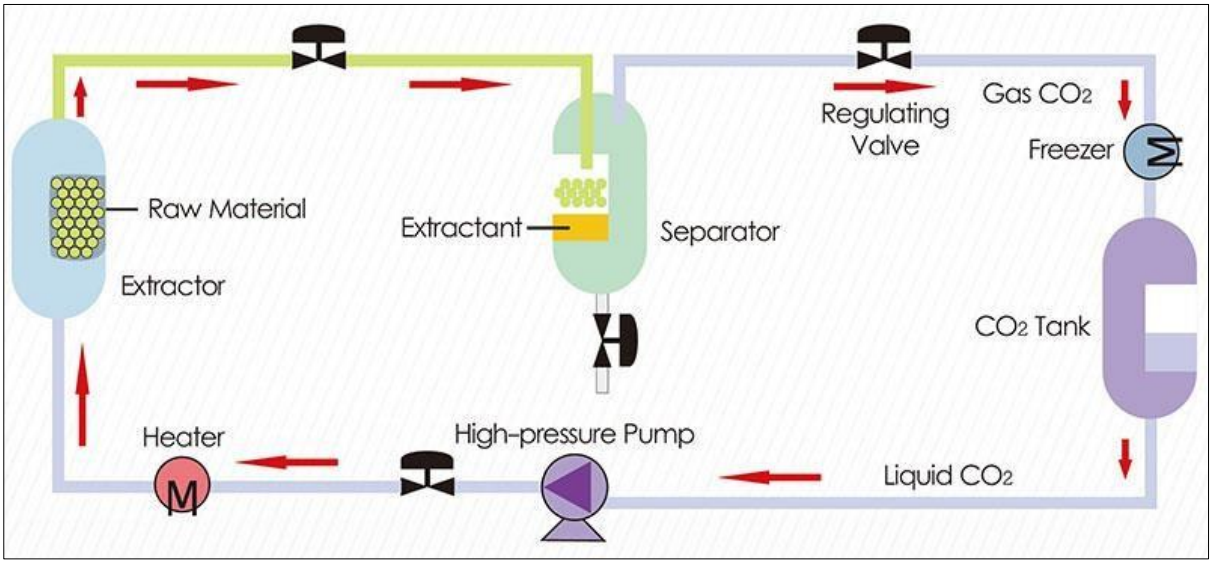


Figure II.4: Flowchart of CO_2 supercritical extraction pathway [6]

II. Analysis of Basil Essential Oil

To ensure the quality of the extracted essential oil, we need to pass through some analysis techniques in which the compounds of our oil are specifically determined.

There are many Chemical analysis methods, and we will discuss one of the most used Method in this field.

II.1 Gas Chromatography Mass Selective (CGMS)

The essential oils were extracted through steam distillation using a **Clevenger-type device**. Their chemical composition was determined through gas chromatography mass selective analysis. The antioxidant properties of these oils were evaluated using 1,1-diphenyl-2- picrylhydrazyl assays, while the tyrosinase inhibition abilities were measured through spectrophotometric analysis. Additionally, the antimicrobial activity of the essential oils was determined using the agar diffusion method, and the minimal inhibitory concentrations were recorded. [7]

II.1.1 Results

The essential oil from O. basilicum var. purpureum primarily consisted of 57.3% methyl- chavicol (estragole), while O. basilicum var. thyrsiflora oil contained 68.0% linalool. O. citriodorum oil, on the other hand, had nerol (23.0%) and citral (20.7%) as its main constituents. [7]

Chapter III

Pharmacology of Basil Oil

I. Introduction

Basil essential oil used as the treatment of various ailments such as anxiety, fever, infections, insect bites, stomach discomfort, coughs, headaches, and constipation . Moreover, it possesses the potential to regulate and reduce blood glucose levels, thanks to its anti- spasmodic and anti-diabetic properties. Previous studies have also demonstrated the oil's antimicrobial, antifungal, and antioxidant activities. Notably, the significant medicinal characteristics of basil essential oil include its antifungal effects, ability to combat nematodes, and antibacterial effects against food-borne pathogenic bacteria. [8]

In this particular chapter, we will touch the pharmacology of our three (3) major components in the extracted essential oil of basil which are: Estragole, Linalool and Eugenol.

Each one of these has its own effects on body as well as the body has its own reactions for each one of them.

II. Eugenol

II.1 Absorption – Distribution - Elimination

After an intraperitoneal injection of a single 450 mg/kg dose of C(14) methoxy labelled eugenol, it was quickly distributed to all organs. Most tissues and excretions contained both ether- and water-soluble materials. Only 0.2-1.0% of the dose was released as expired CO_2. When rabbits were given a lethal dose of eugenol, over 70% of it was found in their urine after death. [13]

II.2 Metabolism / Metabolites

The metabolism of eugenol was investigated in isolated rat hepatocytes, and it was found that incubation of hepatocytes with eugenol led to the formation of conjugates with sulfate, glucuronic acid, and glutathione. The primary metabolite formed was the glucuronic acid conjugate. [14]

The dihydrodiol metabolite of eugenol has been found in the liver homogenates and urine of rats that were pre-treated with eugenol, this metabolite is a result of the metabolism of eugenol in the body. [15]

II.3 Toxicity and carcinogenicity of Eugenol

The topical and herbal use of low concentrations of eugenol and clove extracts has not conclusively demonstrated a connection to liver injury, either through serum enzyme elevations or clinically apparent liver issues. However, when taken in high doses, eugenol can act as a direct cytotoxin. There have been documented cases of severe acute liver and kidney injury resulting from accidental overdoses of herbal products containing eugenol, particularly in children. Overdoses are characterized by symptoms such as agitation, decreased consciousness, and coma occurring shortly after ingestion (typically 10-30 mL of clove oil). [16]

These incidents are often accompanied by acidosis, respiratory depression, and severe hypoglycemia, necessitating ventilation and intravenous glucose. Liver injury manifests 12 to 24 hours post-ingestion, with notable elevations in serum aminotransferase levels and early coagulation abnormalities. Rapid onset of signs of hepatic failure, deepening jaundice, and a clinical presentation resembling acute hepatic necrosis, akin to acetaminophen, iron, or copper overdose, are observed. Although the liver injury worsens initially, it subsequently improves and resolves within 1 to 3 weeks. Renal dysfunction may occur, but intervention or dialysis is rarely required. Long-term effects or injuries have not been reported, and cases in the literature often involve infants who ingested clove oil used by their parents. [16]

III. Linalool

III.1 Absorption - Metabolism – Excretion

III.1.1 Absorption

Based on experiments conducted on rats using a labelled substance called C (14), it has been observed that linalool is rapidly absorbed into the bloodstream after oral ingestion. The delay in fecal excretion indicates that the absorption in the intestines is complete. [9]

III.1.2 Metabolism

Once absorbed, linalool is quickly metabolized, leading to the immediate excretion of C (14) activity in the urine. Additionally, significant amounts of radioactivity were detected in the expired air as CO_2 a few hours after administration, indicating complete intermediary metabolism. The excretion of radioactivity through feces was delayed and primarily occurred between 36 and 48 hours after dosing, suggesting entero-hepato-biliary re-circulation. This re-circulation was confirmed in a subsequent experiment involving the connection of a treated rat and an untreated rat with a biliary-to-intestinal cannula, followed by radio-analysis. [9]

III.1.2.1 Metabolites

After rats were orally given linalool (VII), the urine samples revealed the presence of two metabolites: 8-hydroxy-linalool (VIII) and 8-carboxy-linalool (IX). Following a three-day period of feeding rats with either geraniol or linalool, there was an observed increase in liver- microsomal cytochrome P-450. However, the activities of NADH (Nicotinamide Adenine Dinucleotide Hydrogen) and NADPH (Nicotinamide Adenine Dinucleotide Phosphate Hydrogen)-cytochrome c reductase remained unchanged throughout the six-day treatment duration. Notably, the oral administration of these two terpenoids had no discernible impact on any of the measured lung-microsomal parameters. [9]

During the induction study, male (IISc) strain rats were administered 600 mg of linalool per kilogram of body weight once daily for six days. This was done by administering a suspension of linalool in a 1% methyl cellulose solution through a gastric tube. Control rats were given only the vehicle without linalool. For metabolite identification purposes, a dosage of 800 mg linalool per kilogram of body weight was administered once daily for a duration of 20 days. [9]

The presence of 8-hydroxy-linalool and 8-carboxy-linalool in the urine indicated the selective oxidation of the C8-methyl in linalool. The 8-hydroxylase enzyme, present in both lung and liver microsomes, was found to be mediated by the cytochrome P-450 (CYP450) system. [9]

Following three days of dosing, there was an increase in liver and lung microsomal CYP450 activity. However, the activities of both NADH- and NADPH-cytochrome c reductase remained unchanged throughout the six-day treatment period. It's worth noting that the purity of linalool used in the study was over 99.5%. [9]

III.1.3 Exertion

In total, approximately 60% of the administered dose was excreted in the urine within 72 hours, around 23% was detected in the exhaled air, and roughly 15% was found in the feces. There is no evidence of linalool accumulation in the tissues. The study suggests that in rats, large oral doses of linalool are metabolized through conjugation and excreted in urine and bile, while a significant portion undergoes intermediary metabolism resulting in the formation of carbon dioxide and subsequent pulmonary excretion. Entero-hepato-biliary re-circulation may increase the metabolic burden on the liver for a certain period. [9]

III.2 Toxicity

Sensitization: Linalool, when tested on humans at concentrations up to 20%, did not exhibit sensitization. Sensitization refers to the development of an allergic reaction upon repeated exposure to a substance. In human maximization tests, linalool did not cause sensitization. [10]

Phototoxicity and photoallergenicity: Linalool was also tested for its potential to cause phototoxicity and photoallergenicity in humans. Phototoxic substances can cause adverse skin reactions when exposed to sunlight, while photoallergenic substances can trigger an allergic reaction when exposed to both the substance and sunlight. In human tests, linalool was found to be neither phototoxic nor photoallergenic. [10]

Allergic contact dermatitis: Linalool, however, can cause allergic contact dermatitis. Allergic contact dermatitis is a skin condition that occurs when the skin comes into contact with a substance to which the individual is allergic. In the case of linalool, it can lead to the development of allergic contact dermatitis in susceptible individuals. [10]

Genotoxicity: Linalool was tested for its genotoxic potential using the micronucleus test on peripheral human lymphocytes in vitro. Genotoxicity refers to the ability of a substance to cause damage to genetic material (DNA) within cells. The test results indicated that linalool did not exhibit genotoxic effects when tested on human lymphocytes in vitro. [10]

Linalool is generally well-tolerated by humans at concentrations up to 20% and does not cause sensitization, phototoxicity, or photoallergenicity. However, it is important to note that linalool can cause allergic contact dermatitis in certain individuals. Additionally, based on in vitro testing, linalool did not show genotoxic effects on human lymphocytes. [10]

IV. Estragole (Methyl chavicol)

IV.1 Absorption – Distribution - Metabolism – Excretion (ADME)

In rats and mice, the major metabolic pathways of Estragole have been identified. At low doses of Estragole, the primary pathway involves O-demethylation, which results in the production of CO_2 as the final metabolite. However, as the dose of Estragole increases, the proportion of O-demethylation decreases, and other pathways, particularly 1'-hydroxylation, become more prominent. [11]

In female Wistar albino rats, when single doses of Estragole were administered orally in the range of 0.05 to 50 mg/kg bw (body weight), a significant portion (52-58%) of the administered dose was excreted as CO_2. However, at higher doses of 500 and 1000 mg/kg bw, the excretion of CO_2 accounted for only 28-29% of the administered dose. Instead, the metabolite 1-hydroxyestragole was excreted in the urine, representing 1.3-5.4% of the dose in the lower dose range or 11.4-13.7% in the higher dose range. Similar results were observed in CD-1 mice that were dosed intraperitoneally with Estragole in the range of 0.05 to 50 mg/kg bw. [11]

These findings suggest that at low doses, O-demethylation is the predominant metabolic pathway for Estragole in both rats and mice. However, as the dose increases, 1'-hydroxylation becomes more significant. [11]

Regarding human studies, two volunteers were given oral doses of estragole at a daily dose of 100 µg for 6 months. The excretion of 1'-hydroxyestragole in their urine amounted to 0.2% and 0.4% of the administered dose for each volunteer, respectively. This indicates that in humans, the excretion of the 1'-hydroxyestragole metabolite is relatively low 4compared to the administered dose. [11]

IV.2 Effects of Estragole on Animals

The following Table (Table III.1) shows the effects of Estragole on different animals: [12]

Animal	Dose rate	Major Findings
Mice	Oral Estragole Supplementation at Dosage of 100 mg/kg	Enhance humoral and immune responses of mice infected with T. gondii
Mice	Estragole was given orally at dosage of 30 mg/kg	Inhibits peritoneal vascular permeability and leukocyte emigration.
Mice	Oral Estragole Supplementation at Dosage of 250 - 750 mg/kg	The anti-inflammatory activity of Estragole was demonstrated by stimulating macrophage and phagocytic activity and inhibiting leukocyte recruitment
Dog	Powdered basil (Ocimum basillicum) leaves are mixed with dog food at dosage of 0.025% - 0.05%	It may prevent DM by modulating blood glucose and improving antioxidant status in Rottweiler dogs.
Chicken	Chicken fed a normal diet supplemented 1% to 0.5% OBLP (Ocimum basillicum leaves powder)	Increased HI antibodies to NDV vaccines and show anti- oxidative activity
Chicken	970g corn silage per kilogram combined with 30g dried basil per kg (Ocimum basillicum)	Increased egg production
Broiler	In drinking water, broilers supplemented with 5mg/kg Ocimum basillicum + 75ppm GA3 (Gibberellic Acid)	Basil dramatically enhanced IgG and IgM antibody levels as well as antioxidant activity (SOD, CAT, GSH, GPX, GST)
Broiler	Broilers supplemented with basil (Ocimum basilicum) flour in feed up to 3%	Reduced the mortality in broiler chickens and excreta NH3 and H&S gases
Broiler	Basal diet supplemented with 5 g/kg basil seed (Ocimum basilicum) and 200 mg/kg ascorbic acid.	Improved intestinal villus, increased crude protein (CP). crude fiber (CF), and metabolized energy absorption (ME). Increase white blood cell count, hemoglobin, antibody titer to new castle disease, weight growth, and feed conversion ratio.
Broiler	Basal diet supplemented with basil leaves (Ocimum gratissimum) 5-15 g/bird	Increased FCR, weight, and total feed consumption
Broiler	The base food was treated with 0.3 ml. of fennel oil per kg body weight.	Increase phagocytic index, phagocytic activity, leukocytes, globulins, heterophils, total protein, A/G ratio, T3, T4, IgM, and IgG
Broiler	Feed was added with 0.30% fennel seed powder (Foeniculum vulgare Mill.)	Improved intestinal morphology and promotion of healthy and effective development
Broiler	Basal diet supplemented with 3.2% fennel seeds	Fennel seed powder could be used to enhance the broiler's tolerance during chronic heat stress conditions.
Quail	Basal diet supplemented with basil (Ocimum basillicum L.) essential oil 250 - 500 mg/kg.	Increase body weight, feed intake, quail FCR, total protein, globulin, and albumin, and decrease the overall quantity of dangerous bacteria and the total number of fungi.
Quail	Basal diet supplemented with fennel seeds supplemented at 1% of their body weight.	Improved mean body weight

IV.3 Toxicity and carcinogenicity of Estragole

Estragole's carcinogenicity is believed to occur through a genotoxic mechanism. It is thought to act as a genotoxic hepatocarcinogen in the liver of rodents by forming DNA adducts, which are responsible for its genotoxic effects. [12]

The genotoxicity of Estragole is linked to its metabolism in the liver by cytochrome P450 enzymes, which result in the formation of 1'-hydroxy Estragole and certain DNA-reactive epoxides. [12]

In vitro studies have shown that Estragole epoxides, specifically estragole-2',3'-oxide and 1'hydroxy estragole-2',3'-oxide are hepatocarcinogenic and can form DNA adducts, However, in vivo administration tests of Estragole have not discovered the associated DNA adduct for Estragole epoxide, suggesting that its role in tumorigenicity may be modest. [12]

Estragole isolated from basil, when converted to the carcinogenic 10-sulfoxymetabolite, may exhibit carcinogenic and genotoxic properties. [12]

Some studies have shown that prolonged exposure to high doses of Estragole can induce cell death (apoptosis) and affect the locomotor system of organisms such as (D. melanogaster flies). [12]

Studies in mice consuming low doses of food containing estragole have not shown toxicity. [12]

Overall, while there is evidence suggesting the toxicity and carcinogenicity of estragole, further research is needed to determine the specific doses at which these effects may occur. [12]

Chapter IV
In Silico Study of Basil Oil

I. Introduction

Molecular docking is a computational technique used in structural molecular biology and computer-assisted drug design. It aims to predict the preferred orientation of a ligand (e.g., a small molecule drug) when bound to a target protein to form a stable complex. This technique is valuable in lead optimization, virtual screening of compound libraries, and predicting the binding conformation of small molecule ligands to a target protein. Successful docking methods use scoring functions to rank candidate dockings and can be used to predict the strength of association or binding affinity between the molecules. Molecular docking is widely used in structure-based drug design and has the potential to lead to the development of new therapeutics and medicines. [17]

II. Programs Used

- **MGL Tools:** a software application, utilizes AutoDock tools to predict the binding of small molecules, such as substrates or potential drugs, to a receptor of a known three-dimensional structure.
- **PyMole:** PyMOL is a user-sponsored open-source molecular visualization system maintained and distributed by Schrödinger, It is a popular and widely used software for rendering and animating 3D molecular structures, with a focus on creating high- quality images for scientific publications and presentations.
- **AutoDock Vina:** AutoDock Vina is an open-source program for molecular docking and virtual screening. It is one of the fastest and most widely used docking engines, designed to predict the preferred orientation of a small molecule ligand when bound to a protein receptor. AutoDock Vina is based on a simple scoring function and rapid gradient-optimization conformational search.
- **Biovia Discovery Studio:** BIOVIA Discovery Studio is a comprehensive modeling and simulation software suite for drug discovery and life sciences research, The software suite includes applications for:
    - Molecular modelling
    - Molecular design
    - Macromolecule engineering

III. Mechanism of action of Anti-Inflammatory Molecule

NSAIDs (non-steroidal anti-inflammatory drugs) block a specific enzyme called cyclooxygenase (or COX) used by the body to make prostaglandins. By reducing production of prostaglandins, NSAIDs help relieve the discomfort of fever and reduce inflammation and the associated pain. [18]

III.1 Mechanism of action Eugenol as an Anti-Inflammatory

Eugenol can inhibit enzymes such as cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5- LOX), which are involved in the production of pro-inflammatory prostaglandins and leukotrienes, respectively. By blocking these enzymes, eugenol helps mitigate inflammation. [43]

IV. In Silico study of Eugenol anti-inflammatory Activity

IV.1 Preparation of the COX-2

From the Protein Data Bank (PDB) website, we downloaded COX-2 under its Code (1CX2).

By using Python Molecule viewer (MGL Tools), we dropped COX-2 inside it in order to eliminate water molecules so as not to interfere with interactions, we added polar hydrogens and Colman charges as shows (Figure IV.1) shows:

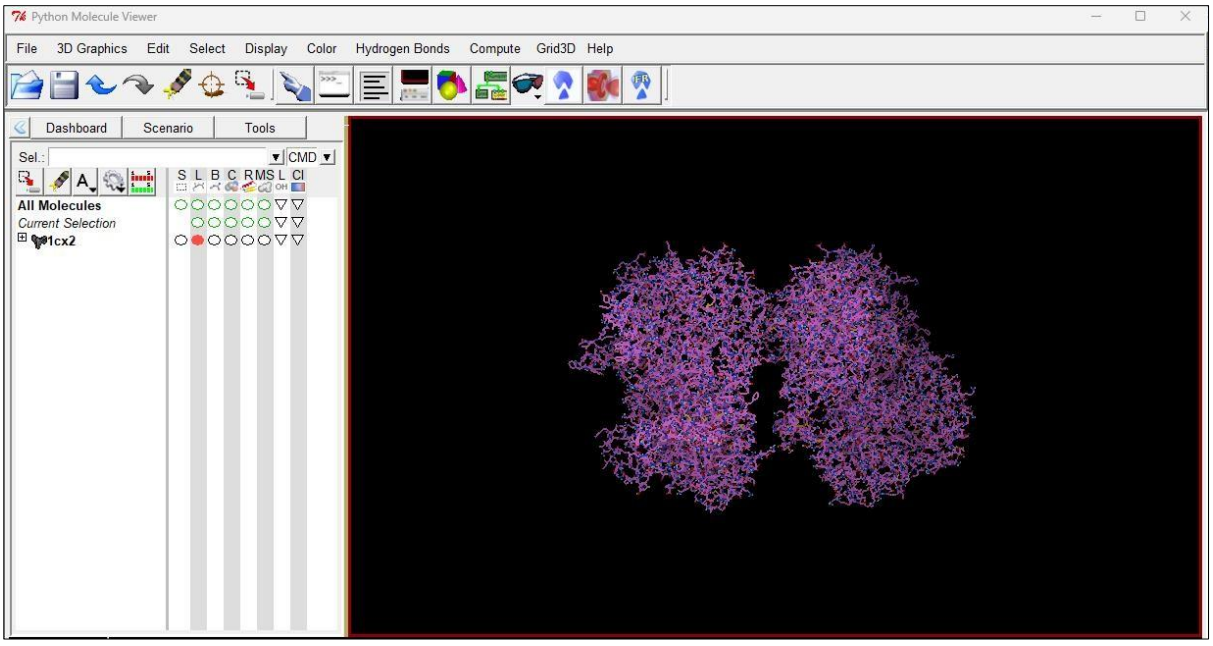


Figure IV.1: COX-2 Preparation in MGL Tools

IV.2 Preparation of Eugenol

We went to PubChem website to download Eugenol the 3D Conformer of Eugenol, then we used PyMol program to convert the file from sdf to pdb.

We went back to MGL Tools to prepare the EUG Molecule by clicking on Ligand > Input > Choose > Eugenol > Select molecule as (Figure IV.2) represents:

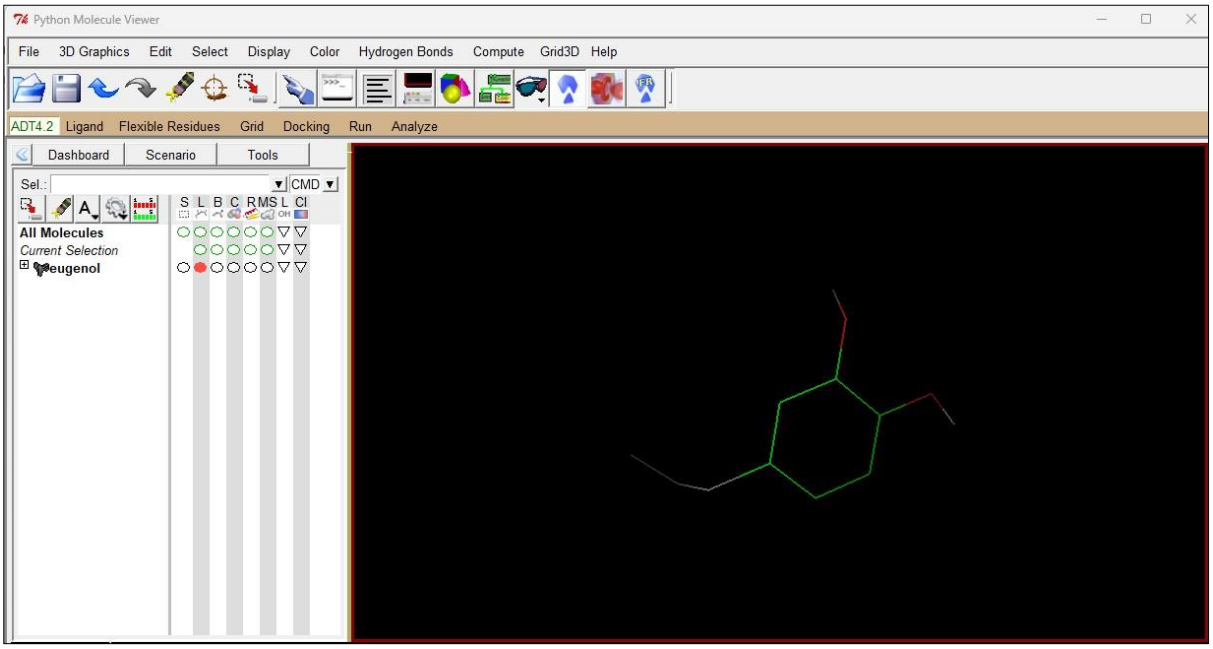


Figure IV.2: EUG Molecule after preparation.

After that, we saved the compound as a PDBQT file in my eugenol file.

IV.3 Interaction

After the preparation of COX-2 And EUG, we moved to the interaction step where we input my two prepared compounds under PDBQT format in MGL Tools as shown below:

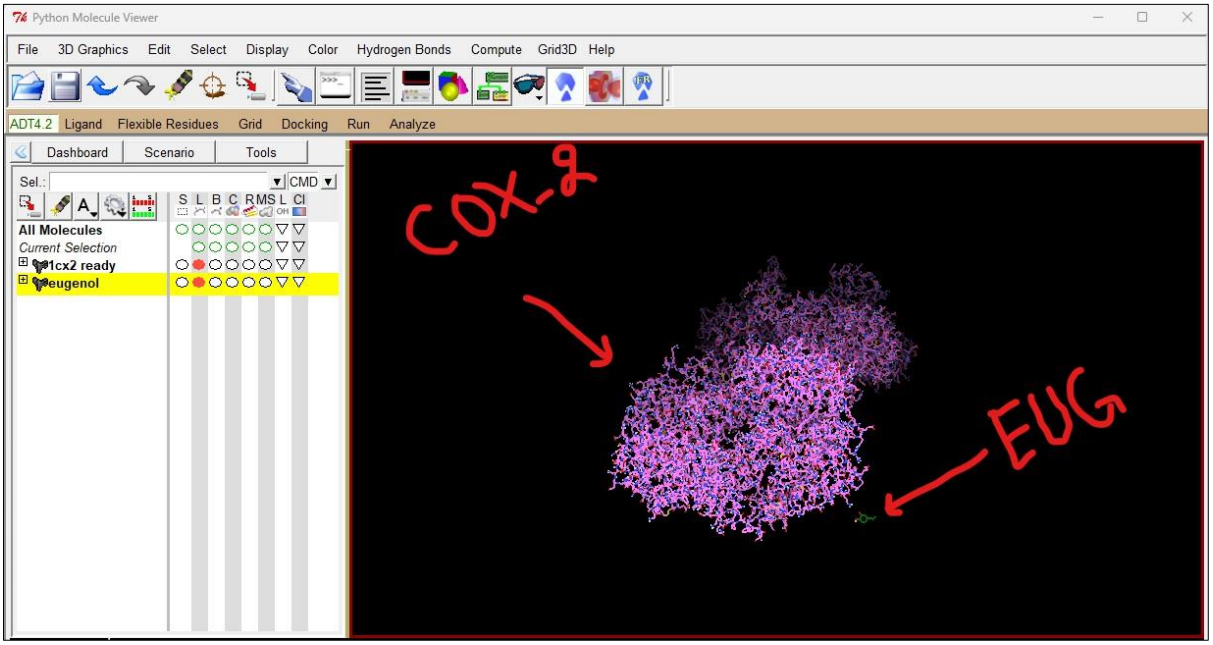


Figure IV.3: COX-2 and EUG Before interaction in MGL Tools.

We couldn't zoom in in MGl Tools program, so in order to zoom in, we switched to Biovia discovery studio, we was able to screen shot how EUG and COX-2 are placed before their interaction as (Figure IV.4) Shows:

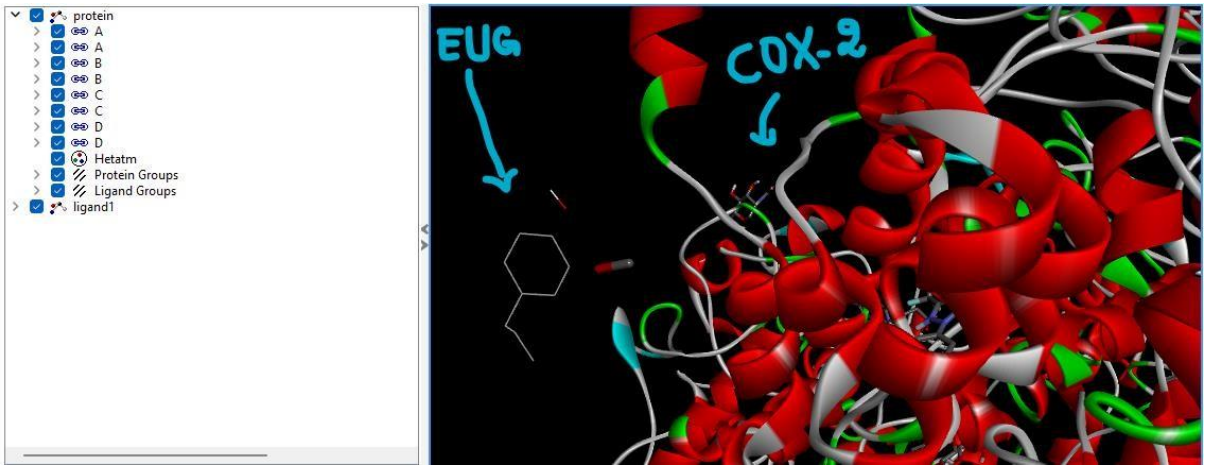


Figure IV.4: COX-2 and EUG Before interaction in Biovia Discovery Studio.

We notice that the two compounds are far from each other, and this is when it comes the step of making the interaction possible by:

Clicking on Grid > Macromolecule > Choose > selection of our COX-2 Molecule > Click on "NO".

We will have this look as (Figure IV.5) illustrates:

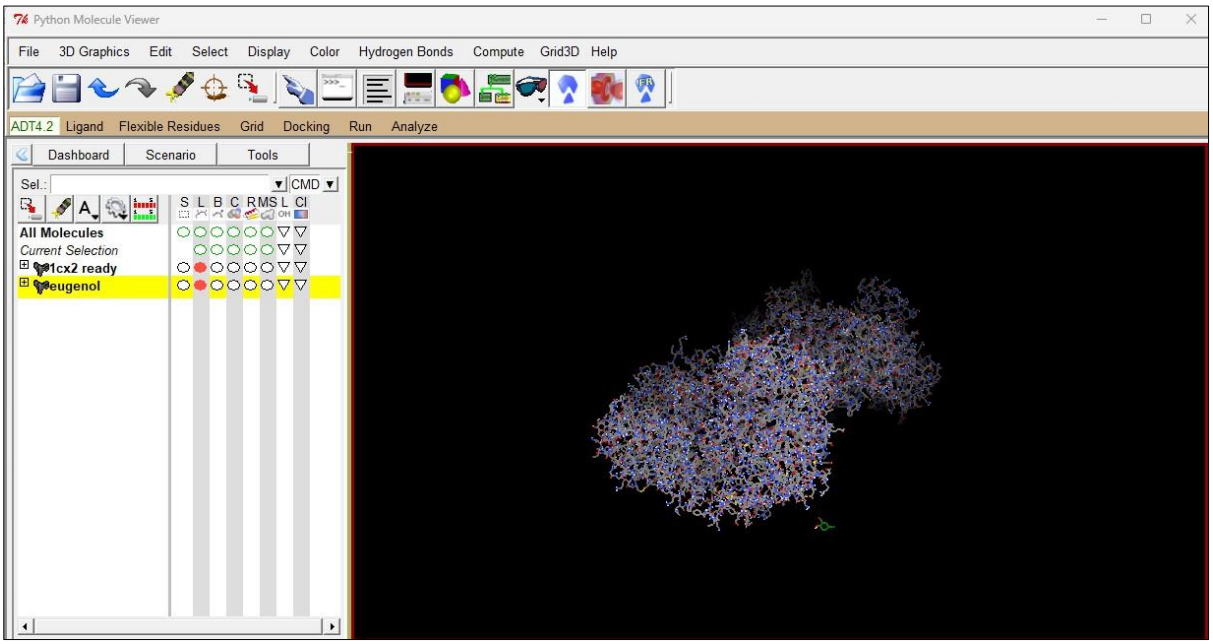


Figure IV.5: Eugenol and COX-2 After preparation.

Now we go to : Grid >Grid Box > File > Output Grid Dimension File > We save our active site's on COX-2 dimensions under Txt Format as shown in (Figure IV.6) below:

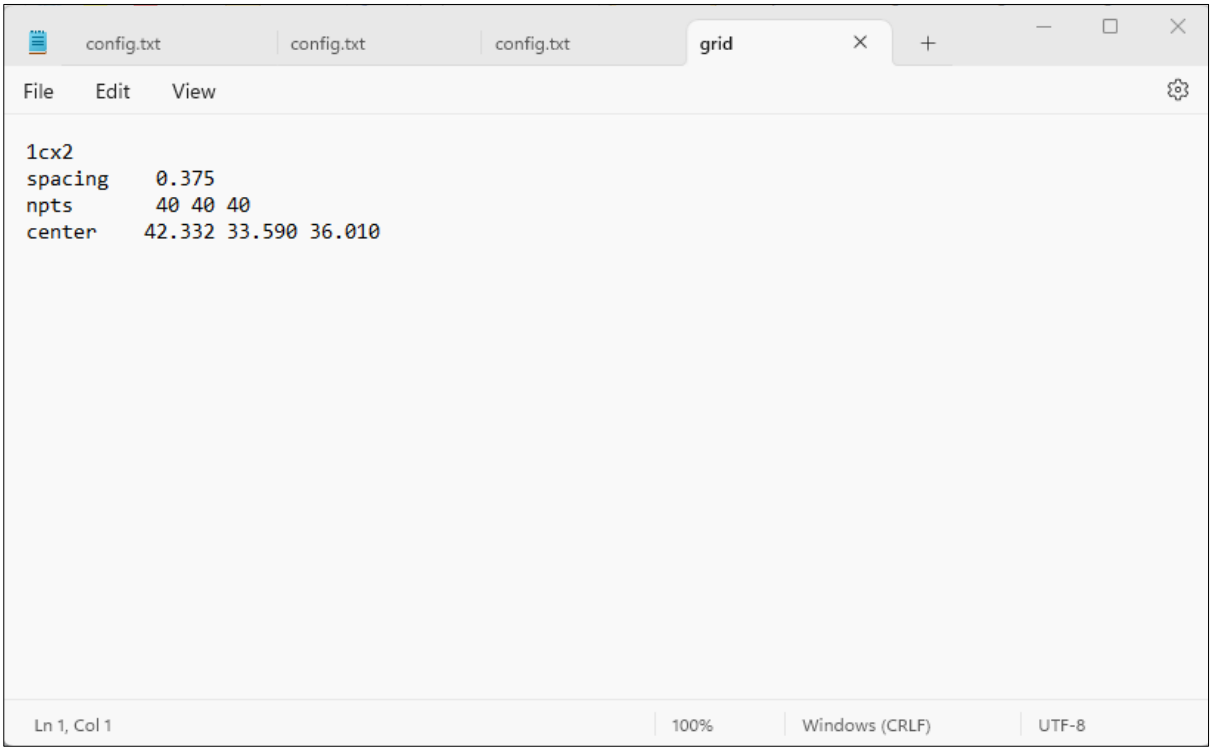


Figure IV.6: Screenshot of the grid file informations for docking

Now we create a new txt file under the name “config.txt”, in this file we type the configuration data of our Docking brought from grid options as (Figure IV.7) below shows:

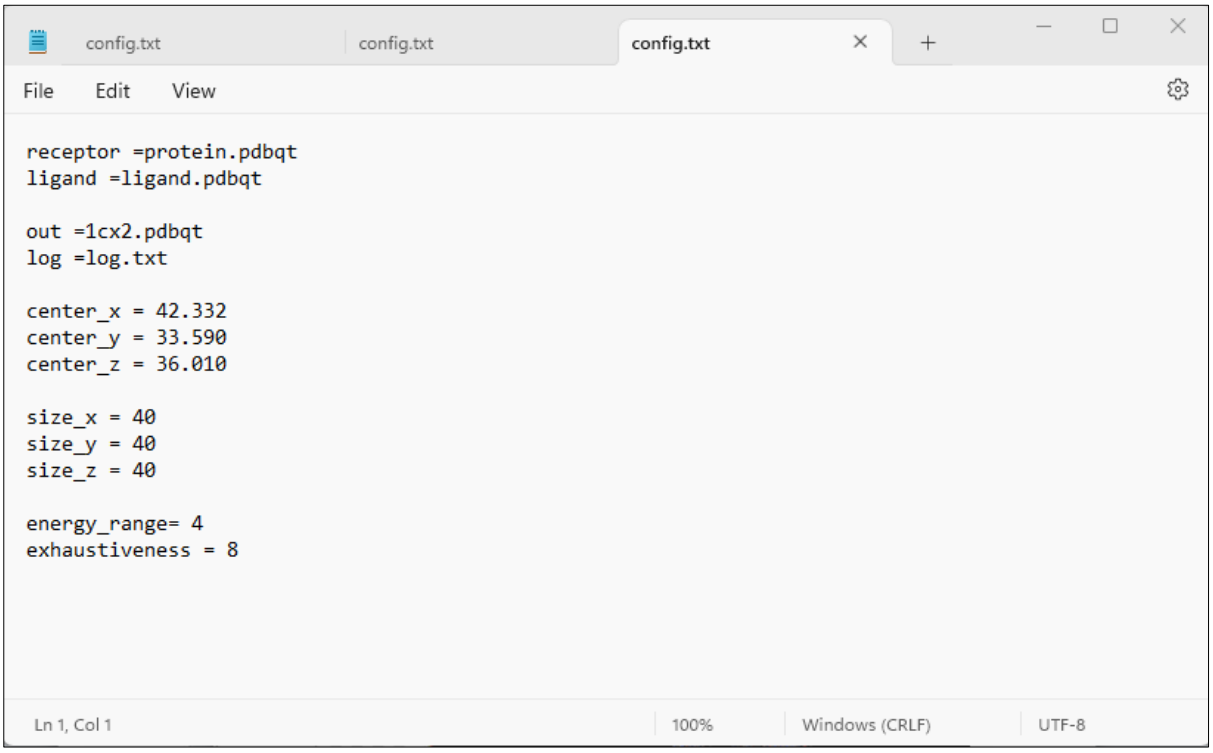


Figure IV.7: Screenshot of “config.txt” file informations

IV.4 Autodocking step using

Now moving to the Autodocking step where we’re using Autodock vina program. In our MGL Tools we go to” RUN” > Run Autodock Vina

A window appears, in vina program pathname we select “**Vina.exe**” which was copied in our eugenol file before, and in config filename we select “**config.txt**” the we created before also, then we click “**Lunch**”:

IV.5 Results

When the processing is completed we will get our results in a window Shown in (Figure IV.8) :

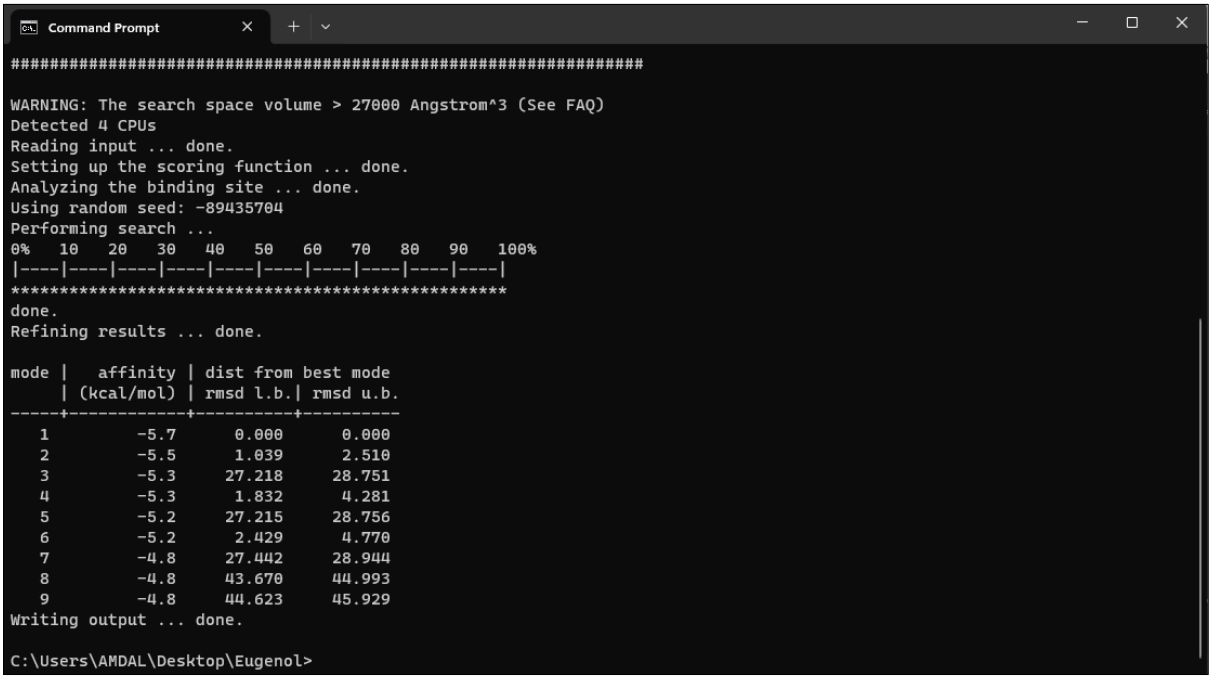
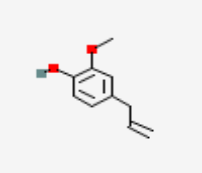


Figure IV.8: Screenshot of the end and results of the docking computation

We will get our "**output.pdbqt"** file which shows us how eugenol attaches to COX-2 in the active site (Figure IV.9) illustrates in **PyMol** Program:

Figure IV.9: Interaction sites between COX-2 & EUG

The affinity value between COX-2 and Eugenol is represented in (Table IV.1):

Enzyme	Inhibitor	*Inhibitor (2D Structure)*	Affinity (kcal/mol)
Cyclooxygenase-2 (1CX2)	Eugenol		-5.7

The interaction between EUG & COX-2 was possible by forming a conventional hydrogen bond between oxygen atom of the alcohol function of eugenol and cysteine number 47 of COX-2, and a carbon hydrogen bond between the carbon atom bound with the alcohol function of EUG and glycine number 135 of COX-2, all of this is represented in a 2D diagram brought from Biovia Discovery Studio program as shown in (Figure IV.11):

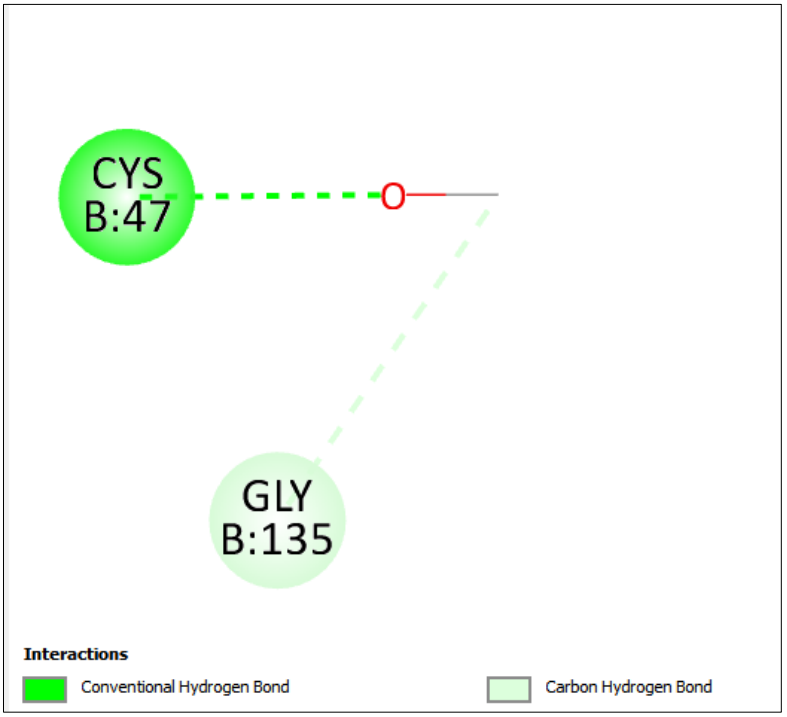


Figure IV.11: 2D Diagram of Eugenol – COX-2 interactions

Biovia Discovery Studio (BDS) is able to give us further information about EUG – COX-2 interaction, results are shown in (Table IV.2):

Name	Distance (Angstrom)	Type (Ligand – Protein)
CYS47	2.07403	O-NH
GLY135	3.51127	C-O

IV.6 Discussion

In order to discuss whether eugenol has some desired therapeutic effects, we are going to study its interaction with TNF-alpha protein, results are going to be compared with the results of ibuprofen and diclofenac.

IV.6.1 In silico study of eugenol-TNF-alpha interactions

IV.6.1.1 what is TNF-alpha?

TNFα serves as a potent pro-inflammatory factor that governs various aspects of macrophage activity. Its swift secretion follows trauma, infection, or exposure to bacterial-derived lipopolysaccharides (LPS), establishing it as among the most prevalent early signaling molecules in inflamed tissue. [33]

IV.6.1.2 molecular docking of eugenol – TNF-alpha

Same steps are followed as before, starting by downloading TNF-alpha protein file from pdb bank, the 3D conformer of eugenol, moving by preparing the two molecules for autodocking using autodock vina program, after that we lunch docking and wait for the computer to process the operation, results brought are shown in (Figure IV.12):

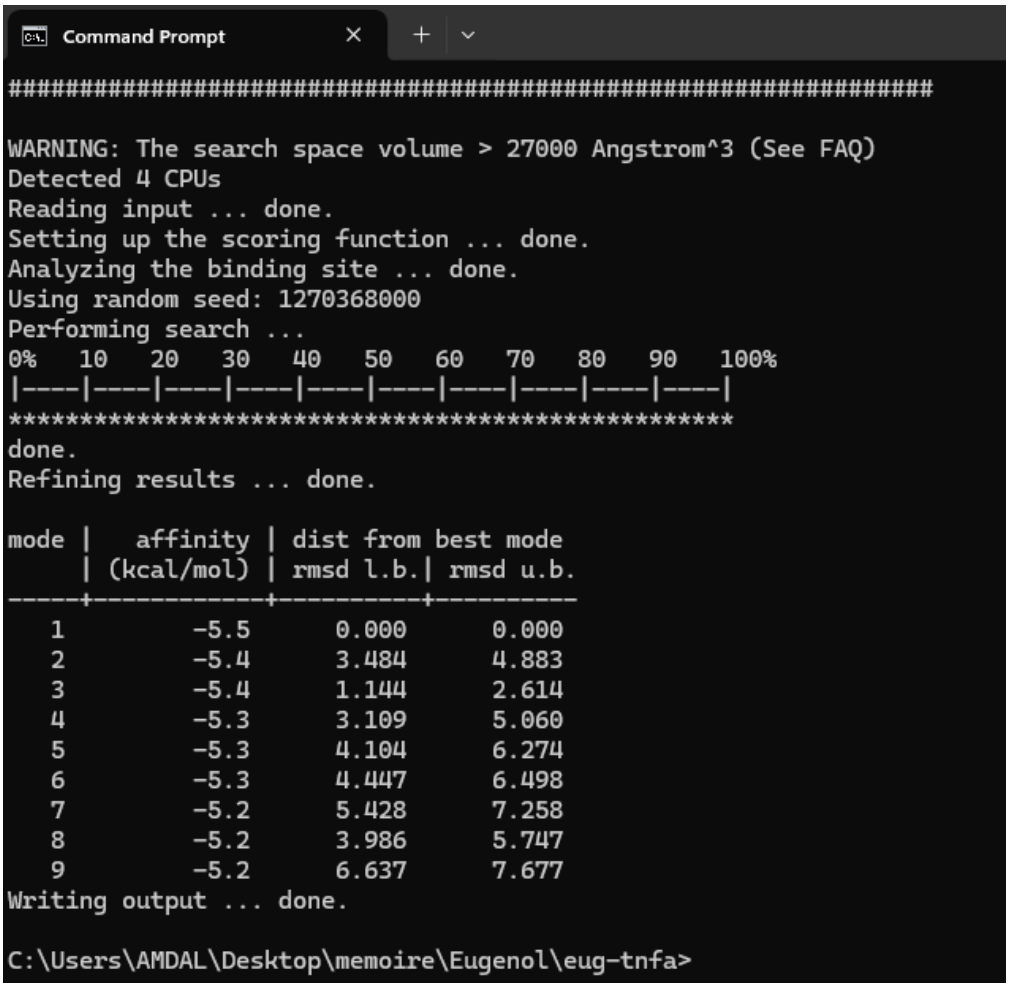
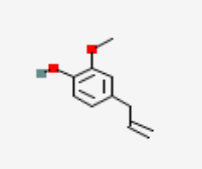


Figure IV.12: Results of Eugenol – TNF-alpha Autodocking

The following table (table IV.3) represents the affinity value between eugenol and tumor necrosis factor alpha:

Protein	Inhibitor	*Inhibitor (2D Structure)*	Affinity (kcal/mol)
Tumor Necrosis Factor – alpha (1tnf)	Eugenol		-5.5

In order to see how eugenol really interacts with TNF-alpha, we are using PyMol program to illustrate that, the following figure (Figure IV.13) shows the active site on TNF-alpha in wich eugenol interacts:

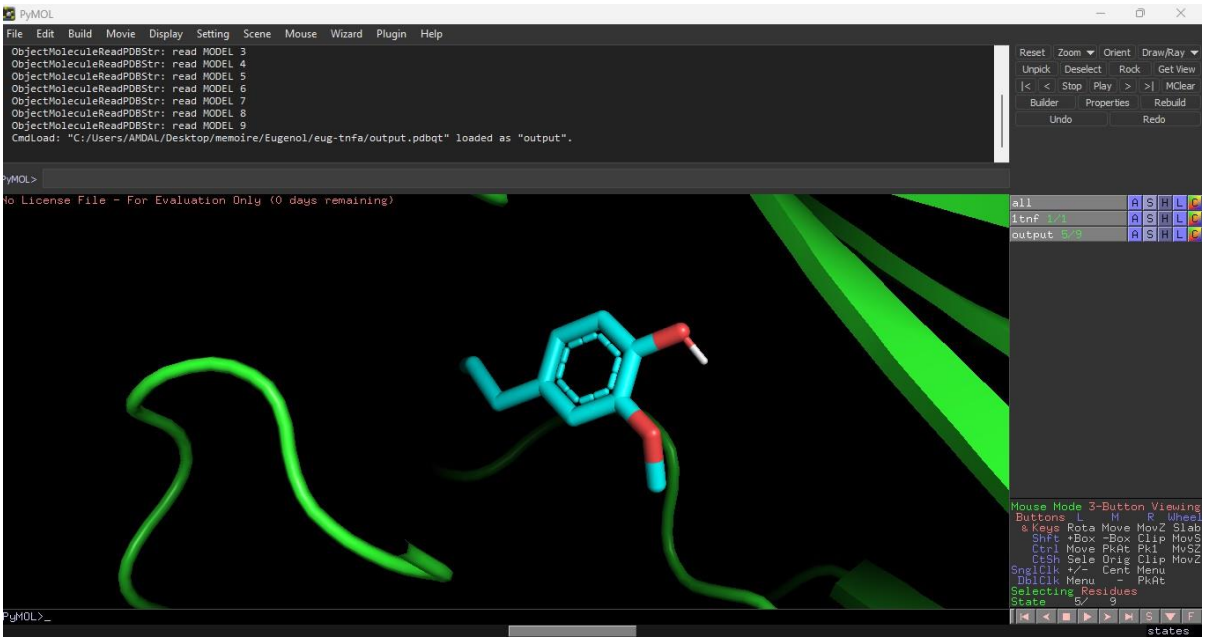


Figure IV.13: Eugenol – TNF-alpha interaction illustration by PyMol

In biovia discovery studio program, we know exactly how and where Eugenol bonds to TNF-alpha, a carbon hydrogen bond is produced between eugenol and proline B:100 as the 2D diagram shows in (Figure IV.14):

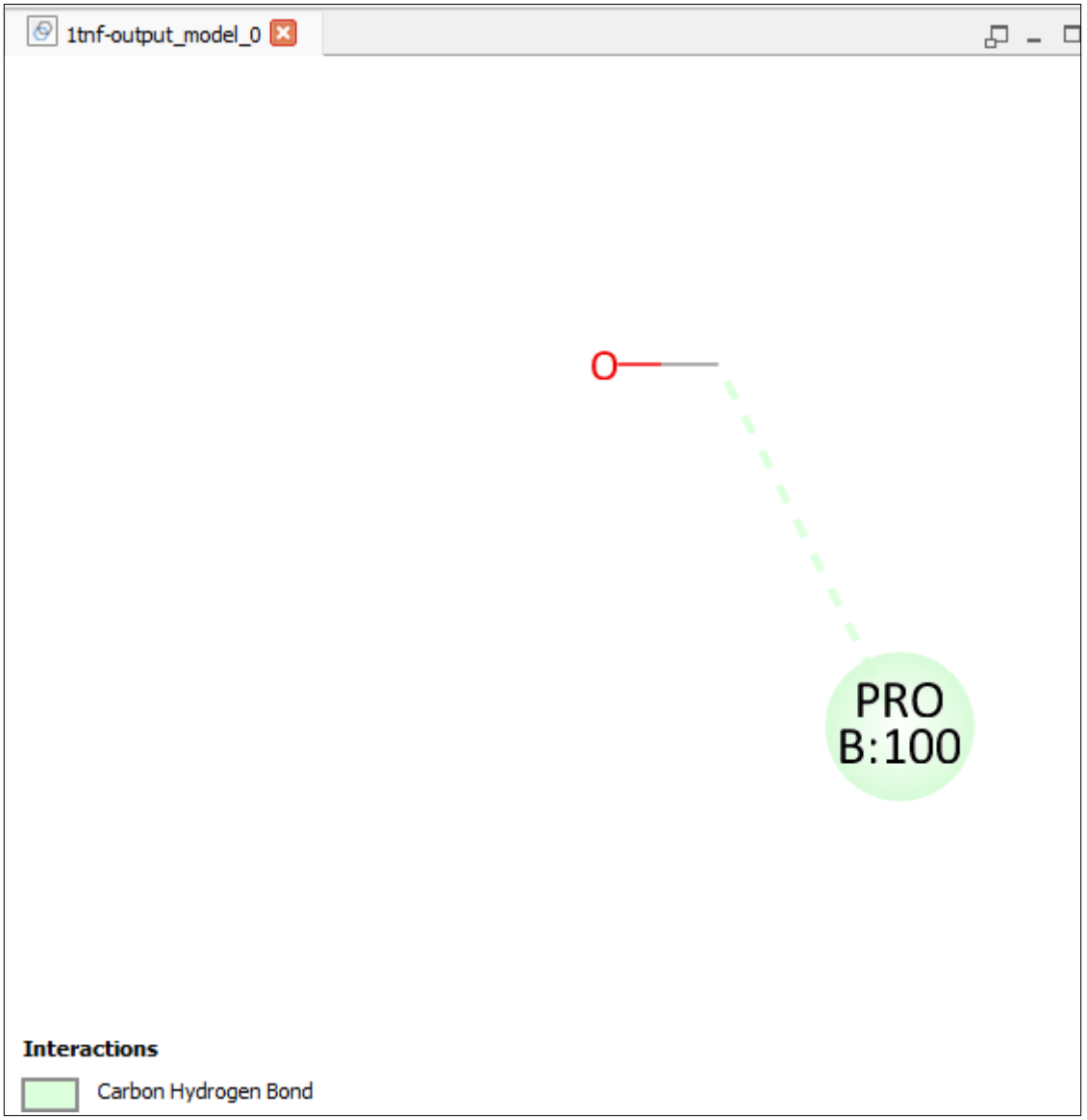


Figure IV.14: 2D diagram of eugenol – TNFalpha interaction by (BDS)

From biovia discovery studio (BDS) program, we were able to take more information about the interaction, these informations are represented in (table IV.4):

Name	Distance(Angstrom)	Type (Ligand – Protein)
PRO B:100	3.42008	C-O

In order to see if eugenol has an efficient anti-inflammatory activity, we need to compare it with the anti-inflammatory activity of a famous drug such as (Diclofenac)

Diclofenac is a molecule that inhibits cyclooxygenase-2 and cyclooxygenase-1 also TNF-a, so first, we are going to start a molecular docking of diclofenac – COX-2 and compare the brought results with the previous results of (Eugenol-COX-2).

IV.6.1.3 Molecular docking of Diclofenac – COX-2

The same steps are followed as before, starting by downloading the 3D conformer of Diclofenac and COX-2 moving by preparing them straight forward to autodocking, the 2d structure of diclofenac is represented in (Figure IV.15):

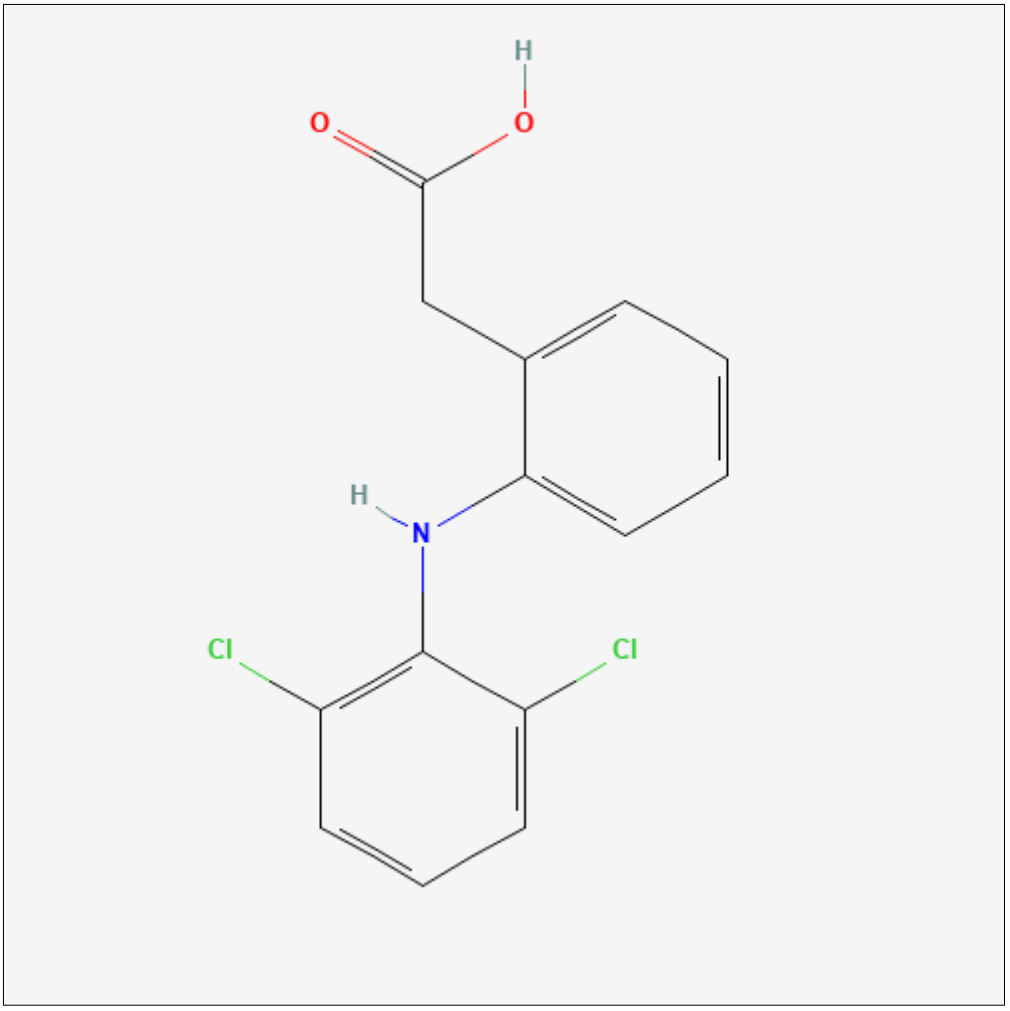


Figure IV.15: Diclofenac 2D structure (PubChem)

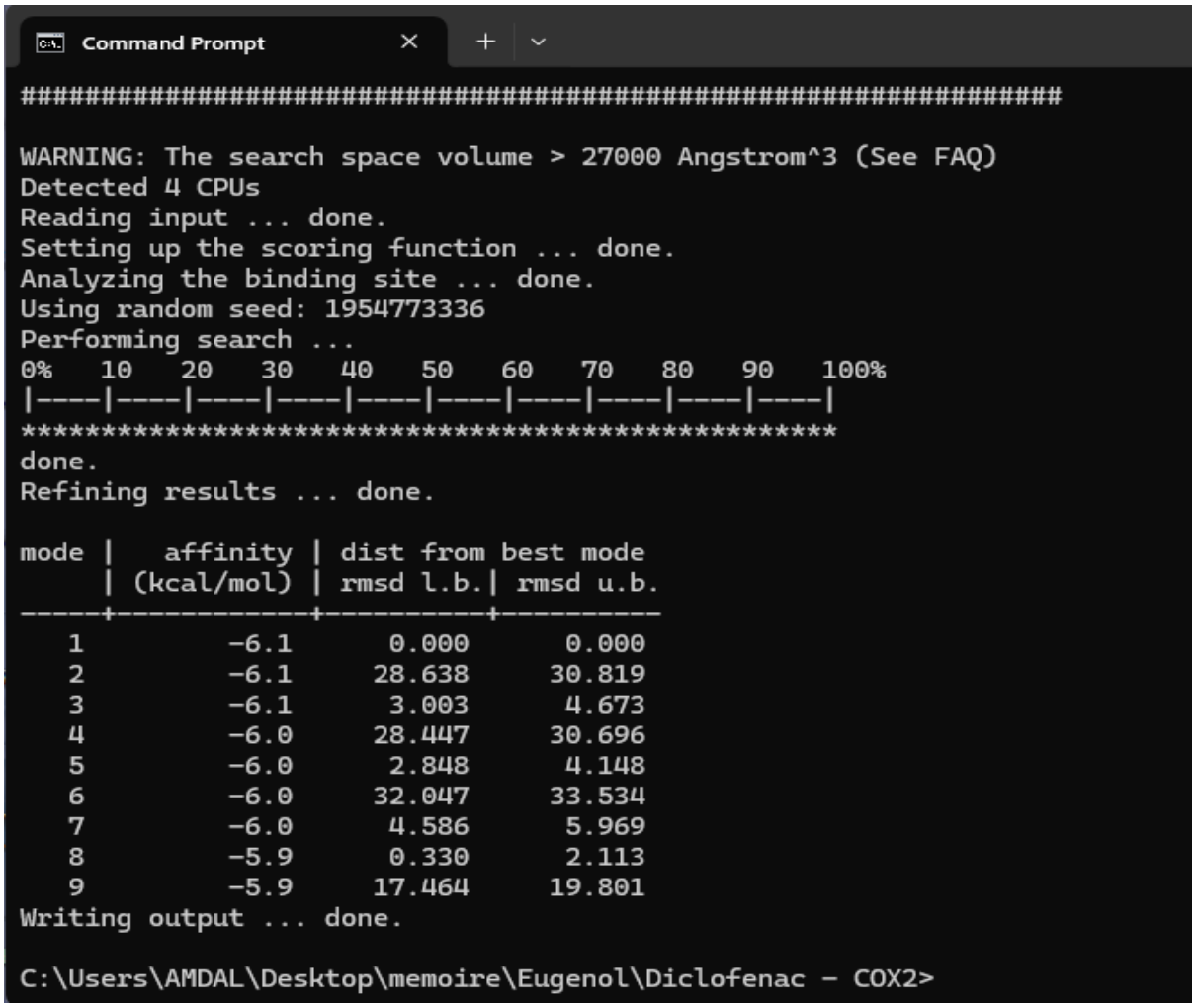
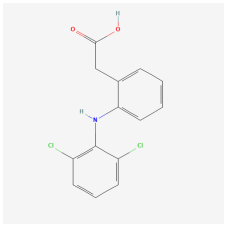


Figure IV.16: Diclofenac – COX-2 Autodocking results

Docking results are shown in (Figure IV.16) above.

The following table (table IV.5) represents the affinity value between Diclofenac and Cyclooxygenase-2:

Protein	Inhibitor	Inhibitor (2D Structure)	Affinity (kcal/mol)
Cyclooxygenase-2 (COX-2)	**Diclofenac**		**-6.1**

In order to see how diclofenac really interacts with COX-2, we are using PyMol program to illustrate that, the following figure (Figure IV.17) shows how Diclofenac interacts with COX- 2:

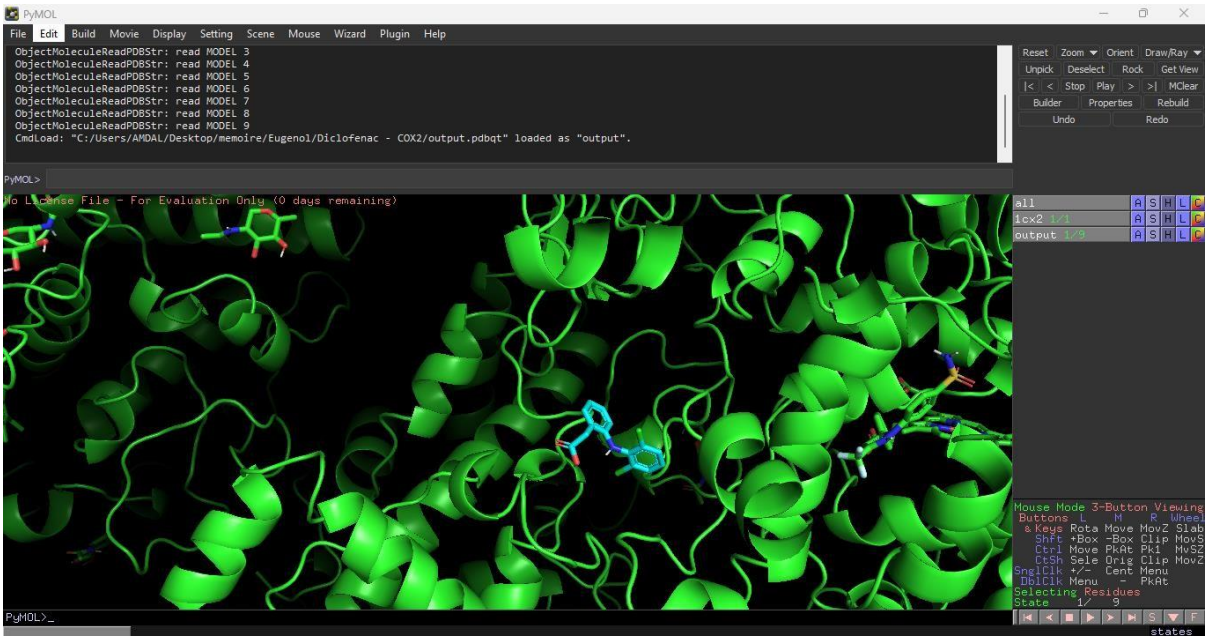


Figure IV.17: Diclofenac – COX-2 interaction illustration by PyMol

In biovia discovery studio program, we know exactly how and where Diclofenac bonds to COX-2, an alkyl bond is produced between Diclofenac and proline A:542 as the 2D diagram shows in (Figure IV.18):

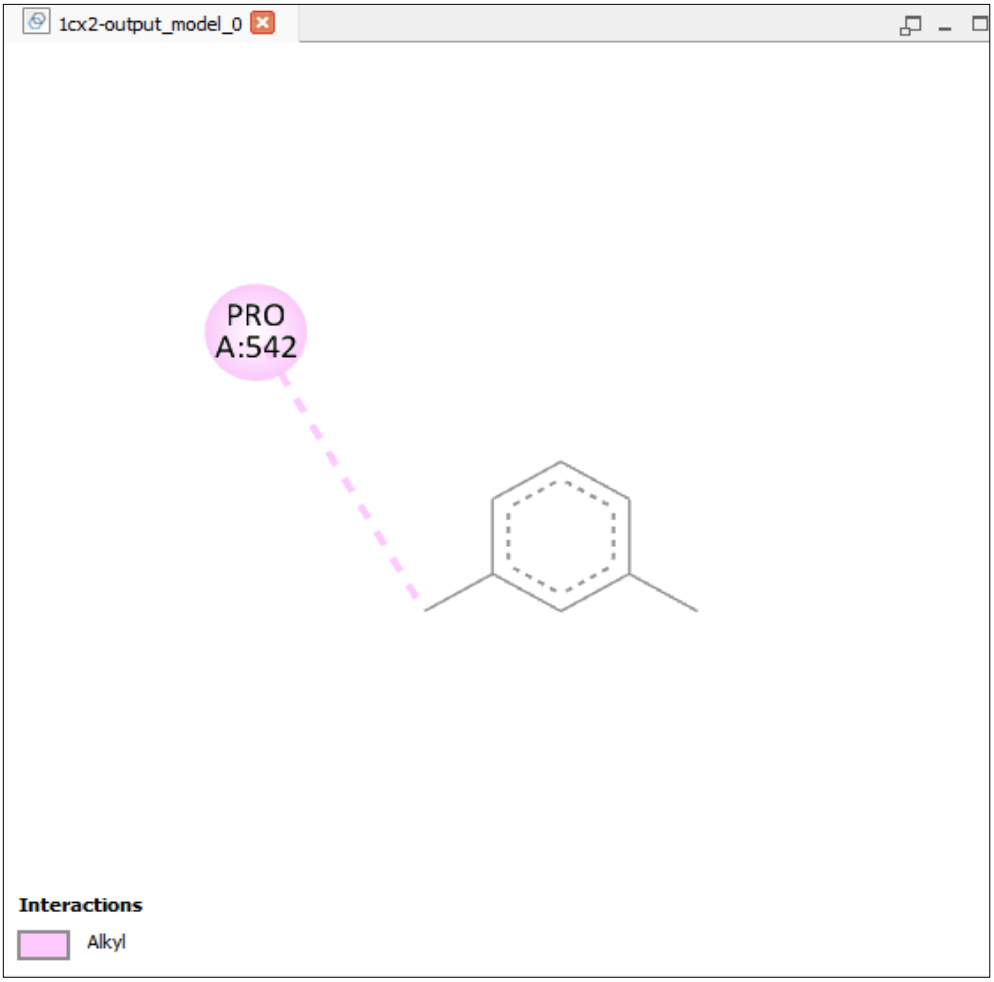


Figure IV.18: 2D diagram of diclofenac – COX-2 interaction by (BDS)

From biovia discovery studio (BDS) program, we were able to take more information about the interaction, these informations are represented in (table IV.6):

Name	Distance(Angstrom)	Type (Ligand – Protein)
PRO B:542	4.42155	C-C

IV.6.1.4 Molecular docking of Diclofenac – TNF-alpha

Same steps are followed as before, TNF-alpha is already downloaded as well as Diclofenac, so we are moving straight forward into preparing them, after that, they are ready for auto docking, results are represented below:

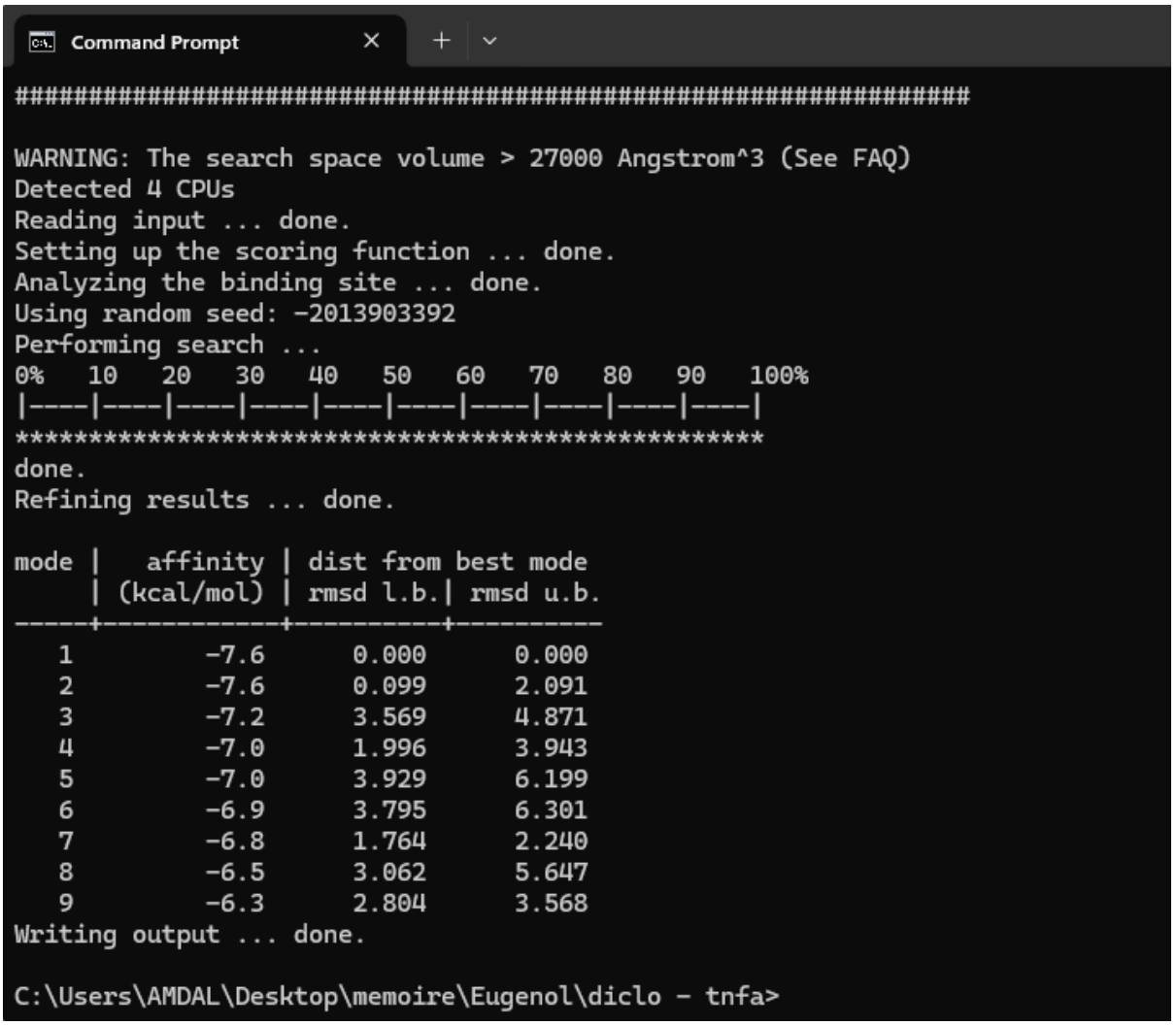
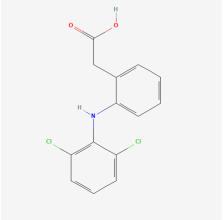


Figure IV.19: Diclofenac – TNF-alpha Autodocking results

The following table (table IV.7) represents the affinity value between Diclofenac and TNF- alpha:

Protein	Inhibitor	Inhibitor (2D Structure)	Affinity (kcal/mol)
TNF-alpha (1tnf)	**Diclofenac**		**-7.6**

In order to see how diclofenac really interacts with TNF-alpha, we are using PyMol program again to illustrate that.

The following figure (Figure IV.20) shows how Diclofenac interacts with TNF-alpha:

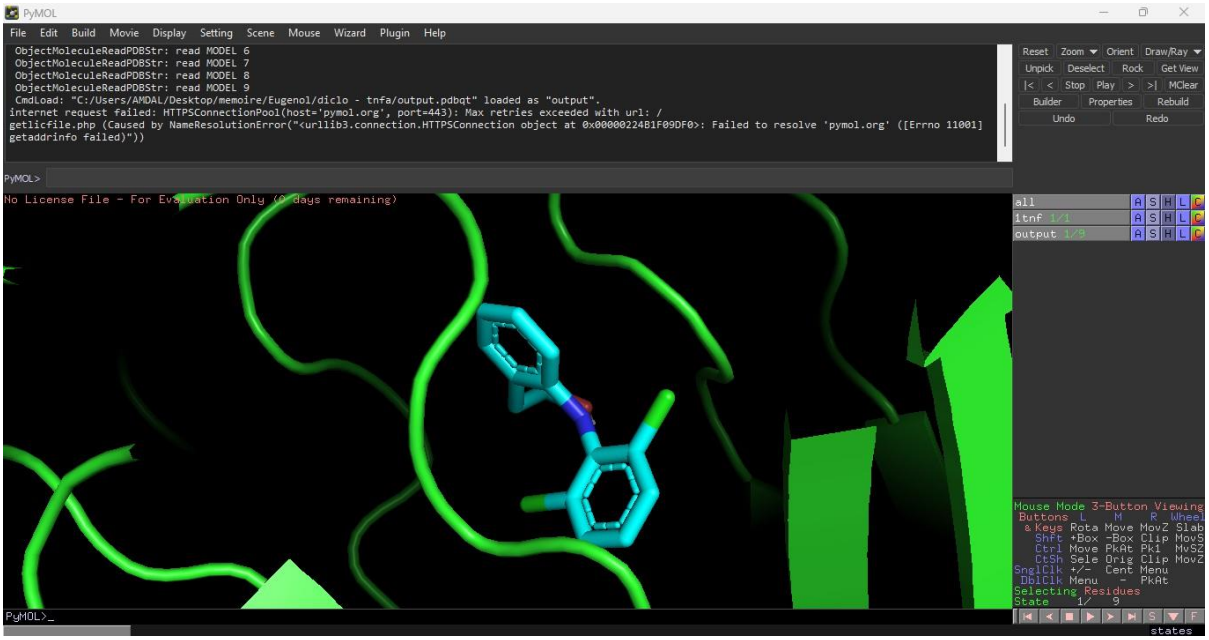


Figure IV.20: Diclofenac – TNF-alpha interaction illustration by PyMol

In biovia discovery studio program, we know exactly how and where Diclofenac bonds to TNF-alpha, three bonds are produced: Pi-Anion, Pi-Alkyl, Alkyl bond between Diclofenac and proline A:100, Tryptophane A:114, Glutamic acid A:116 as the 2D diagram shows in (Figure IV.21):

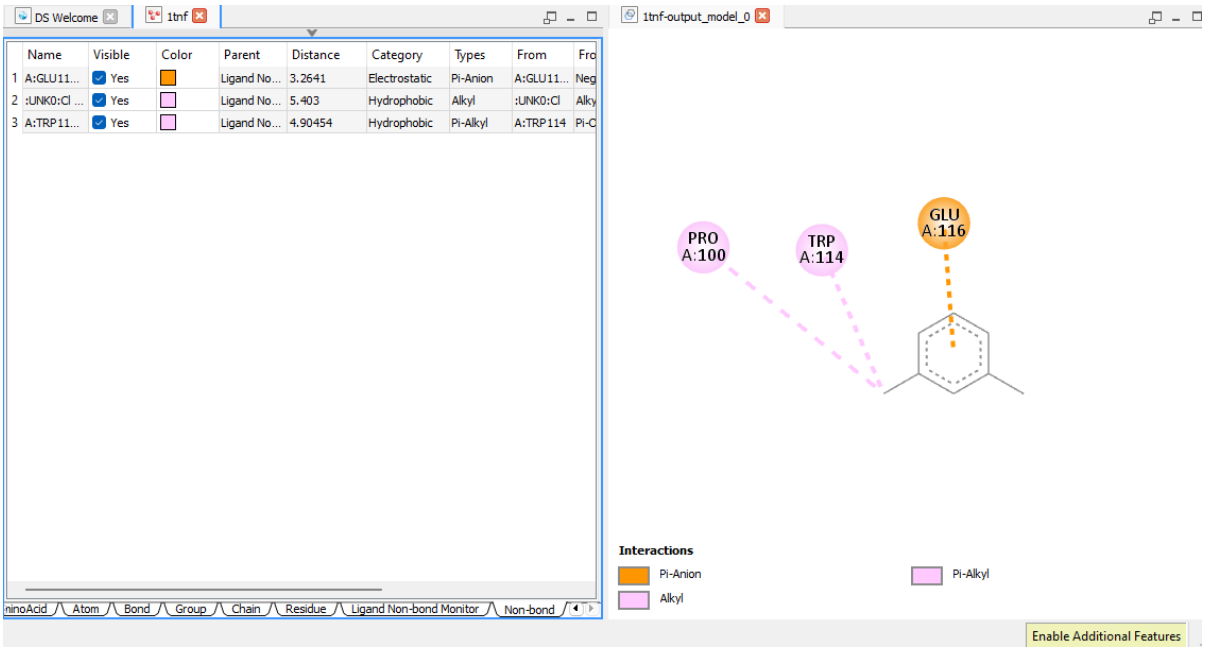


Figure IV.21: 2D diagram of diclofenac – TNF-alpha interaction by (BDS)

From biovia discovery studio (BDS) program, we were able to take more information about the interaction, these informations are represented in (table IV.8):

Name	Distance(Angstrom)	Type (Ligand – Protein)
GLU A:116	3.2641	Electrostatic
TRP A:114	4.90454	Pi-Alkyl (Cl – TRP A:114)
PRO A:100	5.403	Alkyl (Cl – PRO A:100)

We wanted to explore the in vivo anti-inflammatory effect of eugenol brought from a study was made by a group of researchers from Hassan II University of Casablanca, Casablanca, Morocco.

IV.6.1.5 ADMET evaluation

ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties of ligand were estimated by using ADMET-SAR (Structure-Activity Relationship) . This property prediction is one of the most important phases in the drug discovery process. The chosen ligand show excellent human intestinal solubility (HIA) a value of 0.9947 for eugenol, also it is permeable to blood brain barrier (BBB). [43]

The ligands is AMES negative which means that it is nontoxic and can be used in treating anti-inflammatory disease.[43]

(Table IV.9) displays the ligands' HIA, BBB, and LD50 values.

Compounds	HIA	BBB	AMES Toxicity	Carcinogenicity	LD50 in rats (mol/kg)
Eugenol	0.9947	0.5250	Nontoxic	Non carcinogenic	2.02

IV.6.1.6 In vivo study

The anti-inflammatory effects of eugenol or acetyleugenol preparations were examined using a model of DNFB-induced ear edema in mice. The mice were sensitized, challenged with DNFB(2,4-dinitrofluorobenzene), and then injected intraperitoneally with eugenol or acetyleugenol 24 hours after the challenge. The effect of the extract on the progression of inflammation was compared to that in untreated animals. The positive control group (untreated mice) developed edema 48 hours after the challenge, with a significant increase in ear thickness (260 µm) compared to the negative control group (113 µm). [43]

Treatment with eugenol or acetyleugenol significantly ($P < 0.0001$) reduced inflammation in mice at the ear level compared to the positive control group. Ear swelling in the positive control group was 260 µm, while it was 105 µm in eugenol-treated mice and 85 µm in acetyleugenol-treated mice 48 hours after inflammation induction. However, the difference observed between the eugenol and acetyleugenol treated groups was not statistically significant. [43]

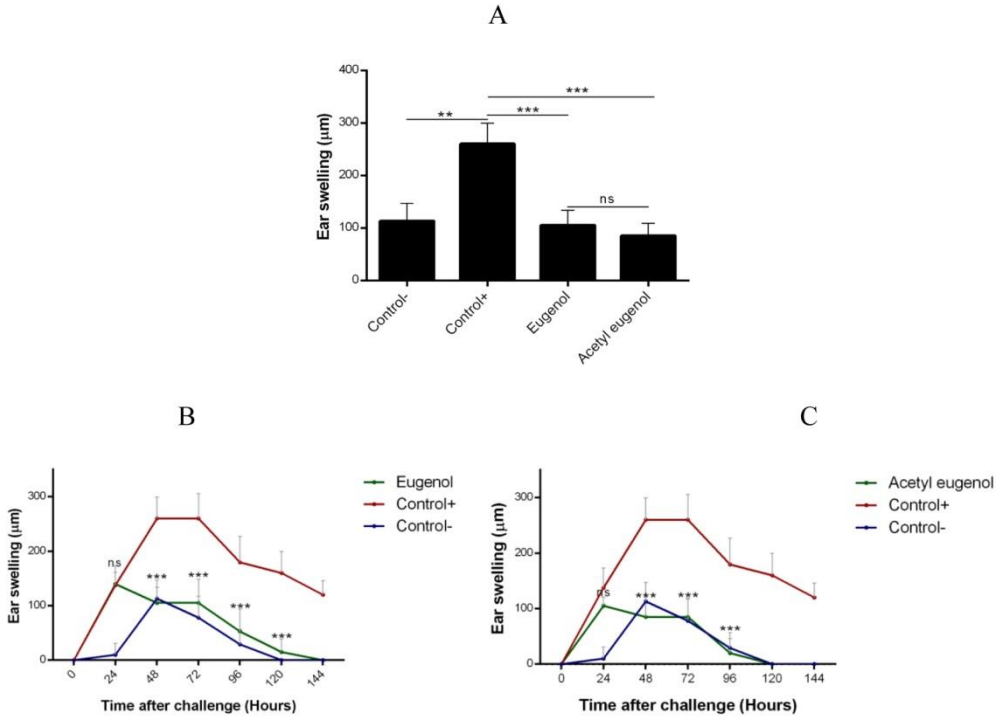


Figure IV.22: Treatment with eugenol or acetyleugenol significantly inhibited the inflammation. [43]

The activity of eugenol or acetyleugenol on the progression of inflammation was assessed after challenge. Ear thickness was assessed every 24 h. Treatment with eugenol or acetyleugenol was able to modulate the inflammatory reaction in mice after 7 and 6 days (144 h and 120 h, respectively) of induction (Figure IV.22: B, C). The results suggest that eugenol or acetyleugenol extract significantly suppressed edema in mice and exhibited anti-inflammatory activity. [43]

IV.6.2 Conclusion

After studying multiple examples of eugenol and diclofenac therapeutic activities, we are able to compare our results, and this is what we have arrived to:

- Eugenol can be considered as a good anti-inflammatory agent in comparison with diclofenac, because the affinity values of the two molecules are close to each other, and both have good affinity values.
- Eugenol cannot be considered as a competitor ligand to diclofenac, because each one of them has its precise bounding site.
- Eugenol has an effective anti-inflammatory activity against ear edema

IV.7 Mechanism of action of Anti-Microbial Molecule

Antimicrobial molecules, such as antimicrobial peptides (AMPs), interact with the membrane and/or cytoplasmic components of microorganisms, leading to changes in their cellular functions, which can result in cellular death or depletion of metabolism. This is influenced by several characteristics of these molecules, including their amino acid composition, amphipathicity (dual polarity), electric charges, and small size. These characteristics facilitate the insertion of the molecules into lipid bilayers, leading to the formation of pores and the leakage of cellular contents. [19][20]

The antimicrobial peptides, or AMPs, bind to the membranes of microorganisms, disrupting their normal function and potentially causing their death or a reduction in their ability to carry out essential metabolic processes. This is due to the unique properties of AMPs, such as their amino acid makeup, dual polarity, electric charge, and small size, which enable them to insert into the lipid bilayers of the microorganism's membrane, ultimately leading to the formation of pores and the release of cellular contents. [20]

IV.8 Mechanism of action Linalool as an Anti-Microbial Molecule

Linalool, a compound found in various essential oils, has demonstrated anxiolytic, anti-cholesterol, and antibacterial properties. The antibacterial activity of linalool can be attributed to several factors, including membrane damage, bacterial metabolic inhibition, and the disruption of cellular processes, In particular, linalool has shown strong antibacterial activity against Campylobacter jejuni and Campylobacter coli, which are important foodborne pathogens. [21]

The antimicrobial effects of linalool can be summarized as follows:

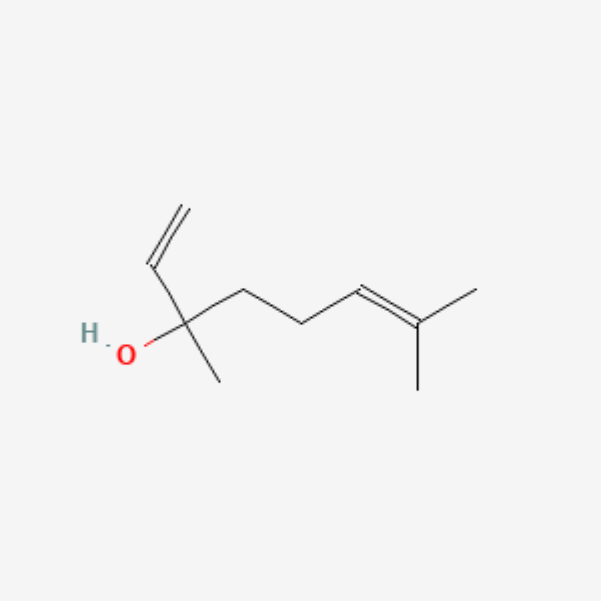
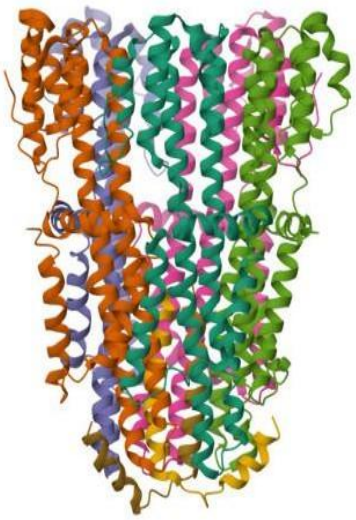
- Membrane damage: Linalool can cause damage to the bacterial cell membrane, leading to the leakage of cellular contents and eventual cell death. [21]
- Bacterial metabolic inhibition: Linalool can inhibit bacterial metabolic processes, which may result in the accumulation of toxic compounds and eventual cell death. [21]
- Disruption of cellular processes: Linalool can interfere with various cellular processes, such as the activity of key enzymes and the integrity of cellular membranes, ultimately leading to cellular dysfunction and death. [21]

Overall, linalool's antibacterial activity is a result of its ability to target multiple cellular processes and structures, making it an effective antimicrobial agent against various bacterial strains, including Campylobacter jejuni and Campylobacter coli. [21]

IV.9 In silico study of Linalool's Anti-Bacterial Mechanism of action

In this study, Campylobacter jejuni has been chosen as a target because all information needed for the molecular docking are available.

The following table (Table IV.10) illustrates the shape of Linalool (2D) and Campylobacter jejuni membrane protein (3D)

Linalool (2D)	Campylobacter jejuni membrane protein (3D)
	

IV.9.1 Creation and Manupulation of the Docking file

Starting by creating a new file that we named "Linalool", we downloaded (Campylobacter jejuni membrane protein) 3D conformer From Protein Data Bank PDB, then we also downloaded Linalool 3D conformer from PubChem website, from now on, all steps are the same as eugenol part, so we will jump straight to our final results.

The following figure represents the autodocking results of linalool- Campylobacter jejuni membrane protein interaction:

```
Detected 4 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: 279603904
Performing search ... done.
Refining results ... done.

mode |   affinity | dist from best mode
     | (kcal/mol) | rmsd l.b.| rmsd u.b.
-----+------------+----------+----------
   1         -5.3      0.000      0.000
   2         -5.3     22.122     23.459
   3         -5.2     26.426     28.437
   4         -5.1      1.066      1.873
   5         -5.1      2.129      4.029
   6         -5.0      1.446      5.017
   7         -4.9     34.487     36.407
   8         -4.8     33.502     34.748
   9         -4.7      1.625      4.865
Writing output ... done.
```

Figure IV.23 : Molecular Docking results of Linalool - Campylobacter jejuni membrane protein interaction (CJMP).

strate that, the following figure (Figure IV.24) shows how Linalool interacts with CJMP:

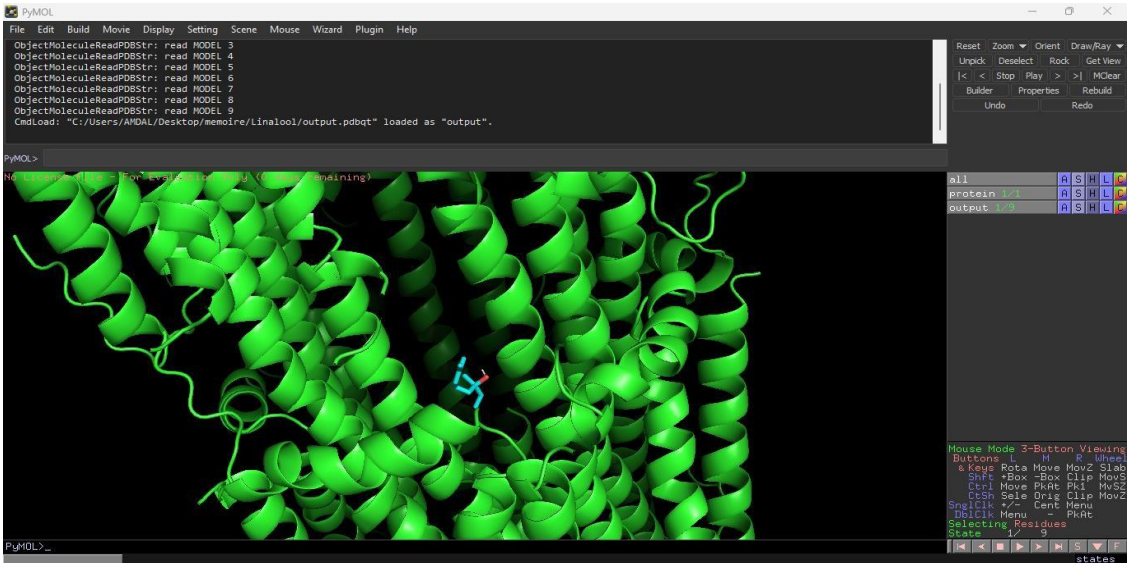


Figure IV.24: Interaction site of linalool and CJMP in PyMol.

IV.10 Results

The affinity value between Linalool and CJMP is represented in (Table IV.11):

protein	The antimicrobial molecule	Affinity (kcal/mol)
Campylobacter jejuni membrane protein	Linalool	-5.3

In order to get more informations about the interaction, we used BDS (Biovia Discovery Studio) program, we were able to get the 2D diagram of the active site:

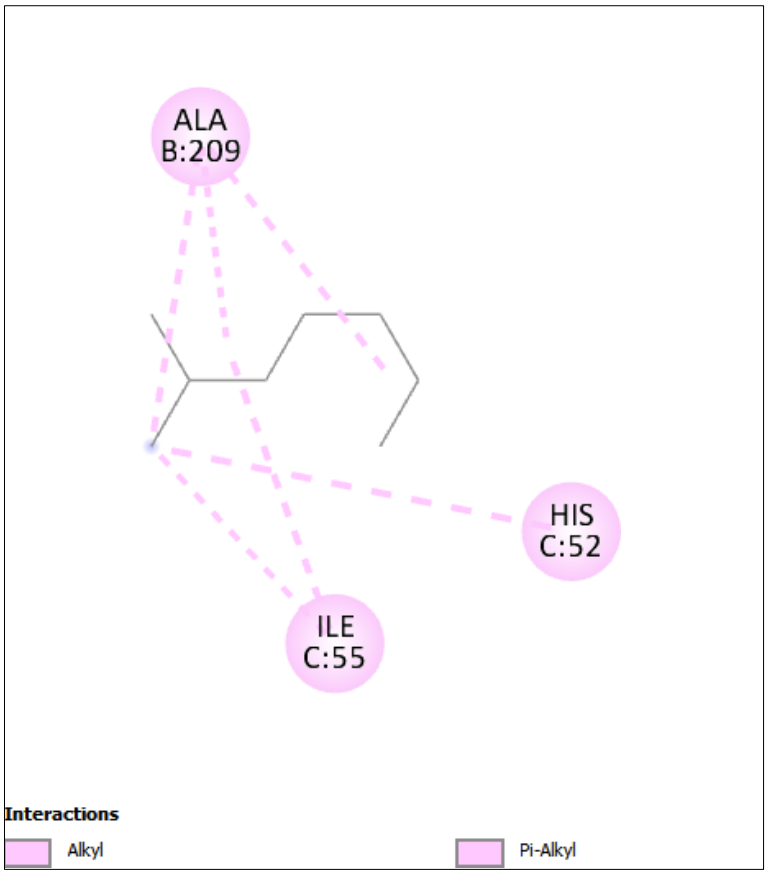


Figure IV.25 : 2D Diagram of the interaction between Linalool and CJMP by (BDS)

There were multiple bonds between linalool and CJMP, fortunately BDS provided the accurate calculations preview.

Informations collected from BDS are represented in (Table IV.12):

Name	**Distance (Angstrom)**	**Types**
ALA B:209	4.55281	Alkyl
ALA B:209	3.93546	Alkyl
ALA B:209	4.0113	Alkyl
ILE C:55	4.81692	Alkyl
ILE C:55	4.78026	Alkyl
HIS C:52	4.8873	Pi-Alkyl

IV.11 Discussion

To know whether Linalool is an efficient anti-bacterial molecule, we need to compare it's activity with a famous anti-bacterial molecule, we choosed amoxicillin for this comparison.

Amoxicillin is an effective antibiotic against a large number of bacteria. The bacterial species targeted by amoxicillin are more or less sensitive to its action depending on whether or not they have acquired resistance to this antibiotic. [34][35]

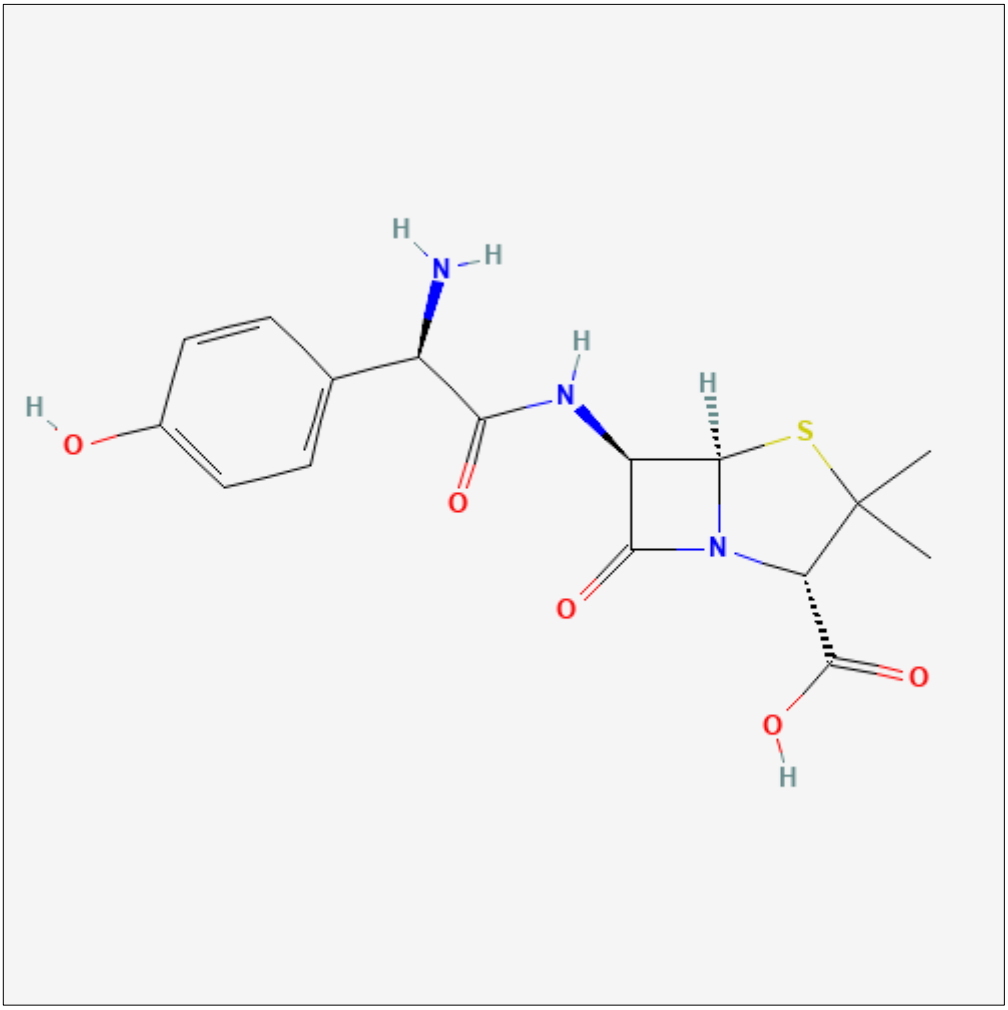


Figure IV.26: 2D structure of amoxicillin molecule [PubChem]

IV.11.1 in silico study of Amoxicillin – CJMP

Starting off by downloading the needed files including the 3D conformer of amoxicillin molecule from PubChem website as an sdf file, then turning it into a pdb file, also the 3D conformer of CJMP file is needed, fortunately it is already downloaded before.

After preparing files by following the exactly same steps as before, we will get the following results:

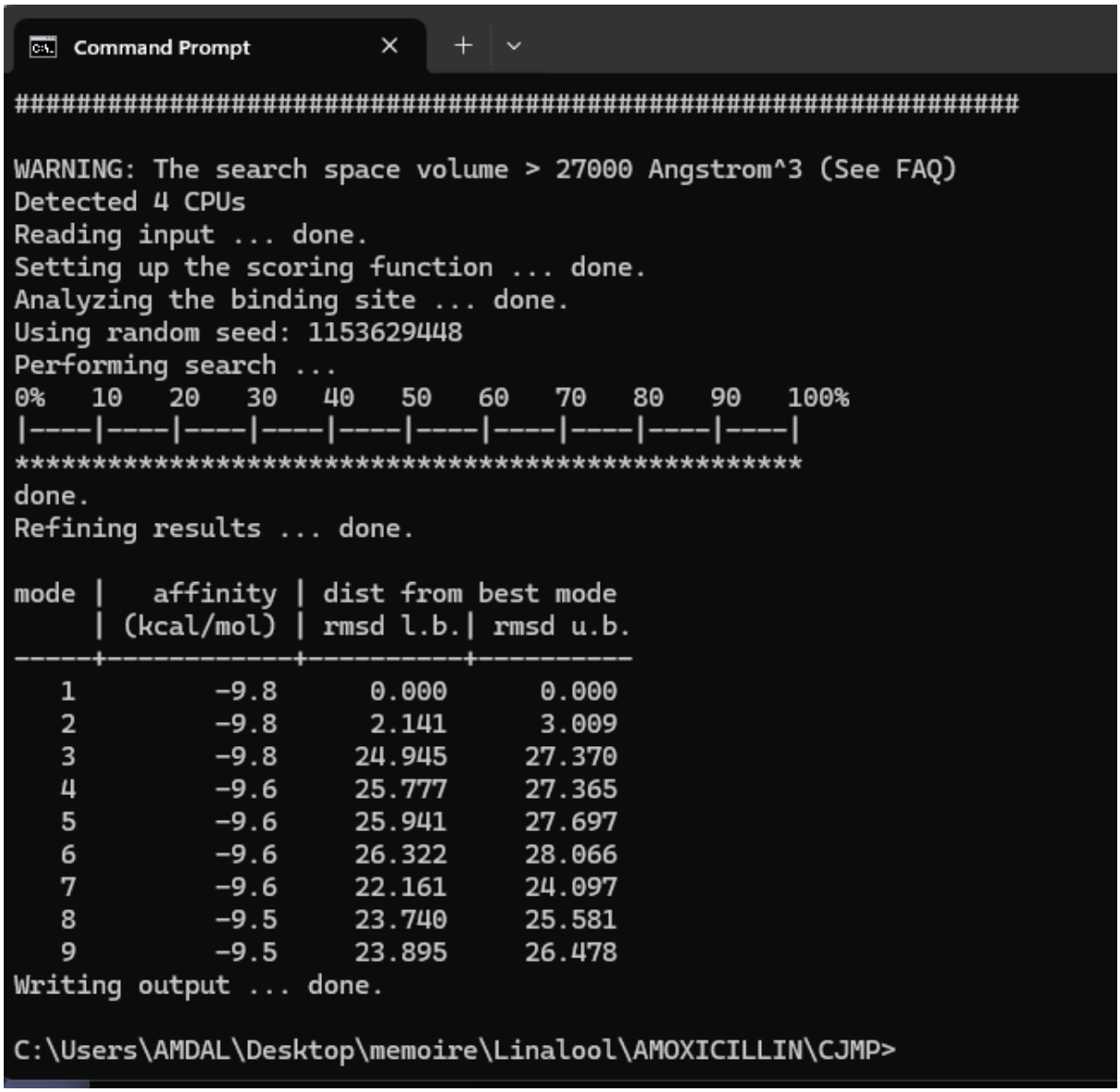


Figure IV.27: Amoxicillin-CJMP interaction results after molecular docking

We notice that Amoxicillin has an excellent affinity with CJMP estimated by (-9.8 kcal/mol) as (Table IV.13) represents:

protein	**The antimicrobial molecule**	**Affinity (kcal/mol)**
Campylobacter jejuni membrane protein	Amoxicillin	-9.8

In order to see how Amoxicillin really interacts with CJMP, we are using PyMol program to illustrate that, the following figure (Figure IV.28) shows how Amoxicillin interacts with CJMP:

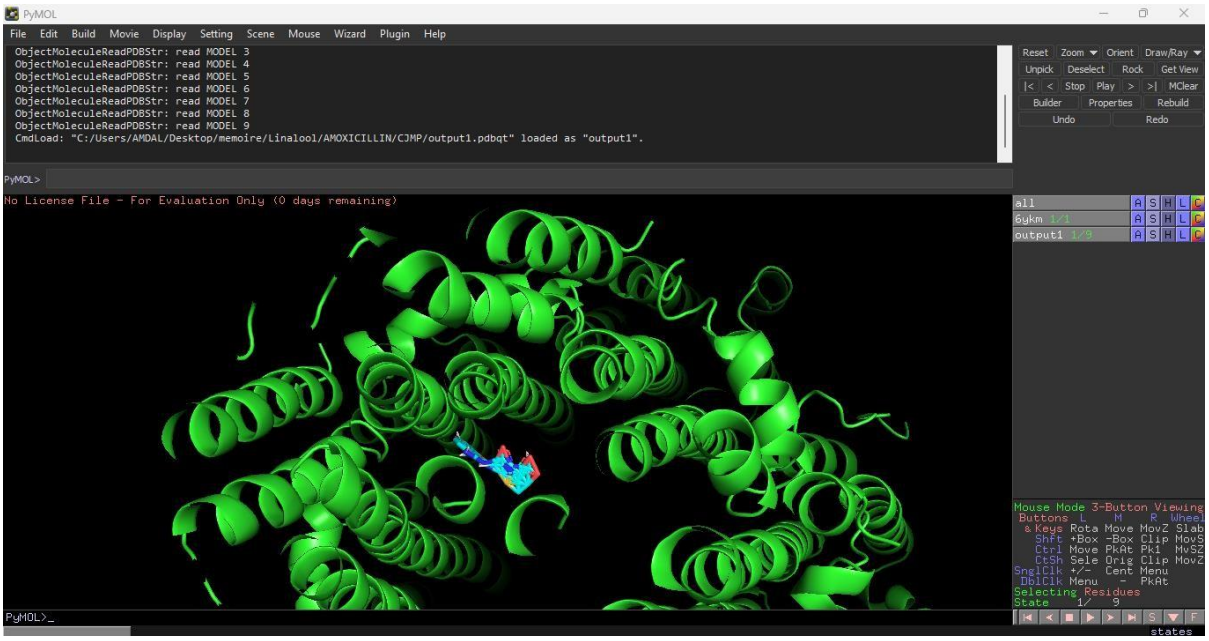


Figure IV.28: interaction illustration between Amoxicillin and CJMP by PyMol

In order to get more informations about the interaction, we used BDS (Biovia Discovery Studio) program, we were able to get the 2Dand 3D diagrams of the active site:

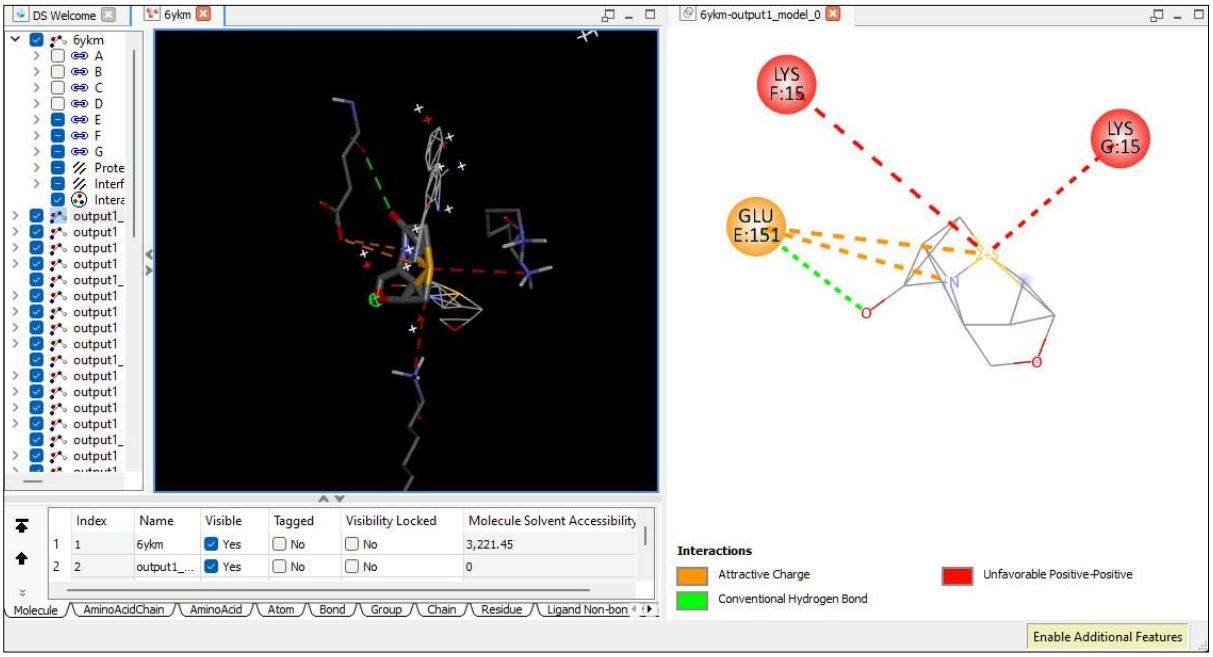


Figure IV.29: 3D and 2D diagrams of Amoxicillin – CJMP binding by (BDS)

The next table (Table IV.14) gives more informations about the last interaction including binding types, distances of bounds as well as types of molecules which participated in the interaction:

Name	Distance (Angstrom)	Types
GLU E:151	4.75211	O-S
	4.15027	O-N
	3.27314	O-O
LYS F:15	5.47373	Unfavorable Positive-positive (N-S)
LYS G:15	4.58189	Unfavorable Positive-positive (N-S)

IV.11.2 In vivo study of linalool

An antibacterial activity of linalool against Pseudomonas aeruginosa study was made by a group of researchers from College of Food Sciences & Engineering, Hainan University and Chunguang Agro-product Processing Institute, the objective of this study was to assess the antibacterial activity and mechanism of linalool against Pseudomonas aeruginosa. [44]

The antibacterial activity was evaluated using the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC). [44]

Additionally, the mechanism of action was investigated through growth curve assays, scanning electron microscopy (SEM), cell membrane permeability tests, membrane potential measurements, and respiratory chain dehydrogenase activity assays.

The MIC and MBC of linalool were found to be 431 μg/mL and 862 μg/mL, respectively. [44]
The growth curve assay demonstrated that linalool inhibited the growth of P. aeruginosa. SEM results indicated that linalool disrupted the normal cell morphology. [44]

The release of nucleic acids and the decrease in membrane potential confirmed that linalool compromised the integrity of the bacterial cell membrane. Furthermore, the respiratory chain was impaired, as evidenced by a decrease in absorbance at 490 nm during the dehydrogenase activity assay. [44]

This research suggested that linalool has the potential to be used as a food preservative by disrupting bacterial cell membranes and inducing cell death. [44]

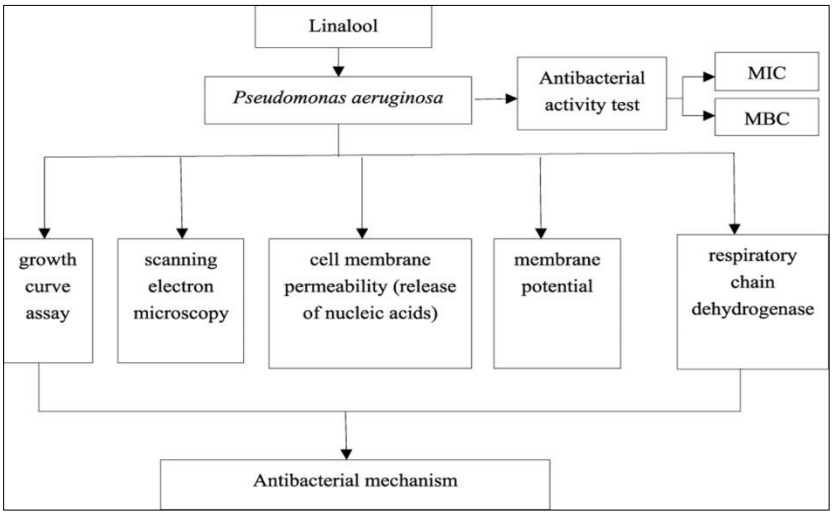


Figure IV.30: antibacterial activity and mechanism of action investigation plan of linalool

IV.11.2 Conclusion

From our previous molecular dockings, we notice that amoxicillin has a much higher affinity value than linalool, but that doesn't mean linalool has no effects on CJMP, (Table IV.16) represents the difference between the two affinity values:

protein	The antimicrobial molecule	Affinity (kcal/mol)
Campylobacter jejuni membrane protein	Amoxicillin	-9.8
	Linalool	-5.3

- The affinity value of linalool can be considered as a good value in which linalool would be an efficient antibiotic for Campylobacter jejuni bacteria.
- Amoxicillin and linalool does not have the same bindings which means that there would not be a competition between these two molecules.
- From the previous in vivo study, Linalool can be suggested as an antibacterial Molecule as results show that the growth of P. aeruginosa decreased after Linalool's intervention.

IV.12 Estragole as an Anti-Inflammatory /Anti-edematogenic molecule

IV.12.1 What is EDEMA?

Edema is a medical condition characterized by swelling caused by an accumulation of fluid in the body's tissues. It can affect any part of the body, but is most commonly observed in the legs, feet, and ankles. Edema can be caused by various factors, including pregnancy, certain medications, heart failure, kidney disease, liver problems, and issues with the lymphatic system. The condition can be managed through lifestyle changes, such as reducing salt intake, elevating the affected body part, and wearing compression garments. In more severe cases, medical treatment and medication may be necessary. If left untreated, edema can lead to complications such as pain, stiffness, skin problems, and decreased blood circulation. [22][23][24][25]

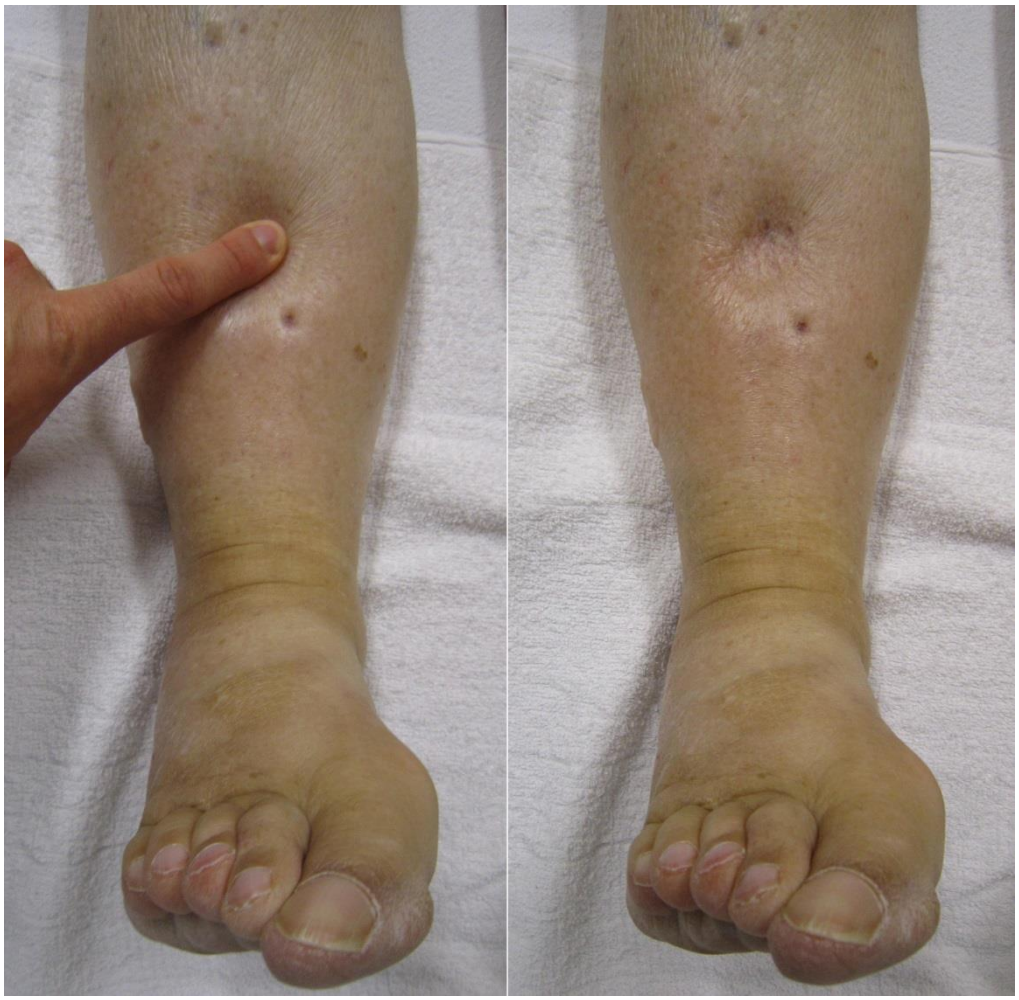


Figure IV.31: Example of leg edema (swelling). [James Heilman photography]

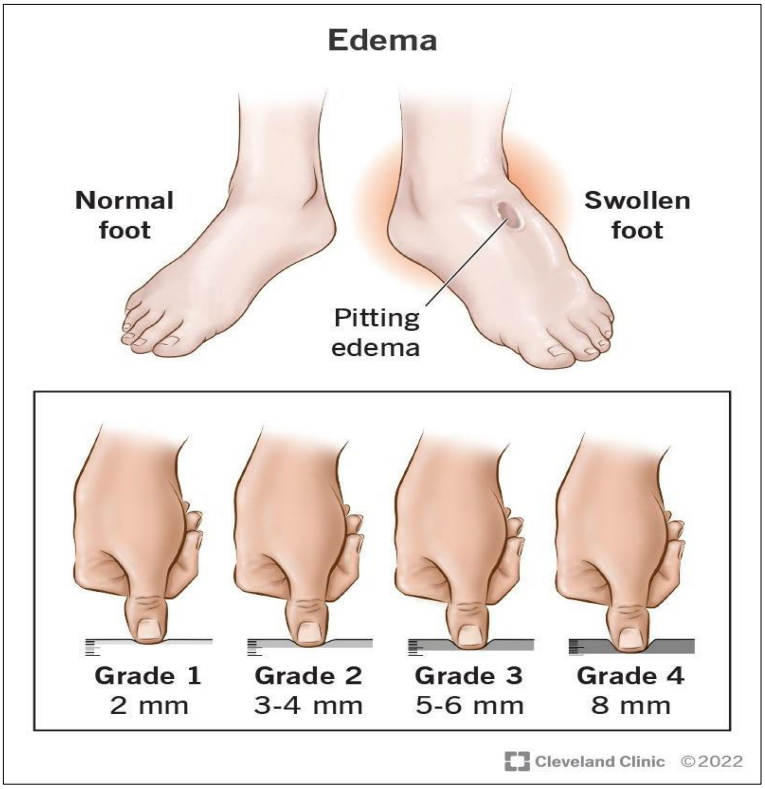


Figure IV.32: Illustration of edema grades. [Cleveland Clinic]

IV.12.2 Mechanism of action Estragole as an Anti-edematogenic

The plethysmometry technique was used to assess the anti-edematogenic properties of anethole and estragole in Swiss mice. [26]

Plethysmometry is a method used to measure changes in the volume of tissue affected by edema (swelling) in Swiss mice. This technique can provide insights into the effectiveness of certain substances or treatments in reducing edema. [26]

Anethole demonstrated inhibition of carrageenan-induced edema across doses of 3, 10, and 30 mg/kg from 60 to 240 minutes post-induction. Conversely, the inhibitory effects of estragole were observed solely from 60 to 120 minutes at 10 and 30 mg/kg doses. Estragole showed inhibition of edema triggered by substance P, bradykinin, histamine, and TNF-a. [26]

IV.12.3 In silico study of estragole anti-inflammatory mechanism of action

In this study we attempt to see the affinity between Estragole and TNF-a protein by using the same methods and tools as our previous molecules, the more the affinity is bigger the more the inhibition is successful.

We downloaded TNF-a protein pdb file from pdb bank and estragole 3D molecule from PubChem website.

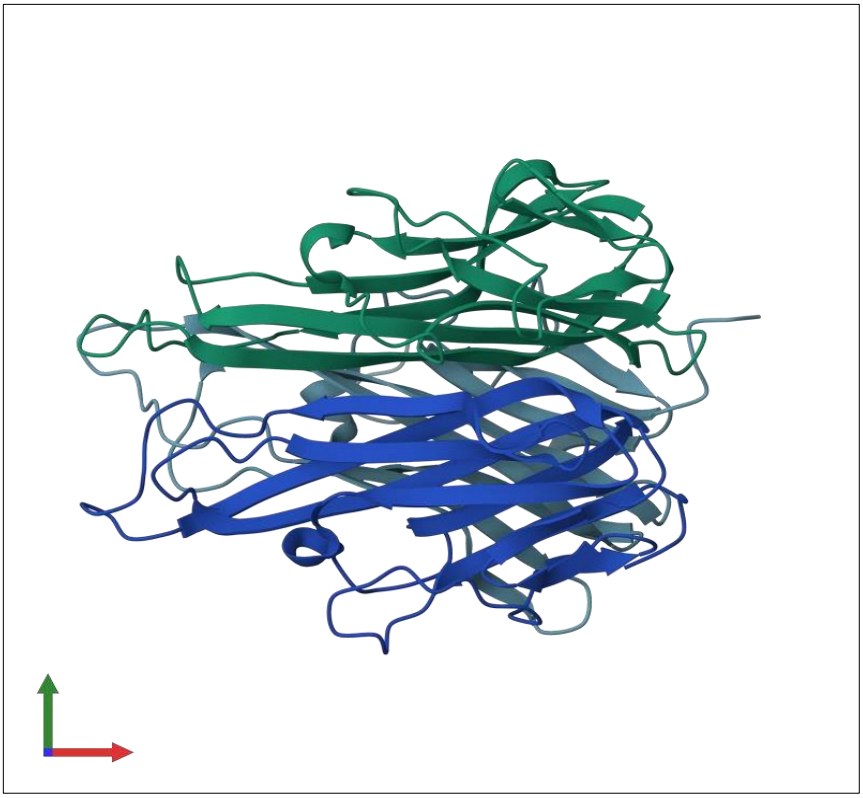


Figure IV.33: TNF-alpha Protein.

Passing through the same steps, the following figure represents TNF-alpha and Estragole as they are ready for docking:

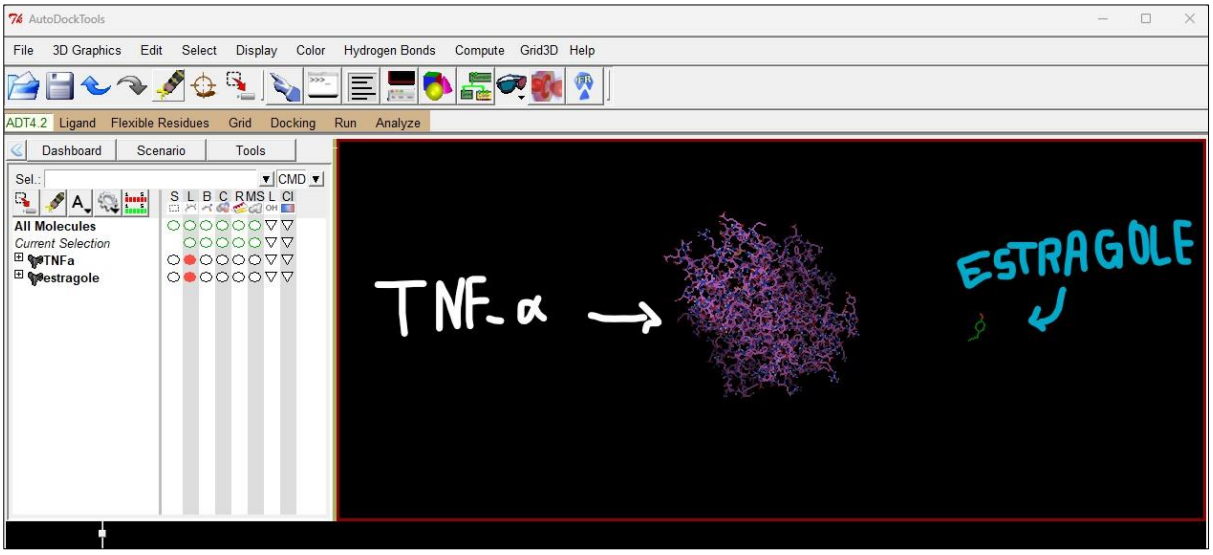


Figure IV.34: TNF-a & Estragole prepared for docking

IV.13 Results

After preparing files by following the exactly same steps as before, we will get the following results:

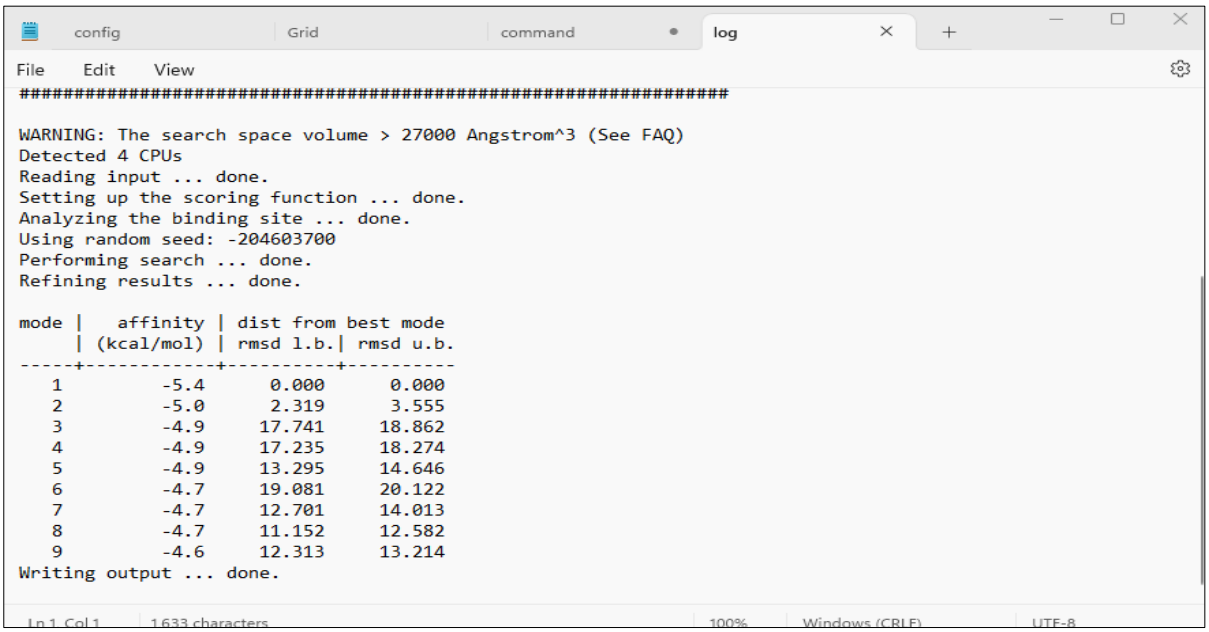


Figure IV.35: Results of the affinity value between TNF-alpha and Estragole using Autodock.

We notice that the affinity value brought from our molecular docking is (-5.4 kcal/mol) as (Table IV.15) shows:

Protein	Ligand	Affinity(Kcal/mol)
TNF-alpha	Estragole	-5.4

Using PyMol App, we were able to see the site of binding between estragole and TNF-alpha as the figure below shows:

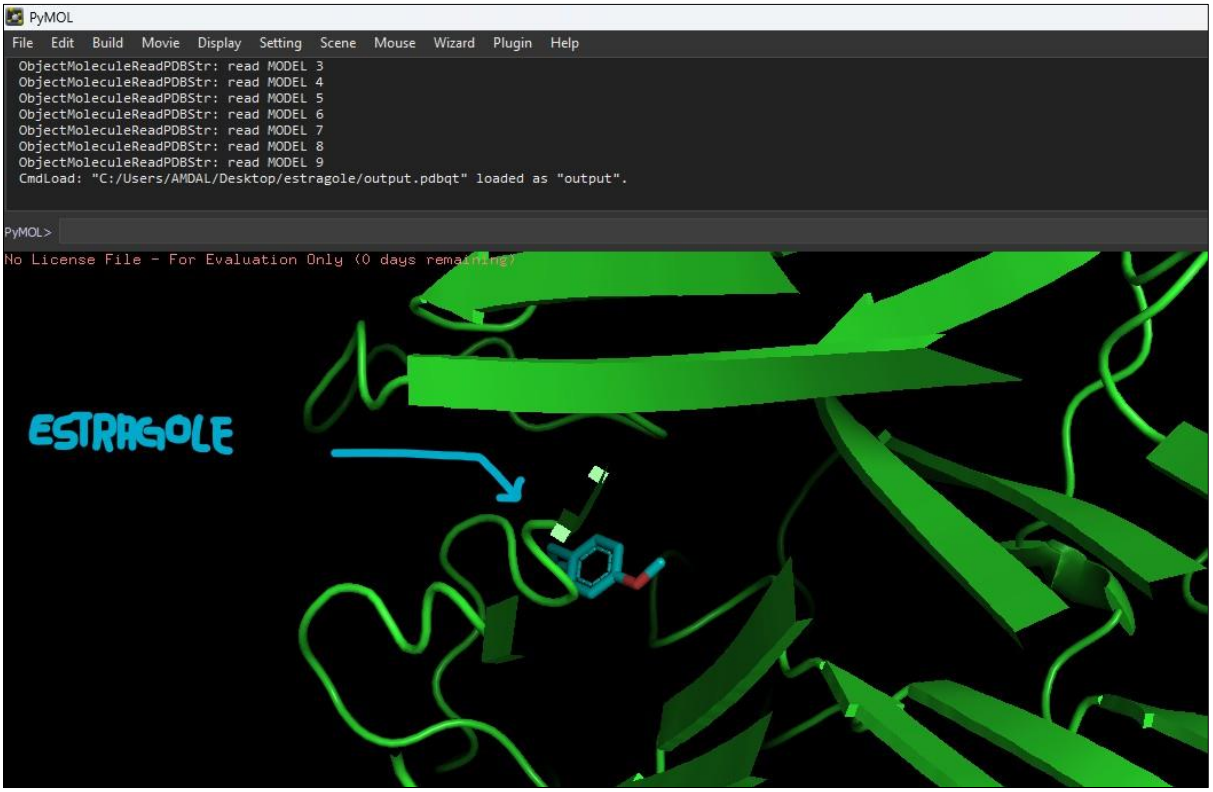


Figure IV.36: Estragole TNF-a binding site on PyMol App

In order to get more informations about the interaction, we used BDS (Biovia Discovery Studio) program, we were able to get the 2D diagram of the active site:

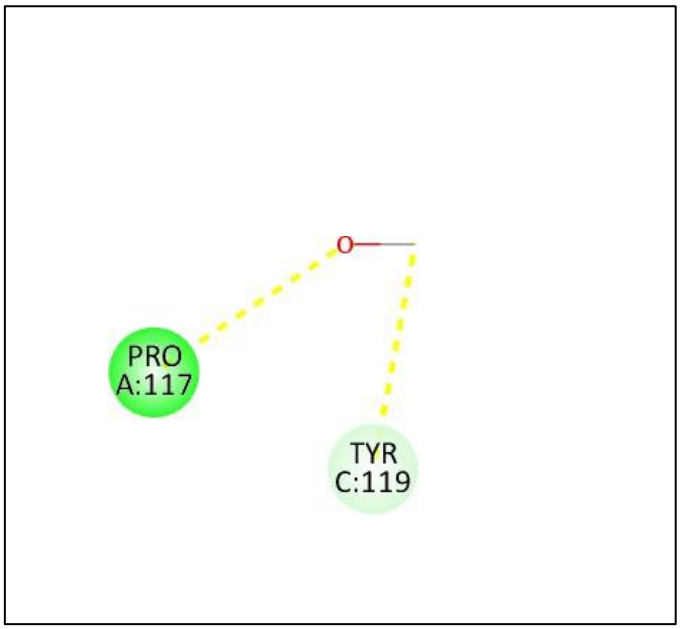


Figure IV.37: 2D diagram of Estragole – TNF-alpha bindings by (BDS).

Biovia Discovery Studio (BDS) provided us with more informations in (Table IV.16):

Name	Distance (Angstrom)	Types (Ligand – Aminoacid)
PRO117	3.1587	O-O
TYR119	2.99893	C-O

IV.14 Discussion

Molecular docking studies indicate that binding affinities in the range of -5.9 kcal/mol to -11.08 kcal/mol show strong binding affinity. [27][28]

To discuss our results, we need to compare them with results of other reliable anti-inflammatory molecules, we choose ibuprofen and diclofenac.

IV.14.1 The Anti-inflammatory activity of ibuprofen

Ibuprofen stands as the most commonly utilized and frequently prescribed NSAID.[36][37]

It functions as a non-selective inhibitor impacting both cyclo-oxygenase-1 (COX-1) and Cyclooxygenase-2 (COX-2). [38]

While its anti-inflammatory attributes might not be as potent as certain other NSAIDs, it plays a significant role as an analgesic and antipyretic agent. Its mechanism of action involves inhibiting cyclo-oxygenases, pivotal enzymes in prostaglandin synthesis.[39] Prostaglandins play a crucial part in pain, inflammation, and fever generation.[39]

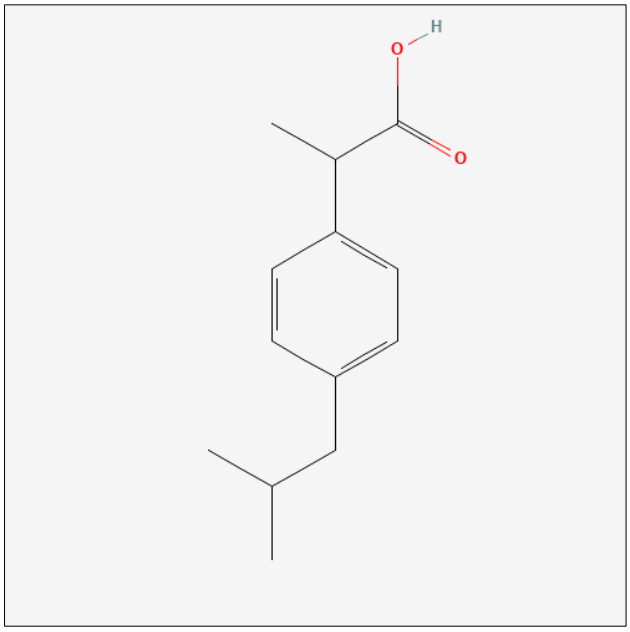


Figure IV.38: Ibuprofen 2D structure. [PubChem]

IV.14.2 In silico study of Ibuprofen – COX-2 interaction

Same steps are followed as in the previous molecular docking, starting by downloading the files, preparing them and finally starting the autodocking.

Results of the autodocking of ibuprofen-COX-2 interaction are represented below:

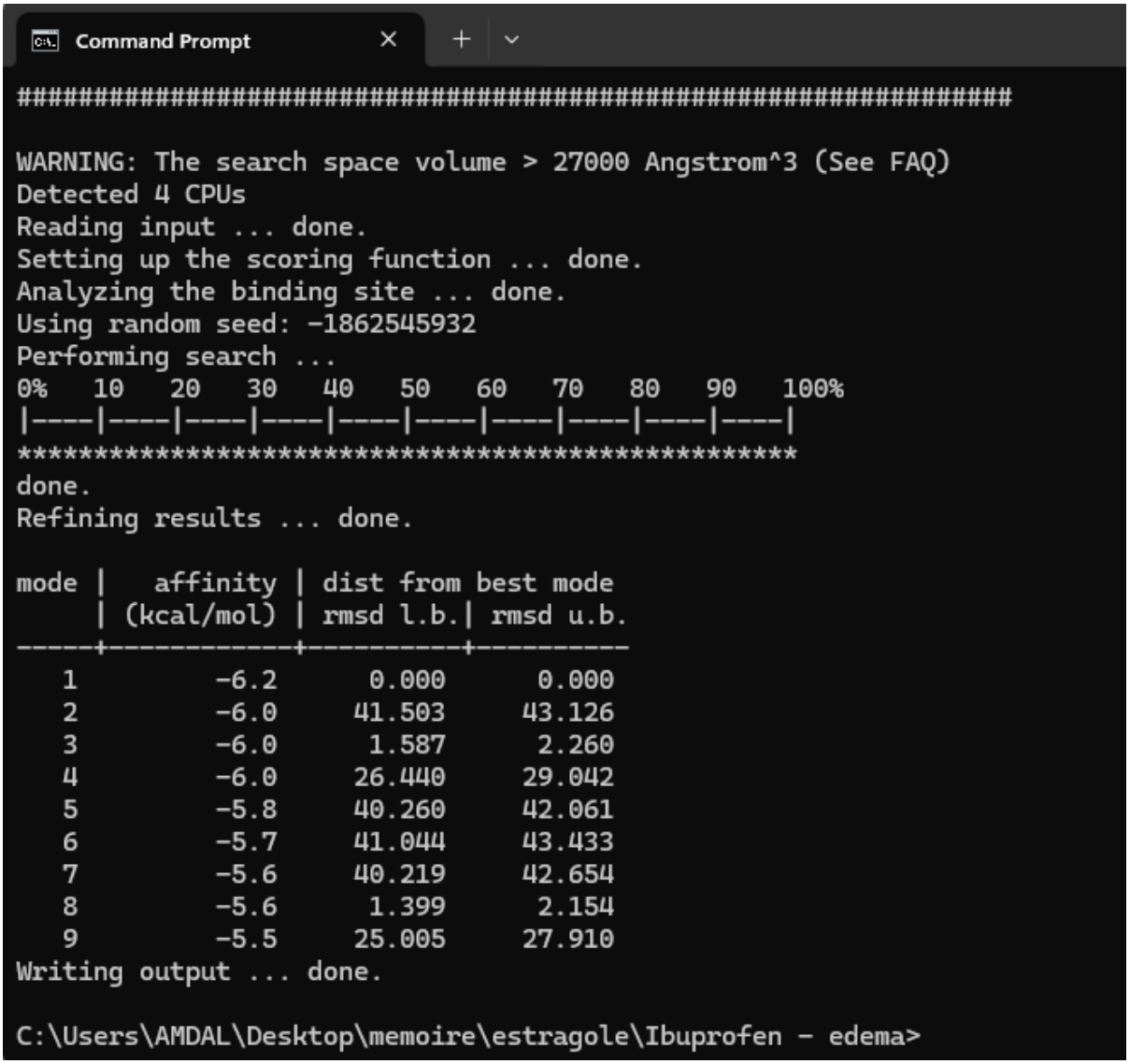


Figure IV.39: Ibuprofen-COX-2 autodocking results.

We notice that the affinity value brought from our molecular docking is (-6.2 kcal/mol) as (Table IV.17) shows:

Protein	Ligand	Affinity(Kcal/mol)
COX-2	Ibuprofen	-6.2

Using PyMol App, we were able to see the site of binding between Ibuprofen and COX-2 as the figure below shows:

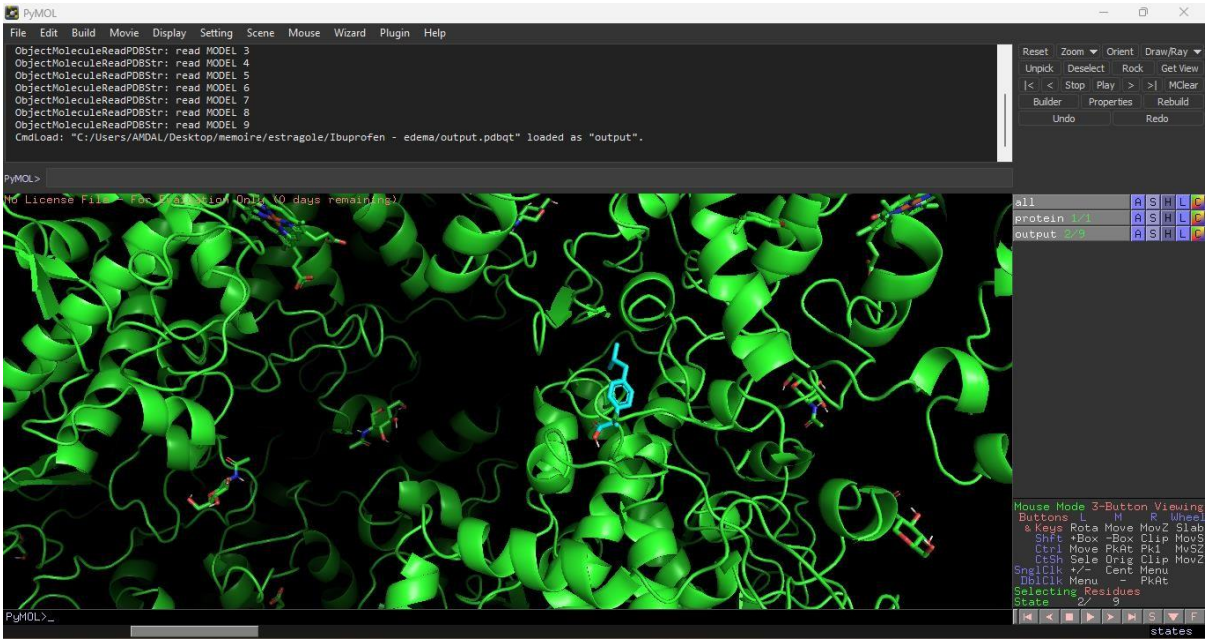


Figure IV.40: Ibuprofen - COX-2 binding site on PyMol App.

In order to get more informations about the interaction, we used BDS (Biovia Discovery Studio) program, we were able to get the 3D diagram of the active site:

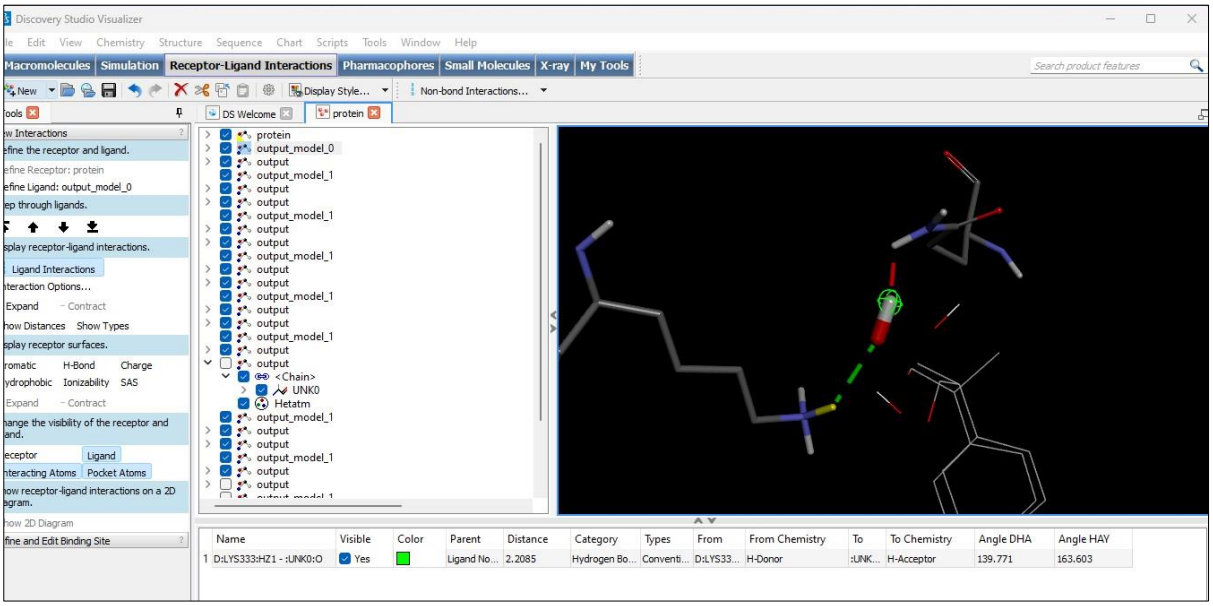


Figure IV.41: 3D diagram of Ibuprofen – COX-2 bindings by (BDS).

Biovia Discovery Studio (BDS) provided us with more informations in (Table IV.18):

Name	Distance (Angstrom)	Types (Ligand – Protein)
LYS D:333	2.2085	Conventional Hydrogen Bond (O-HZ1)

IV.14.3 In silico study of Ibuprofen – TNF-alpha interaction

ibuprofen has been shown to inhibit tumor necrosis factor-alpha (TNF-α) production. Research studies have demonstrated that ibuprofen, along with other nonsteroidal anti- inflammatory drugs (NSAIDs), can affect TNF-α levels. For example, a study published in The Journal of Infectious Diseases found that pre-treatment with ibuprofen augmented circulating TNF-α levels during acute endotoxinemia. [40]

Additionally, another study highlighted in the Turkish Journal of Immunology showed that ibuprofen significantly decreased the TNF-α response in a dose-dependent manner. [41]

These findings suggest that ibuprofen can inhibit TNF-α production, which is a key mechanism underlying its anti-inflammatory actions.

After we know that ibuprofen can inhibit TNF-alpha, we will do an autodocking about the interaction of it with TNF-alpha, results are shown below:

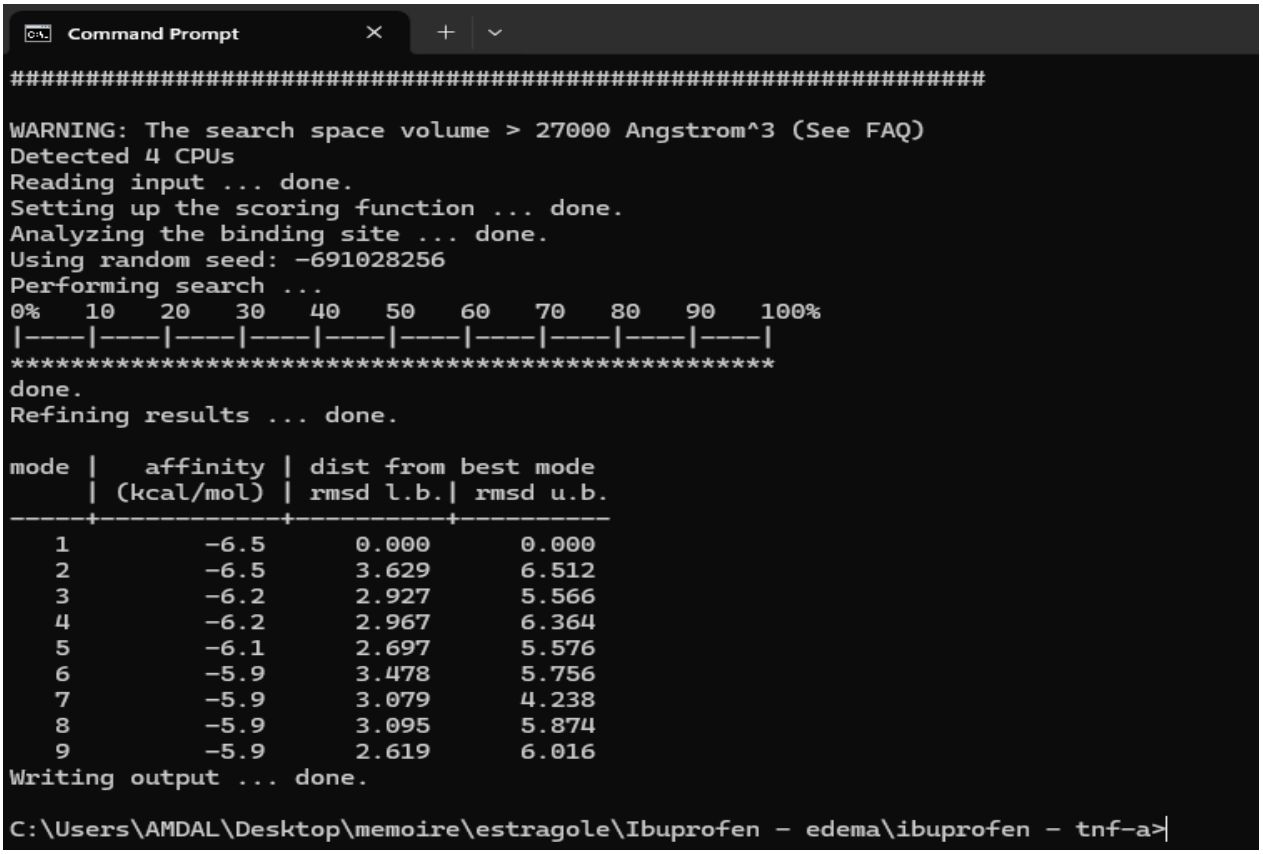


Figure IV.42: Ibuprofen- TNF-alpha autodocking results.

We notice that the affinity value brought from our molecular docking is (-6.5 kcal/mol) as (Table IV.19) shows:

Protein	Ligand	Affinity(Kcal/mol)
TNF-alpha	Ibuprofen	-6.5

Using PyMol App, we were able to see the site of binding between Ibuprofen and TNF-alpha as the figure below shows:

Figure IV.43: Ibuprofen – TNF-alpha binding site on PyMol App.

In order to get more informations about the interaction, we used BDS (Biovia Discovery Studio) program, we were able to get the 3D diagram of the active site:

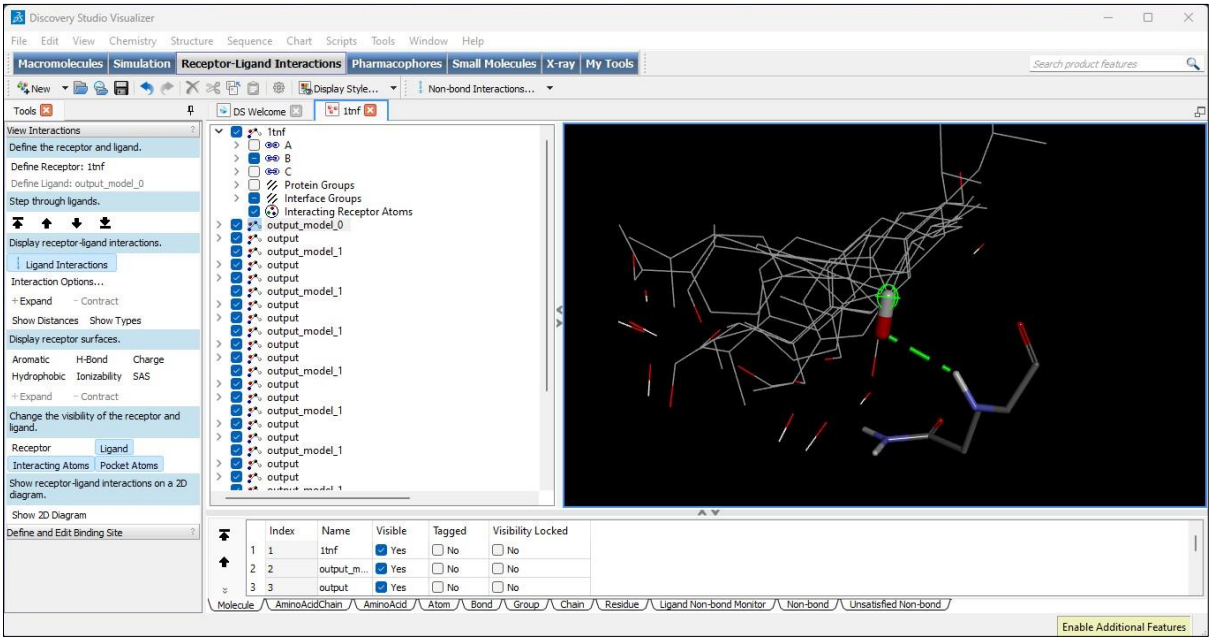


Figure IV.44: 3D diagram of Ibuprofen – TNF-alpha bindings by (BDS).

Biovia Discovery Studio (BDS) provided us with more informations in (Table IV.20):

Name	Distance (Angstrom)	Types (Ligand – Protein)
GLN B:102	2.27565	Conventional Hydrogen Bond (O-HN)

Now after we know that Ibuprofen can inhibit both COX-2 and TNF-alpha, we will study the interaction between Estragole and COX-2 in order to compare our results.

IV.14.4 In silico study of Estragole – COX-2 interaction

estragole has been found to inhibit cyclooxygenase-2 (COX-2) expression. Research studies have shown that estragole exhibits anti-inflammatory activity by regulating the expression of COX-2. For instance, a study published in the Korean Journal of Pharmacognosy demonstrated that estragole significantly inhibited COX-2 expression, along with other inflammatory mediators, through specific pathways like NF-κB (Nuclear factor kappa B) and MAPK (Mitogen-activated protein kinase). [42]

This indicates that Estragole has the potential to be used therapeutically in various inflammatory conditions due to its ability to inhibit COX-2 and other inflammatory pathways.

After we know that Estragole does inhibit COX-2, we will do the autodocking study, results are shown below:

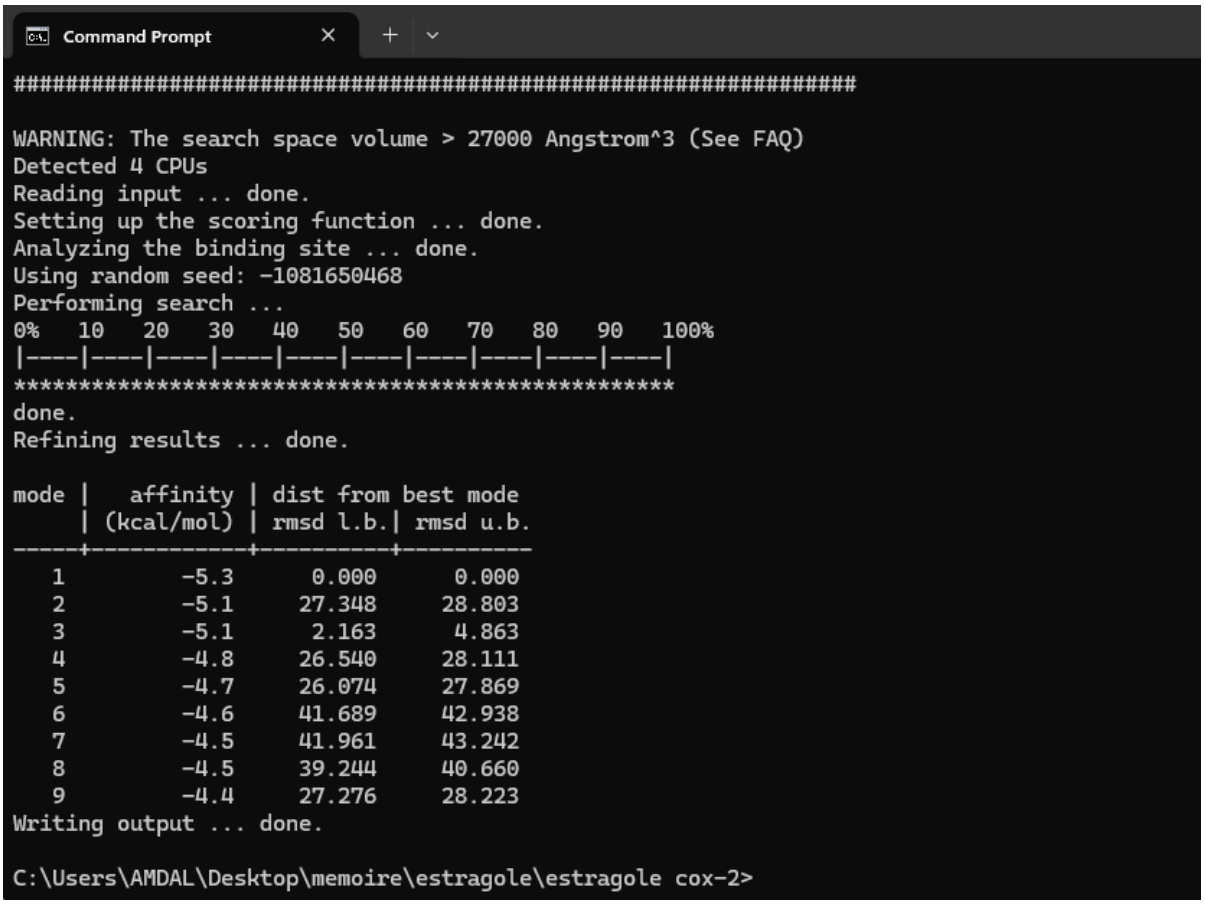


Figure IV.45: Estragole – COX-2 autodocking results.

We notice that the affinity value brought from our molecular docking is (-5.3 kcal/mol) as (Table IV.21) shows:

Protein	**Ligand**	**Affinity(Kcal/mol)**
COX-2	Estragole	-5.3

Using PyMol App, we were able to see the site of binding between Estragole and COX-2 as the figure below shows:

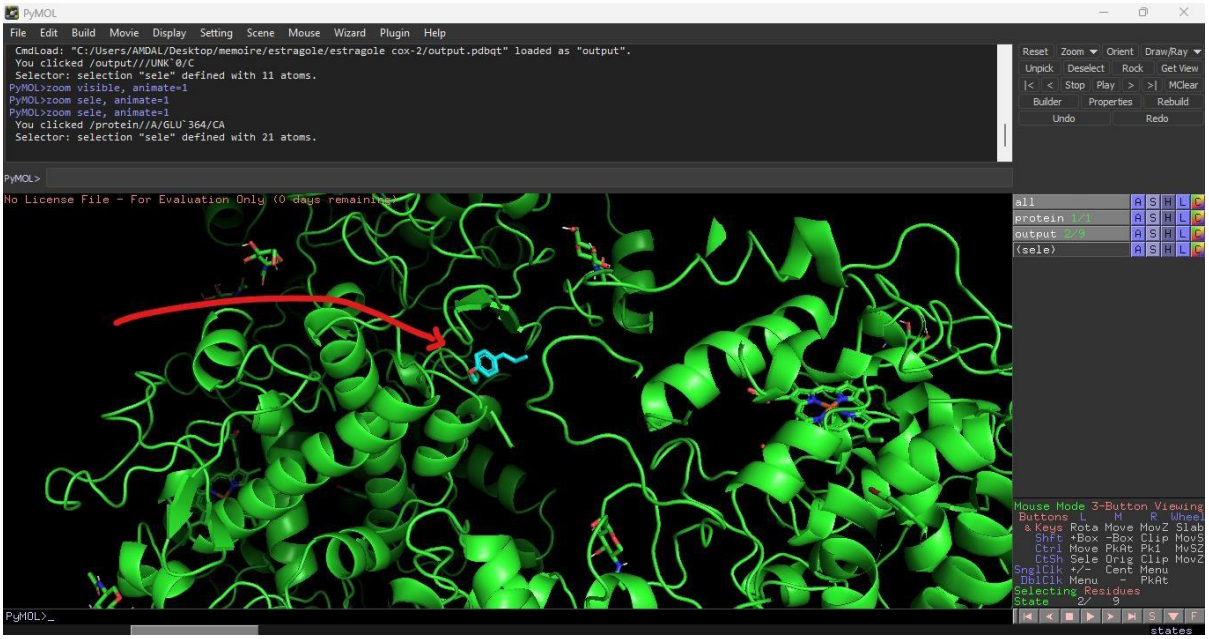


Figure IV.46: Estragole – COX-2 binding site on PyMol App.

In order to get more informations about the interaction, we used BDS (Biovia Discovery Studio) program, we were able to get the 3D and 2D diagram of the active site:

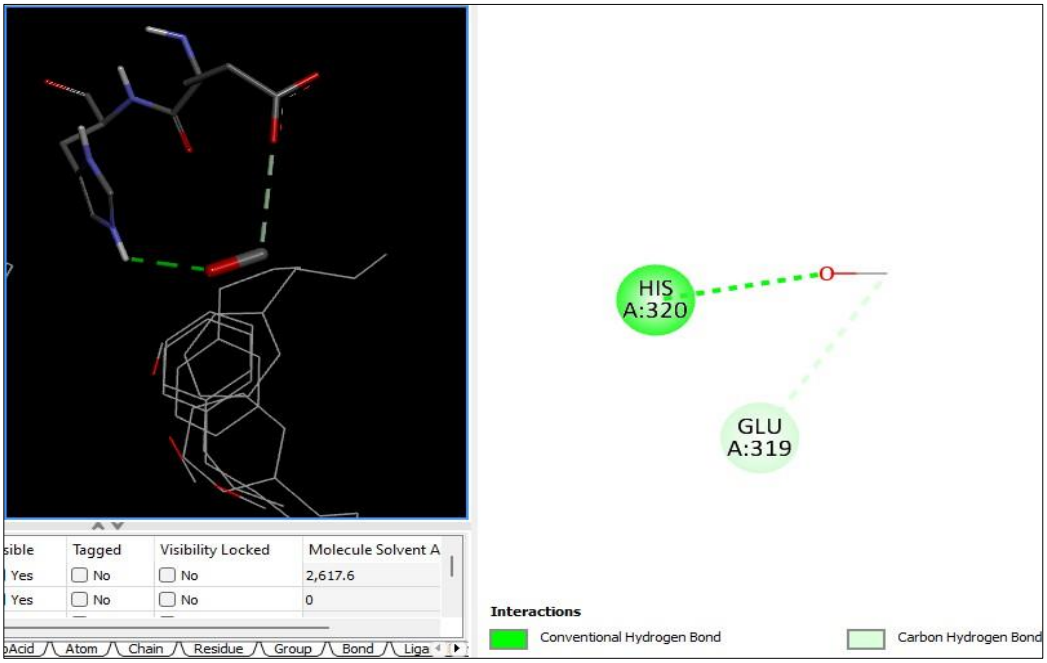


Figure IV.47: 3D diagram of Estragole – COX-2 bindings by (BDS).

Biovia Discovery Studio (BDS) provided us with more informations in (Table IV.22):

Name	Distance (Angstrom)	Types (Ligand – Protein)
HIS A:320	2.50147	Conventional Hydrogen Bond (O-H)
GLU A:319	3.38649	Carbon Hydrogen Bond (C-O)

IV.14.5 In vivo Study of Estragole

A study was made by a group of researchers under the title (Anti-inflammatory and antiedematogenic activity of the Ocimum basilicum essential oil (EOOB) and its main compound estragole: In vivo mouse models) in some universities of Brazil including Regional University of Cariri and Federal University of Sergipe. [45]

The in vivo test results indicate that treatment with EOOB (100 and 50 mg/kg) and estragole (60 and 30 mg/kg) significantly reduced paw edema induced by carrageenan and dextran. [45]

The lowest doses of EOOB (50 mg/kg) and estragole (30 mg/kg) were effective in reducing paw edema induced by histamine and arachidonic acid, inhibiting vascular permeability, and reducing leukocyte emigration in the peritoneal fluid. These doses were also able to mitigate the chronic inflammatory process. [45]

The results observed between EOOB and estragole demonstrate their efficacy in anti-inflammatory activity, with the essential oil proving to be more effective in both acute and chronic anti-inflammatory actions. This study confirms the therapeutic potential of this plant and supports its use in traditional medicine. [45]

IV.15 Conclusion

According to previous results of the in silico comparison between the anti-inflammatory activity of Ibuprofen, Diclofenac and Estragole shown in (Table IV.23) below we can declare that:

- Estragole has an interesting affinity value in comparison to ibuprofen and diclofenac.
- Estragole can be considered as a good anti-inflammatory molecule for edma and swelling.

Protein	**Ligand**	**Affinity (Kcal/mol)**
COX-2	Estragole	-5.3
	Diclofenac	-6.1
	Ibuprofen	-6.2
TNF-alpha	Estragole	-5.4
	Diclofenac	-7.6
	Ibuprofen	-6.5

IV.15 General Conclusion

In conclusion, the accumulated evidence from this in silico study strongly suggests that basil oil indeed possesses noteworthy therapeutic effects. From its long history of traditional medicinal use to modern scientific investigations, basil oil has demonstrated diverse pharmacological properties, including antimicrobial, anti-inflammatory, antioxidant, and analgesic activities. Its rich chemical composition, particularly dominated by compounds like eugenol, linalool, and methyl chavicol, contributes to its versatile therapeutic potential. Clinical studies and experimental research further support its efficacy in addressing various health conditions, ranging from respiratory ailments and skin disorders to stress and anxiety management. However, while the existing body of literature underscores the promising therapeutic benefits of basil oil, continued research is essential to elucidate its mechanisms of action, optimize dosage regimens, and explore its potential synergistic effects with other therapeutic agents. Overall, the findings suggest that basil oil holds considerable promise as a valuable addition to the armamentarium of natural remedies, offering a holistic approach to health and wellness.

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