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Thesis

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Theme:

Pharmacoinformatics-based identification process of phytochemicals against PARP-1 , HDACs and MDR as potential bioactive inhibitor compounds

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Dedication

I dedicate this work

To my amazing parents who supported and encouraged me during my studies.

My father who raised me, educated me and encouraged me to become the person I am today

My mother who always stood by my side and stayed up nights to illuminate my path and shared my joys and sorrows to the spring of kindness and tenderness, the most wonderful woman in existence, I love you.

To my brothers. Mohamed .Nasreddine .Kamel and Littel Ismail.

To my only sister Iméne, she warmly supported me and encouraged me throught my journey.

To my family, my loved ones and those who give me love and vitality.

To my friends Douaa Hassiba.Rahima Feriel and Rayane .

To my best friends Amel.and Ikram.

I wish for all of them more success.

I love You all.

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First I want to thank me for believing in me, I want to thank me for doing all this hard work all this years. I wanna thank for having no days off 5 years gone. I wanna thank me for never quitting. I wanna thank me for always being a giver and trying to give more than I receive. I dedicate this work

To my amazing parents who supported and encouraged me during my studies.

My father who raised me to become the person I am today

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To my only younger brother Mohamed and my only sister Aya

To him my You; His support and vigilance, his presence to relieve fatigue, and his companion for late nights at work

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To my greatest friends Douaa Hassiba.Rahima Feriel.

To my best soul mate and my partner of work Hana

I wish for all of them more success.

I love you all.

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Abstract

Heart failure and Colon cancer diseases are among the most common diseases in the world while time. Treating these two diseases by targeting three specific proteins using molecules from different plants will be a promising potential process. The object of this study is to inhibit the PARP-1; HDACs and MDR receptors which are responsible for one of these two diseases respectively. After screening of more than 21 plants original from chlef State, Algeria. We selected the *citrus peels* ; *Thymus vulgaris* ; and *Curcuma longa* . The best ethanolic extracts yield is *Citrus lemon peels* (24.10%) and *Thymus Vulgaris* have the best inhibitory activity against *Staphylococcus aureus* ATCC6538 (Gram positive) with D=25mm. All the plants have antioxidant activity: IC50(*Citrus*)=0.198mg/ml , IC50(*Curcuma*)=0.206mg/ml, IC50(*Thymus)*=0.187mg/ml .GC-MS analysis indicated several components in extracts. The study conducted a pharmacoinformatics analysis "*in silico"* of 12compounds from 25 bioactive compounds. *In silico* studies reveal that the inhibitor ligand *apigenine* with PARP-1, forming 8 amino acid bonds with ΔG = -8.8 kcal/mol and *thymonine* interacts with HDAC1 forming 7 amino acid bonds with ΔG = -7.6 kcal/mol . We finished our work with a new formulation which is emulgel.

KEYWORDS: pharmaco-informatics; *citrus peels; thymus vulgaris; curcuma longa*; PARP-1 ; HDACs; MDR; molecular docking; GCMS.

الملخص

يعد مرضا قصور القلب وسرطان القولون من أكثر الأمراض شيوعًا في العالم في الوقت الحالي. سيكون علاج هذين المرضين عن طريق استهداف ثلاثة بروتينات محددة باستخدام جزيئات من نباتات مختلفة عملية محتملة واعدة. ويتمثل الهدف من هذه الدراسة في تثبيطPARP-1 ؛ وHDACs ، ومستقبلات MDR المسؤولة عن أحد هذين المرضين على التوالي. بعد فحص أكثر من 21 نبتة أصلية من ولاية الشلف بالجزائر. وقع اختيارنا على قشور الحمضيات؛ والزعتر البري؛ والكركم . كانت أفضل المستخلصات الإيثانولية هي قشور الليمون الحمضي (24.10%) ونبات الزعتر له أفضل نشاط مثبط ضد المكورات العنقودية الذهبية (ATCC6538) (موجب الجرام) مع D=25 مم. جميع النباتات لها نشاط مضاد للأكسدة :قشر الليمون[IC50] = 0.198 ملغ/ مل، الكركم[IC50] = 0.206 ملغ / مل و الزعتر[IC50] = 0.187 ملغ / مل، وأشار تحليل GC-MS إلى عدة مكونات في المستخلصات. أجرت الدراسة تحليلاً صيدلانيًا " الانسيليكو" لـ 12 مركبًا من اصل 25 مركبًا نشطًا بيولوجيًا. كشفت الدراسات الانسيليكو أن الرابط المثبط *apigenine* معPARP-1 ، مكونًا 8 روابط من الأحماض الأمينية مع ΔG = -8.8 كيلو كالوري/مول ويتفاعل *thymonine* مع HDAC1 مكونًا 7 روابط من الأحماض الأمينية مع ΔG = -7.6 كيلو كالوري/مول. أنهينا عملنا بتركيبة جديدة وهي الهلام المستحلب.

الكلمات المفتاحية: المعلوماتية الدوائية ؛ قشور الحمضيات؛ الزعتر البري ,كركم ؛ PARP-1؛ HDACs؛ MDR ؛ الالتحام الجزيئ ,CGMS .

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List of abbreviations

- PARP-1: Poly (ADP-ribose) polymerase 1
- HDACs : Histone deacetylases
- MDR: Multidrug resistance
- Uv: ultra violet radiation
- Ic50: concentration of inhibition 50
- Dpph: 1,1-diphenyl-2-picrylhydrazyl
- Y: yield
- ATCC: American Type Culture Collection
- ABCB1: P-glycoprotein gene 1
- ABCA1: Multidrug resistance-associated protein 1
- LD50: Median lethal dose
- GCMS: Gas Chromatography-Mass Spectrometry
- logP: The partition coefficient
- 3D:3 dementionnel
- 2D: 2 dementionnel
- ADME: absorption, distribution, metabolism, and excretion
- HA: hydrogen acceptor
- HD: hydrogen donor
- GJF: Gaussian Job File
- Abs: absorption
- T.Vulgaris: thymus vulgaris
- C.Longa:curcuma longa
- PLIP: protein–ligand interaction profiler
- PPI: protein protein interaction

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Introduction

For thousands of years, civilizations around the world have used medicinal plants to treat a variety of diseases [133] today; traditional medicinal plants still constitute the main source of healthcare in many healthcare's in many developing countries and rural areas. Studies have proven to be very useful in identifying biologically active plants.

Much research has been done on the activities and chemical composition of ethno medicinal plants [134].Medicinal plants and plant-derived medicinal products are widely used in traditional cultures around the world and are becoming increasingly popular in modern society as natural alternatives or supplements to synthetic chemicals. [135]

Today, in many countries around the world, aromatic and medicinal plants have received increasing attention [136]

In Algeria, many researchers have studied and explored the bioactive components in the secondary metabolism of aromatic and medicinal plants with more than 3139 species [137]**;** among these plants, a large number of aromatic and medicinal plants grow spontaneously and are a source of substances with different therapeutic virtues, used since ancient times. In our region chlef ; The collected results show an important floristic diversity, 84 species distributed in 80 general have been identified, 44 are aromatic and medicinal species. [61]

On our research made on 27 plants founding in our region to inhibit the receptor choosing. At the end; we select 3 plants according to their founding and there best interaction in inhibitions of receptors with their molecules.

According to the hierarchy and distribution of aromatic and medicinal plants in our state, in the theory part; we have selected the most commonly used ancient and modern plants, including *curcuma longa ; thymus vulgaris* and *citrus Limon peels.*

Among the biological activities of medicinal plants, attention in recent years has focused on antioxidant activity due to the role it plays in the prevention of chronic diseases.[139]

In this study, we use AI and pharmacoinformitics as new process in phytochemicals screening leading to the development and discovering of new drugs, any process lead to development or discovering of drugs is pharmaceutical process engineering.

An Indian study of Akram khan show that the compound 5-isopropyl-2-methyl-1,4-benzoquinone is known as thymoquinone shown is the major active principle of the oil of Nigella sativa to inhibit gastrointestinal cancer ; at the same time this plants is too expensive in Algeria we found the same doses in *thymus vulgaris* [138].

The selected plants were studied in terms of family, classification and areas of inhibition on the receptors of selected diseases such as colon cancer and heart failure. The best interaction is identified with *apeginine ; hesperidin* ; *curcumin* ; and *rutin* .

Plants act at multiple sites and many receptors, depending on the degree of effect and the report of binding of the receptor to ligand interaction stability in the medicinal effect side.

We have identified the receptors studied as the most sensitive receptors for the medicinal effect stable interactions of the selected plants and major of multi-components in a plant that support all at the same time, including PARP-1; HDACs and MDRs, among others.

We studied the receptors carefully and in depth, knowing the physiological role of each receptor and studying what each receptor can cause diseases as a result of its malfunction or its appearance in very high concentrations that exceed the normal limit.

We start the experimental study with extraction of molecules from plants ; second the analysis of GCMS and others to explain the physio-chemical analysis and antibacterial test

The experimental study is on second part an *in silico* study of interaction ligand molecule ; the best interaction aims by the number of acido amine interact during hooking; such as rutin with PARP-1 and thymonine with HDACs .

Our work specifically aims to study the inhibition of the receptors PARP-1. HDACs. and MDR responsible for the colon cancer disease and heart failure in vitro and in silico in addition to its evaluation; antioxidant and antibacterial activities of bioactive compounds of plants .

To make our work more important we use *In silico* detailed study with an important new step optimization of our molecules.

- **Critical review**

As well as researcher said; Curcumin the major active ingredient of turmeric (*Curcuma longa)*. [132] or like citrus peels and derived extracts have demonstrated potent efficacious properties against various cancers due in large part to the rich content of flavonoids present in citrus peels. [131] .in this study; we see that they researched all mechanism of action of *Curcuma longa* inhibition of colon cancer and identified the molecular docking of ligand and receptors .The review it could be completed with optimization molecule part to get the best interaction value.

At the final; molecular docking present large part as reducer of in vivo studies in animals ; as long as the molecular docking show details of interaction ; it is a change to the site study how show in each time deferent acid amine in each interaction; therefore ; we fount optimization part is more essential in this study, we can see the value of dynamic part but also cant be done during the little time of study and the need of specific materials .

- **Problematic**

Can inhibiting receptors HDACS. PARP-1 and MDR completely cure diseases colon cancer and heart failure? Can we cure this disease completely? Or just prevent them? or just minimize their symptoms?

Does artificial intelligence reduce the reliance on experimentation, and can we consider its results as a viable method and a solution to the long duration of experimentation?

- **Purpose**

This research is primarily aimed at the treatment of colon cancer and the prevention of cardiac insufficiency using medicinal plants indigenous to Chlef Algeria. Based on pharmacoinformatics and artificial intelligence.

The novelty of the research lies in the initiative to adopt a completely new technology that has never been used before to treat two intractable diseases at the same time, by using the principle of selective and precise targeting of proteins on the surface of diseased cells that have become dysfunctional and cause the transmission of pathogenic cellular signals by inhibiting

them; leading to a strategic medical objective of significantly reducing the incidence of colon cancer and heart failure.

This leads to the strategic medical goal of significantly reducing treatment costs and minimizing the serious side effects caused by the use of multiple drugs at the same time .which can cause other more complex diseases or metastases.

Bibliographic synthesis

I-Introduction

Common cancers of the digestive system include gastric cancer (GC) and colon and rectal cancer (CRC) [1,2]. Both entities' prognoses remain dismal despite advancements in earlier identification, multimodal treatment, and surgical management [3].

Colon cancer is the second leading cause of cancer-related deaths worldwide. In 2020, more than 1.9 million new cases of colorectal cancer and more than 930 000 deaths due to colorectal cancer were estimated to have occurred worldwide [4].

Following the 2020 National Cancer Registries Network annual meeting in Algiers, Pr Bouzid declares that colorectal cancer is the most prevalent cancer in women over 40, second after breast cancer, and the first common cancer in men, far more common than lung cancer[5].

The majority of the receptors that cause colon cancer to spread quickly include: Interleukin (IL)-4 and -13; Eotaxin-2; PARP-1 ; HDACs ; and MDR (ABC receptors).

I-Epidemiological study of diseases receptors

1-PARP-1

Inhibition of poly(ADP-ribose) polymerase-1 (PARP-1) has turned out an innovative approach for cancer therapy due to its involvement in DNA repair pathways. Although several potent PARP-1 inhibitors have been identified, they exhibit high toxicity, resistivity and diverse pharmacological profile in clinical trials, which necessitate for extensive investigation and development of selective inhibitors [6].

Poly ADP-ribose polymerase (PARP) enzymes play a key role in a number of cellular processes, such as DNA repair, genome maintenance, and cell death. [7]. The best characterized member of the PARP family is PARP1, which was first identified for its role in the recognition and repair of single-strand DNA breaks (SSB) [8,9]. Since then, PARP1 has also been shown to have a role in a number of DNA damage response (DDR) pathways, including base excision repair (BER), homologous recombination (HR), non-homologous end joining (NHEJ), and DNA mismatch repair[8].

1-1- PAPR-1 ACTIVITY

PARP-1 has a modular architecture with DNA binding, catalytic, and regulatory functions distributed among six independently folded domains (Figure 1).Structures of each of the individual domains of PARP-1 in the absence of DNA have been determined [9].

The crystal structure of PARP-1 essential domains (Zn1, Zn3, WGR-CAT) bound to a DNA double-strand break has provided the first views of how the multiple domains of PARP-1 assemble on DNA to form the active enzyme [10].

A recent structure of human PARP-1 domains in complex with a DNA double-strand break demonstrated a PARP-1 interface with DNA damage that is distributed over three domains(see the figure 01) [11], providing new insights into the DNA binding activities of PARP-1[12].

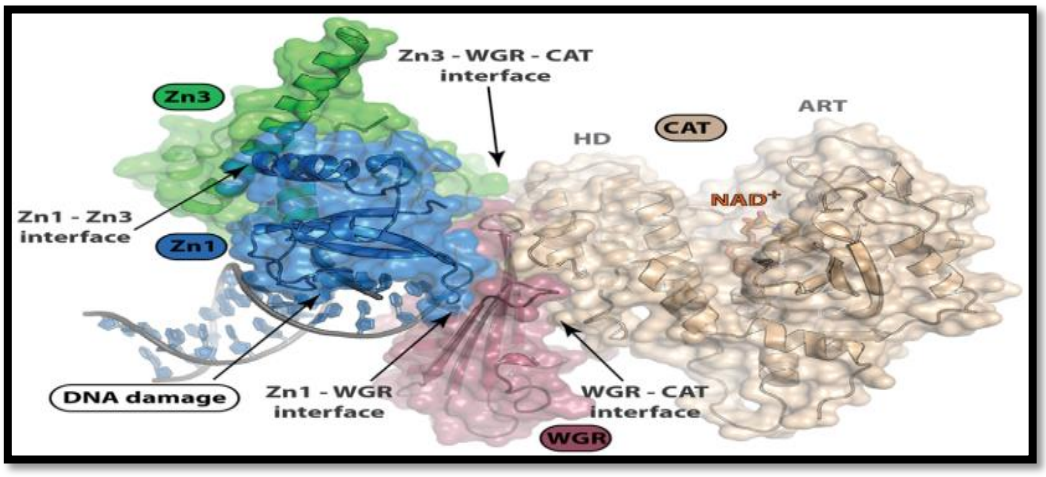


FIGURE 01: Structure of PARP-1 in complex with DNA damage. [12].

Poly(ADP-ribose) polymerase-1 (PARP-1) is an ubiquitous human enzyme that catalyzes the synthesis of the branched polymer poly(ADP-ribose) using NAD+ as the building block. This posttranslational modification typically occurs on the glutamate residues of the target proteins. Many protein substrates, including histones, transcription factors, DNA repair proteins, and PARP-1 itself have been identified. [13]. The cases are identified in figure 02.

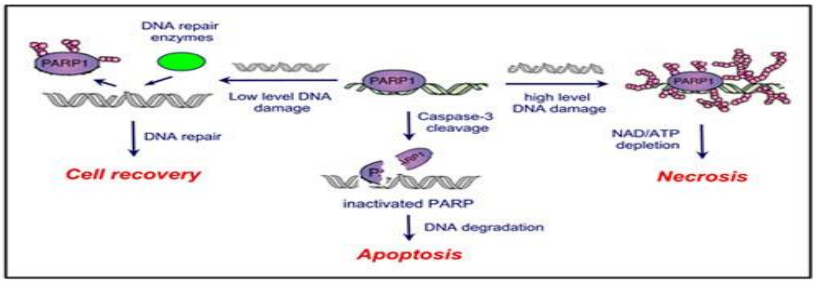


Figure 02: The different cases and damage effect on parp-1 activity. [13].

1-2- PARP-1 and colon cancer activity

PARP-1 is overexpressed in several human cancers, including colorectal cancer [,10,11], in which the expression of PARP-1 seems not to be homogenous in tumor cells, but it is highly expressed in colon cancer [14], indicating that PARP-1 could regulate programming [15]. Explaining the activity in figure 03

The fact that PARP-1 is overexpressed in tumor cells and contributes to vital processes for them suggest the idea of the use of PARP-1 inhibitors to fight colon cancer**.** [16]

Recently, a study revealed that PARP-1 seems to protect against the carcinogenic process but once it is initiated, PARP-1 expression facilitates tumor progression [17,18]

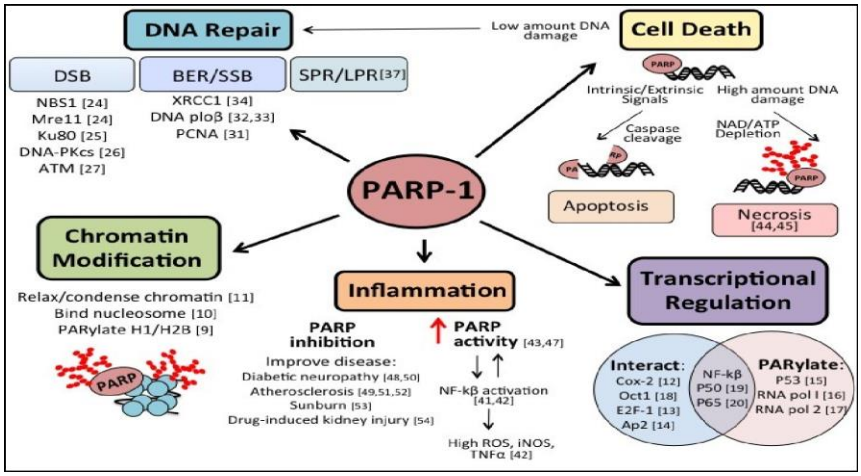


Figure 03: Activity of parp-1 in cell death methods in colon cancer**. [**19]

PARP-1 detects and binds to sites of single strand DNA damage via the DNA-binding domain. It then synthesizes poly(ADP) ribose (PAR) and transfers it to acceptor proteins. PR recruits other repair proteins to the damaged DNA site. **[**19]. PARP1 hyper activation induces depletion of NAD+ and ATP, resulting in cell death by necrosis or apoptosis **[**20].

1-3- PARP-1 and Heart Failure

Growing evidence reveals that poly(ADP-ribose) polymerase-1 (PARP1) is involved in the progression of many cardiovascular diseases. [55] Pharmacologic inhibitors of PARP-1 hold great promise in the treatment of heart disease. We see in figure 04 PARP inhibition or genetic deletion has been shown to be protective in animal models of cardiac and cerebral ischemia-reperfusion injury [56].

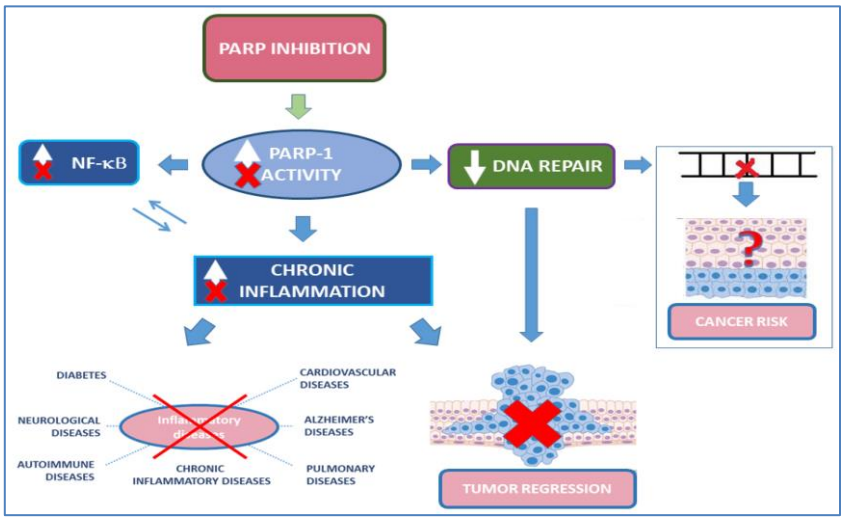


Figure 04: Schematic representation of the effects of PARP-1 inhibition on cancer and inflammatory-related disease. [57].

2- HDACs ;

Histone deacetylases (HDACs) are enzymes that balance the activities of histone acetyltransferases (HATs) in chromatin remodeling and thereby play an essential role in gene transcription to regulate cell proliferation, migration and apoptosis, immune pathways and angiogenesis. [21].

The role of HDACs in cancer is not restricted to their contribution to histone deacetylation, but also to their role in deacetylation of non-histone proteins. [22].

These HDACs play important roles in the regulation of gene expression, stress responses, DNA repair, cell cycle, genomic stability, etc., [23].

2-1 HDACs ACTIVITY

HDACs are a family of enzymes to control the acetylation of lysine residues making up the histones. The balance of acetylation and deacetylation then determines the post-translational acetylation of histone and other non-histone proteins. **[**24].

To date, 18 HDACs have been identified in mammals made up of four classes according to their sequence identity and catalytic activity. **[**25].

Class I HDACs (HDAC1, 2, 3 and 8) share sequence homology with the exception of HDAC3, no nuclear export signal . **[**26].

Class II HDACs (HDAC4–7, 9 and 10) are larger proteins than class I because they contain additional regulatory domains. Class II HDACs are further subdivided into class IIa (HDAC4, 5, 7, 9) and IIb (HDAC6, 10) with class IIa having relatively low enzymatic activity compared to class I possibly to allow them to efficiently process restricted sets of specific, unknown natural substrates.[27/28]

HDAC11 is the sole member of class IV. It is localized in the nucleus and has a catalytic domain in the N-terminal region. HDAC11 has been demonstrated to regulate the balance between immune activation and immune tolerance in CD4+ T-cells. [29]see all the HDACs on figure 04

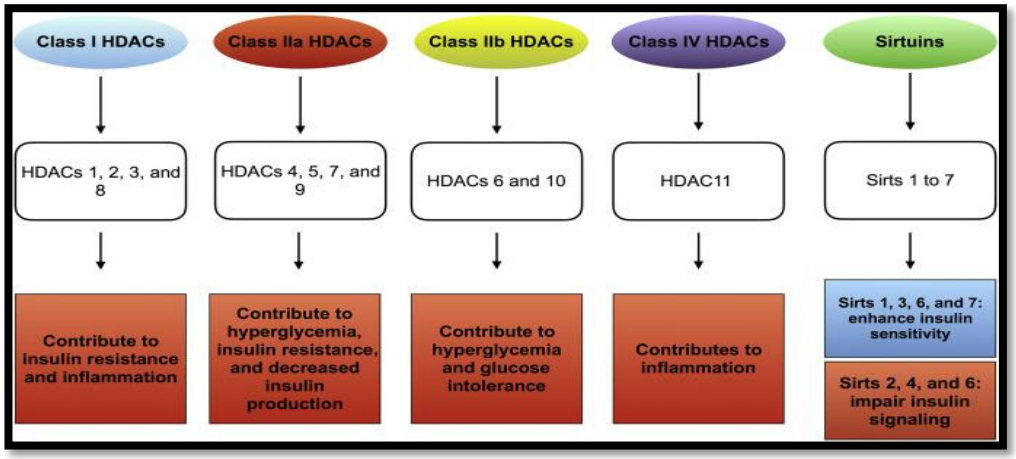


Figure 05: HDACs all classes . [30]

A typical characteristic of human cancer is the deregulation of DNA methylation and posttranslational histone modifications, in particular histone acetylation, the method on figure 06 which has the fatal consequence of gene transcription-deregulation. . [31]

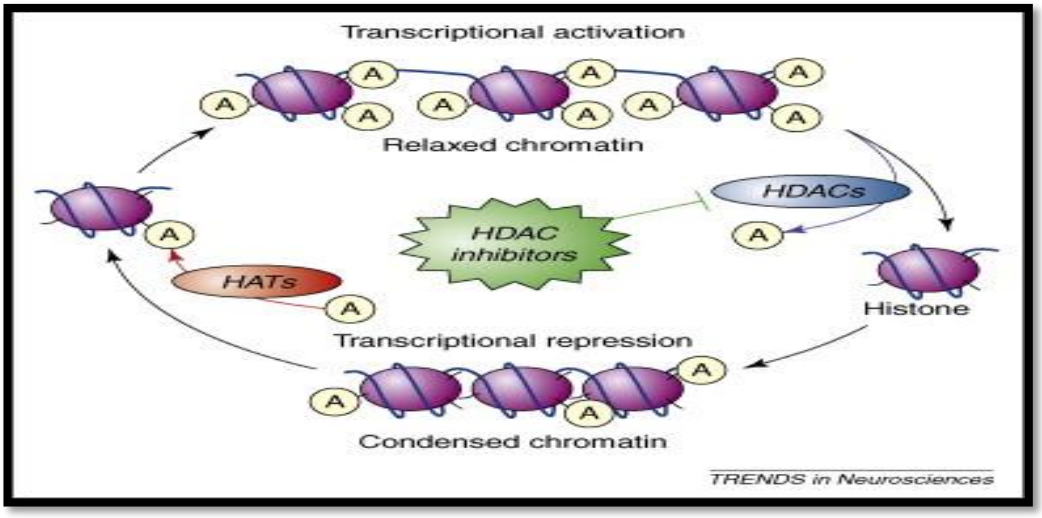


Figure 06 : HDACs activation methods . [32]

2-2- HDACs and colon cancer

Serval studies have also examined HDAC expression in colon tumors. [33]Most of these studies have reported increased expression of the class I HDACs, HDAC1,HDAC2 ; HDAC3and HDAC8, in colon tumors relative to adjacent normal mucosa. [34]

Increased expression of HDACs 1, 2 and 8 in colon tumors has been demonstrated at both the protein and mRNA level, suggesting transcriptional activation may be a likely mechanism of overexpression. [35]

Overexpression of HDACs however, is not observed in all colon tumors. For example, a study identified the presence of a truncating mutation within repeat in exon 1 of the HDAC2 gene. [36]

Knockdown of HDACs 1, 2 and 3 induce p21 expression in colon cancer cell lines, while their overexpression represses basal as well as HDACi-mediated p21 induction. [37] (see the method of inhibition in figure 07)

Mechanistically, the pro-proliferative effects of HDACs in colon cancer cells have been linked to transcriptional repression receptors inhibitor. [38]

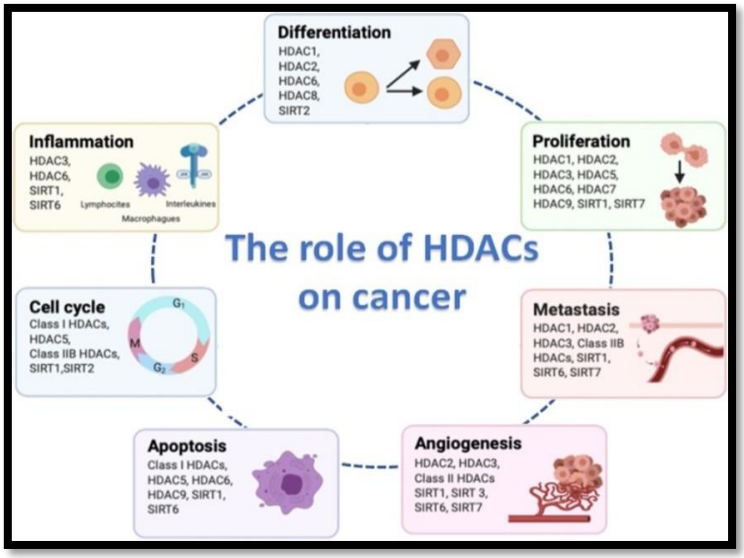


Figure 07: HDACs role in colon cancer inhibition. [38]

3- MDR

The development of simultaneous resistance to multiple drugs, with varying chemical structures and targets, is a major obstacle to effective cancer therapy. [39]

Multidrug resistance (MDR) is a kind of acquired resistance of microorganisms and cancer cells to chemotherapy drugs that are characterized by different chemical structures and different mechanisms of action. MDR is the consequence of the overexpression of a variety of proteins. [40]

The ABC transporter family is a protein superfamily with 49 different members categorized by gene sequence and structural similarities. These ABC transporter families, expressed in various tissues such as the liver, colon, intestine, kidney, and brain. [41]

3-1-MDR activity

ATP-binding cassette (ABC) systems are universally distributed among living organisms and function in many different aspects of bacterial physiology. ABC transporters are best known for their role in the import of essential nutrients and the export of toxic molecules, but they can also mediate the transport of many other physiological substrates. [42]

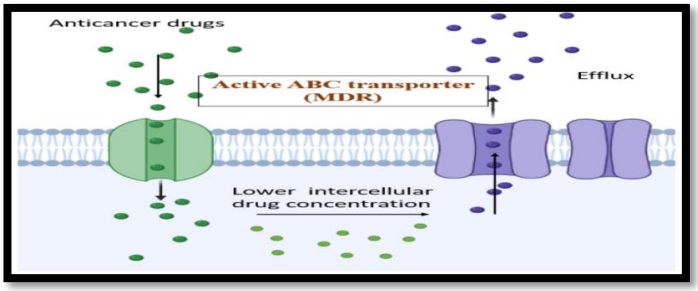


Figure 08: MDR receptors activity mechanism.

Figure 07 present MDR mechanisms in cancers were studied extensively and identified as multifactorial and complex phenomenon. [44]

MDR induction is related to the changes of some molecular pathways[45]. The loss of drug transporter proteins on the surface of the cell, change or mutation in drug specific targets [46] , DNA damage repair, decrease in apoptosis and increase in energy dependent efflux of hydrophobic drugs affect these molecular pathways [47]. There are 2 groups of "resistance to anti-cancer drugs":

1. Those that disturb drug delivery to cancer cells.

2. Those that emerge in cancer cells through genetic and epigenetic changes and affect drug sensitivity.

3-2- MDR AND COLON CANCER

One of the most difficult obstacles to overcome at the moment is multidrug resistance mechanisms, Patients may initially respond fully or partially to the first course of treatment, but later the cancer progresses or returns [48]. With continued therapy, cancers frequently develop cross-resistance to both structurally related and unrelated classes of cytotoxic medicines, in addition to the individual chemotherapy agent being used [49]. The primary cause of resistance developed in colorectal cancer cells [49].(see the colon cancer resistance on figure 09)

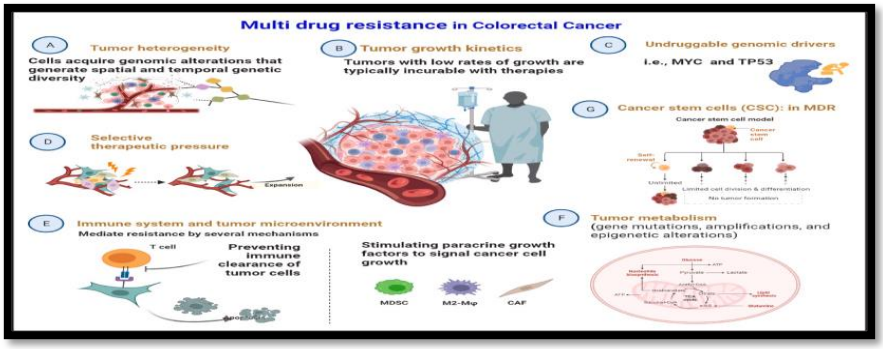


FIGURE 09: MDR in colon cancer activity [50].

II – Identification of Plants uses and here effect on studied diseases:

Researchers to prove and develop new drugs. Generally, using plants in the treatment of cancer has a long history and thus plants have been primary resources for producing traditional drugs effective in the treatment of cancer[51].

Moreover, researchers have reported a large number of plant species that have been used in the treatment of cancer since ancient times, and today, the tendency toward the use and evaluation of therapeutic effects of plants and their compounds as potential anticancer drugs are increasing**.** [52].

There are more than 25 000 phytochemicals in different plants that often have biological effect [53].

Phytomedicines are plant-based pharmaceuticals or extracts used to treat different ailments. 2019 data taken from the World Health Organization[54].

Algerian populations hold enormous traditional knowledge and practices on natural products that are being used for the treatment and management of various ailments including cancer [55]. We see the deferent of plants use histogram in figure 10. [60].

This precious knowledge was handed down through apprenticeship from earlier generations to descendants and through the intermingling of diverse ethnicities and civilizations in North Africa [58]. The classification of plants based on the form uses: seeds; spore; or peels; etc. (see figure 11 [60].)

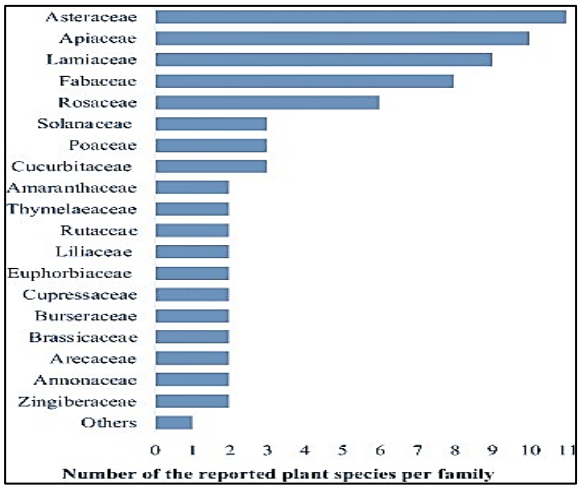


Figure 10: Plant families employed for cancer in Algerian plants .

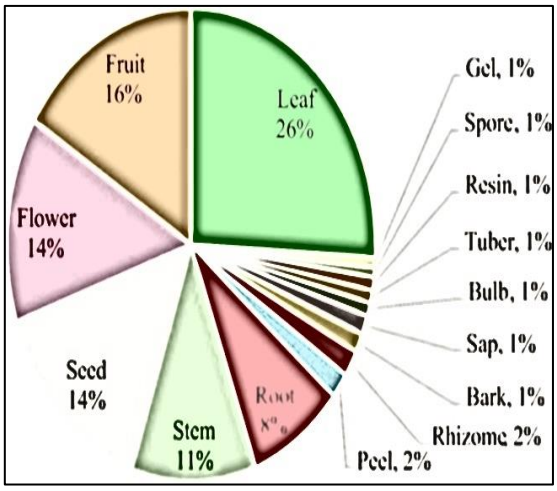


Figure 11: Frequency of plant parts used for therapy tradition way in Algeria.

In our region a census of local aromatic and medicinal plants was carried out in collaboration carried out in collaboration with the population southeast region of Chlef through an ethnobotanical which targeted different social categories (men or women, old or young old or young, civil servants or unemployed) has resulted in a rich and varied of wild medicinal flora and their therapeutic virtues. Showing in table 01: 84 species divided into 80 generation and 48 botanical families of which the *Lamiaceae* and *Asteraceae* [61].

TABLE 01: List of medicinal and aromatic species found in the south-east of Chlef [60].

Plant family: Species	**Common name**	**Local name**	**Plant part used**	**Application**	**Cancer type**
Vitaceae	-------	--------	----------	----------	----------
Vitis vinifera L.	Grape vine	Zbib	Leaf, fruit	Raw, powder	Breast
Curcuma longa L.	Turmeric	Curcum	Rhizome	Powder, decoction	Breast, bone, lung, digestive, uterus
Zingiber officinale Roscoe	Ginger	Zanjabyl	Rhizome	Powder, infusion	Breast, colorectal, liver, lung
Zygophyllaceae	----------	----------	----------	----------	----------
Peganum harmala L.	Harmel	Harmel	Seed	Raw, powder	Breast, brain
Rosaceae	----------	---------- --	----------	----------	----------
Crataegus azarolus L.	Azarole	Zaârour	Leaf, fruit	Infusion, decoction	Colorectal
Prunus armeniaca L.	Apricot	Mechmech	Root, leaf, flower, seed	Decoction, powder, oil	Breast, digestive, liver
Prunus amygdalus Batsch	Almond	Louz el mor	Root, leaf, seed	Raw, powder, decoction	Breast, bladder, colorectal, oral, uterus
Prunus persica (L) Batsch	Peach	Khoukh	Root, leaf, seed	Raw, powder, decoction	Breast, digestive
Rubus fruticosus G.N.Jones	Blackberry	Allaiak	Root, leaf, fruit	Raw, powder, decoction	Colorectal, oral
Rubus idaeus L.	Raspberry	Toute barri	Leaf, fruit	Raw, powder, decoction	Colorectal, oral, ovary

Rubiaceae	----------	----------	----------	----------	----------
Coffea canephora Pierre ex A. Froehner	Robusta coffee	Qahwa	Seed	Raw, infusion	Colorectal, oral, prostate
Rutaceae					
Citrus limon (L.) Osbeck	Lemon	Limone	Fruit, peel	Juice, infusion	Breast, digestive, lung, liver, skin
Ruta chalepensis L.	Fringed rue	Fidjel	Stem, leaf, flower	Decoction	Colorectal, prostate
Thymus vulgaris L.	Thyme	Zaâtar	Entire plant	Infusion	Digestive
Origanum floribundum Munby	Oregano	Zaâtar berri	Stem, leaf, flower	Infusion, inhalation	Breast, digestive, kidney, lung
Cinnamomum verum J.Presl	Cinnamon	Qarfa	Peel, bark	Decoction	Breast, lung

Regardless of the many medicinal plants in our region of Chlef, we chose to study three plants to inhibit our three receptors taking before: *thymus vulgaris*, *citrus lemon*, and *curcuma longa*.

1- *Thymus vulgaris*

Thymus vulgaris commonly known as "thyme" has been used for many centuries for its flavoring, culinary, and medicinal properties. The name thyme derives from the Greek word 'thymos' which means courage or strength. In the first century, thyme was used mainly as a medicinal plant, which was mentioned in Discords' work. However, in the Mediterranean region, it was used mainly as spice and then spread all over the world. [62].

Thymus vulgaris is a flowering plant of the family Lamiaceae commonly known as thyme, native to Southern Europe, and has a worldwide distribution**.** [63].

The plant is indigenous to the Mediterranean and neighboring countries, Northern Africa, and parts of Asia. In Africa, the plant has been cultivated in Egypt, Morocco, Algeria, Tunisia, Libya [64].

Figure 12: *T. vulgaris* fresh (A) and dried (B) leafy branches.

The plant is flowering and grows up to 15–30 cm high. Thyme is a tiny perennial shrub, with stems becoming woody with age[65] (*thymus vulgaris* botanic view on figure 12). The flowers are light violet, two-lipped, 5-mm long with a hairy glandular calyx, and borne with leaf-like bracts in loose whorls in axillary clusters on the branches or in terminal oval or rounded heads [64].

1-1- Vernacular names[66].

- **English**: Garden Thyme, Common Thyme, English Thyme, Thyme.
- **German**: Garden Thyme, Pot-Herb Thyme, English Thyme, Tomillo.
- **French**: Garden Thyme, German Thyme, Serpyllum.
- **Spanish**: Tomillo.
- **In Arabic** : called زعتر البري.
- **Other Names**: Black Thyme, Winter Thyme

1-2- Classification [67].

- Kingdom: Plantae
- Subkingdom: *Tracheobionta*
- Superdivision*: Spermatophyta*
- Division: *Magnoliophyta*
- Class: *Magnoliopsida*
- Subclass: *Asteridae*
- Order: *Lamiales*
- Family: *Lamiaceae*
- Genus: *Thymus L.*
- Species: *Thymus vulgaris L.*

1-3- Chemical compound

Many studies have been conducted on thyme species to identify their chemical composition. A wide variety of chemical compounds as well as essential oils constitute the main composition of thyme that varies with climate and geographical area. Investigations have reported that thyme contains 56.53% monoterpenes, 28.69% monoterpene hydrocarbons, 5.04% sesquiterpene hydrocarbons and 1.84% oxygenated sesquiterpenes [68].

Thymol is an important phenolic component mainly responsible for thyme's antioxidant activity. Thyme is rich in many flavonoids and phenolic antioxidants like zeaxanthin, Lutein, Apigenin, Naringenin, Luteolin and Thymoquinone[69].

The thyme's flowered stem contains flavonoid derivatives such as apigenol and luteolol, phenolic acids such as cafeic and rosmarinic acids, and tannins [70].

The phytochemical constituents of thyme include phenolics, terpenoids, and mostly thymol, eugenol, and saponins [71].

Thyme essential oil showed a high content of oxygenated monoterpenes (56.53%) and low contents of monoterpene hydrocarbons (28.69%), sesquiterpene hydrocarbons (5.04%), and oxygenated sesquiterpenes (1.84%)[72]. See more details in table 02

Tabel 02 : Chemical composition of thyme essential oils[67].

Component	Formula	Relative Concentration (%)
3-Hexanol	**C6H12O**	**0.1**
α-Tujene	**C10H16**	**1.52**
α-Pinene	**C10H16**	**1.31**
Camphene	**C10H16**	**0.75**
Sabinene	**C10H16**	**0.84**
3-Otenol	**C8H16O**	**0.36**
3-Otanone	**C8H16O**	**0.2**
B-Myrcene	**C10H16**	**0.67**
Thymoquinone	**C10H12O2**	**0.26**
α-Pellandrene	**C10H16**	**0.1**
δ-3-Carene	**C10H16**	**0.11**
α-Terpinene	**C10H16**	**2.36**
ρ-Cymene	**C10H14**	**7.61**
Sylvestrene	**C10H16**	**0.34**
1,8-Cineol	**C10H18O**	**0.57**
cis-Oimene	**C10H16**	**0.22**
β-Oimene	**C10H16**	**0.2**
ɤ-Terpinene	**C10H16**	**9.5**
cis-Sabinene	**C10H8O**	**0.1**
Thymol	**C10H14O**	**54.26**
Carvacrol	**C10H14O**	**4.42**
Octadienoic acid	**C18H12O**	**0.1**
Geranic acid	**C10H16O2**	**0.3**
Thymonene	**C18H16O8**	**0.21**

1-4- ***Thymus vulgaris*** **inhibition diseases:**

The thyme plant extract industry infiltrates the food industry since plants have been used historically as food preservatives. Plants contain phytochemicals that protect them from microbial contamination and spoilage [73].

A review of major biological and therapeutic effects of thyme and its main constituents is presented below, with a focus on antioxidant, anti-inflammatory, anticancer, and antimicrobial properties [74].

Thymus vulgaris was recently reported to inhibit the proliferation of human cervical and squamous cell carcinoma. However, whether T. vulgaris modulates the malignant phenotype of human colon cancer cells remains unknown [75].

There are several preclinical studies pointing to the anticancer potential of *T. vulgaris*. For example, the aforementioned herb has demonstrated significant free radical scavenging activity and proapototic effects [76] in the human BC T47D cell line. In a colorectal HCT116 cancer cell model, *T. vulgaris* extract was shown to inhibit proliferation in a concentration- and time-dependent manner [77].

2. *Curcuma longa*

Curcuma longa. (*C. longa*), popularly known as turmeric, belongs to the Zingiberaceae family and has a long historical background of having healing properties against many diseases. *Curcuma longa* has been used for liver obstruction and jaundice, and has been applied externally for ulcers and inflammation. [78].

Curcumin is the yellow pigment that is found in the rhizomes of *Curcuma longa*. This pigment is the main phytochemical with anticancer properties found in turmeric and belongs to the family of polyphenols[79].

Curcuma longa is native to Southeast Asia, and is extensively cultivated in Alleppey, Madras, and Bengal in India. Nowadays, it is also cultivated in many countries worldwide including Pakistan, Thailand, China, Taiwan, Indonesia, Malaysia and the Caribbean. Nevertheless, India remains the largest producer of turmeric in the world. [80].

Therefore, the aims of the present work were to investigate the local knowledge, the importance of use of C. longa in Algeria, and the factors affecting its utilization in herbal medicine. [81].

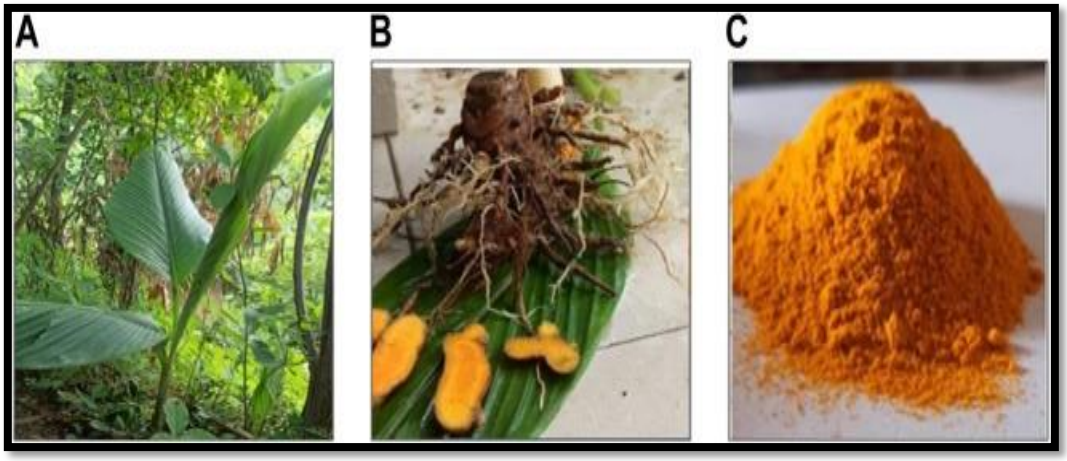


Figure 13: Important parts of *C. longa*. **(A)** *C. longa* in natural habitat, **(B)** medicinally important part of *C. longa* (rhizome), and **(C)** powder of dried rhizome of *C. longa* [82].

It typically grows to 3-4' tall in a foliage clump of ornamentally attractive, canna-like, pleated, elliptic to lanceolate green leaves (each to 3 1/2' long). Short dense spikes of pale yellow flowers are produced in summer. This plant is most noted for its thick, branched rhizomes which are the source of the bright yellow-orange powdery spice known round the world as turmeric. It has a pungent somewhat bitter flavor [83]. Different phase of curcuma mature are defined in figure 13.

2-1- <u>Vernacular names</u> [84].

- In various languages, turmeric goes by different names:
- *Belarusian, Bulgarian*, and *Macedonian*: *куркума*
- Bosnian: *kurkuma*
- Catalan: *Curcuma*
- *In Arabic :* كركم
- Croatian, Czech, Dutch, Finnish, and German: *kurkuma*
- Spanish, French, and Italian: Also referred to as *Curcuma*

2-2- **<u>Classification</u>**[85].

- Phylum: *Angiosperms*
- Class: *Monocots*
- Order: Zingiberales
- Family: Zingiberaceae
- Genus: *Curcuma*
- Species: *C. longa*

2-3- Chemical compounds

Curcumin, demethoxycurcumin and bisdemethoxycurcumin collectively known as curcuminoids (3-6%) are major polyphenolic compounds in turmeric rhizomes. [86].

The main coloring principle of turmeric rhizome was isolatedin 19th century and named as 'Curcumin'. Other phenolic compounds present in turmeric rhizome are(show in table 03) 1-hydroxy-1, 7-bis (4-hydroxy-3- methoxyphenyl)-(6E)-6-heptene-3, 5-dione; 1-(4- hydroxy-3, 5-dimethoxyphenyl)-7-(4- hydroxy-3-methoxyphenyl)-(1E, 6E)-1, 6- heptadiene-3, 4-dione; 1, 5-bis (4-hydroxy- 3-methoxyphenyl)-penta-(1E, 4E)-1, 4-dien- 3-one; 1-(4-hydroxy-3-methoxyphenyl-5-(4- hydroxyphenyl)-penta-(1E, 4E)-1, 4-dien- 3-one; 1-(4-hydroxy-3-methoxyphenyl)-7- (3, 4-dihydroxyphenyl)-1, 6-heptadiene-3, 5-dione and 1, 7-bis (4-hydroxyphenyl)-1[87].

Table 03: Major chemical composition of *curcuma longa* [88].

No.	Compound Name	Compound Type
1	curcumin (curcumin I)	Diarylheptanoid
2	demethoxycurcumin (curcumin II)	Diarylheptanoid
3	1-(4-hydroxy-3-methoxyphenyl)-7-(3, 4-dihydroxyphenyl)-1, 6-heptadiene-3, 5-dione	Diarylheptanoid
4	1-(4-hydroxypheny1)-7-(3,4-dihydroxypheny1)-1,6-heptadiene-3, 5-dione	Diarylheptanoid
5	bisdemethoxycurcumin (curcumin III)	Diarylheptanoid
6	tetrahydroxycurcumin	Diarylheptanoid
7	5-hydroxyl-1-(4-hydroxy-3-methoxypheny1)-7-(4-hydroxypheny1)-4,6-heptadiene-3-one	Diarylheptanoid
8	5-hydroxyl-1,7-bis(4-hydroxy-3-methoxypheny1)-4,6-heptadiene-3-one	Diarylheptanoid
9	1,7-bis(4-hydroxypheny1)-1-heptene-3,5-dione	Diarylheptanoid

2-4- *Curcuma longa* and deases inhibition

Curcumin, the primary polyphenol component in turmeric, has many biological actions. It works as an anti-cancer drug by modulating molecular targets that aid cell signaling processes. Transformation, proliferation, and apoptosis are all suppressed by it [89].

Curcumin has shown to arrest the cell cycle by the inhibition of multiple pathways involved in colorectal cancer. It acts as an anti-inflammatory agent by modulating the expression of key

proteins involved in chronic inflammatory pathologies leading to cancer development. In the intestine, curcumin downregulates self-renewal pathways in cancer stem cells, and anti-apoptotic genes/proteins. Its effect on these pathways has been demonstrated in colon cancer cells, mechanism show in figure 14 [91].

In vivo study in mice with colorectal cancer demonstrated an improved response to radiation therapy when combined with curcumin due to its ability to target nuclear factor [92].

Curcumin is a natural product derived from turmeric that appears to have cardiovascular benefit through a number of mechanisms. In this review, we have assessed the mechanisms by which curcumin may exert its effects in different models [94].

In the treatment of heart failure, curcumin can inhibit myocardial fibrosis and inhibit the activation of myocardial fibroblasts [93].

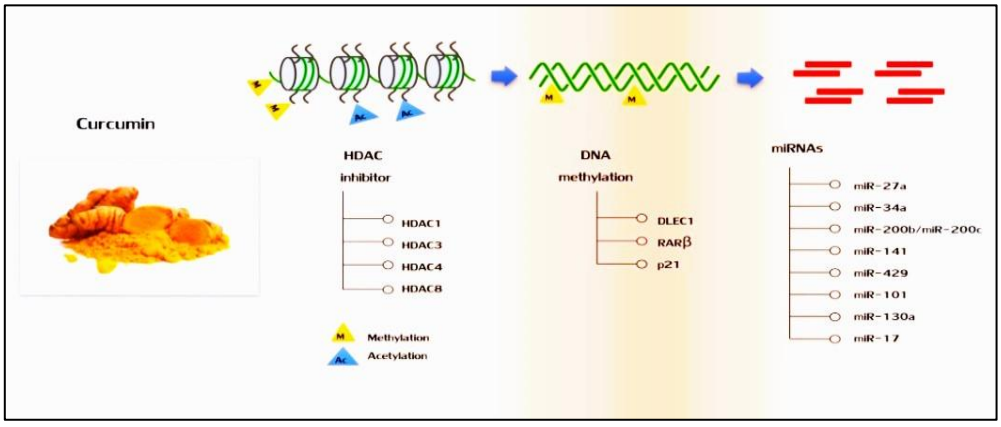


Figure 14: *Curcumins* HDACs inhibitors .

Curcumin as an epigenetic modulator and suppressor of cancer stem cell in colorectal cancer [94].

3. CITRUS PEELS

Citrus all over the world is famous for its nutritional and medicinal worth, and their peels are major origin of flavanones and many poly-methoxylated flavones that are very rarely available in other plants. [95].

Lemon peel contains the high presence of phytochemicals and has the greater antimicrobial and antioxidant activity. The *lemon peel* is a significant antimicrobial agent along with an astringent property [96].

Medicinal property most important specie of the citrus of wastes and byproducts which found major source of pectin, water soluble and insoluble antioxidants and essential oils.

Traditionally lemon is used in medicine for known abundant significant kidneystones, bring down a fever, balance pH because it contains citral, limonene, terpineol, geranyl acetate, linalyl [95].

Figure 15: *Lemon citrus peels* dray methods .

Chlef is one of the wilayas included in the national plan for self-sufficiency in agricultural produce, and ranks second in the country in terms of the area under *citrus* fruit cultivation. A number of varieties were exhibited on the *citrus* train, including *clementines, citrus limon, green lime, mandarin*, etc [96].

3-1- **Vernacular names of *citrus lemon*** [97].

- **English**: Eureka Lemon, Imperial Lemon, Lemon, Sweet Lime[1].
- **Spanish**: Limón[1].
- **Tunisian**: قارص (Qars)[2].
- **Algeria:** الليمون

3-2- **Classification of *citrus limon*** [98].

- **Kingdom:** Plantae
- **Phylum:** *Streptophyta*
- **Class:** *Equisetopsida*
- **Order:** *Sapindales*
- **Family:** *Rutaceae*
- **Genus:** *Citrus*
- **Species:** *Citrus limon*

3-3- Chemical compounds

Citrus peels accumulate in secretary cavities scattered throughout the flavedolayer of *citrus* fruits; The most important group of bioactive compounds like flavonoids such as: flavanones: eriodictyol, hesperidin, hesperetin, naringin; flavones: apigenin, diosmin; flavonols: quercetin; and their derivatives [99]. (See figure 16 and table 04)

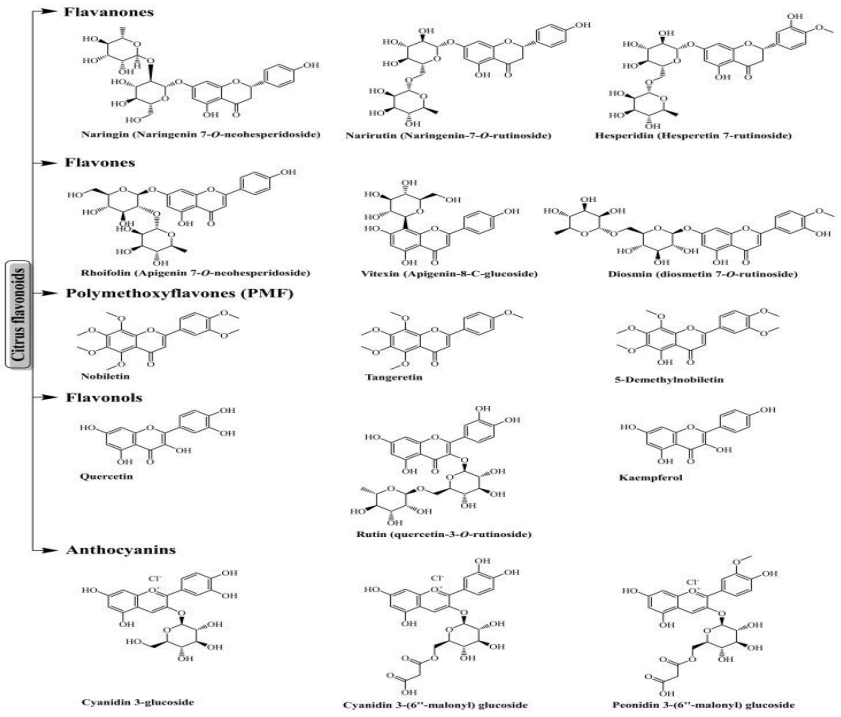


Figure16: Chemical structure of flavonoids characteristic of *C. Limon* [99].

Table 04: Composition of *Citrus Limon* fruits extracts and peels [99].

Group of Compounds	Part of Fruit	Metabolites
Flavonoids	Whole fruit (pulp, seed and peel)	flavonones: eriocitrin, eriodiktyol, hesperidin, naringin, neoeriocitrin, neohesperidin
		flavones: apigenin, diosmetin, diosmin, homoorientin, luteolin, orientin, vitexin
		flavonols: isoramnethin, quercetin, limocitrin, rutoside, spinacetin
Limonoids	Whole fruit (pulp, seed and peel)	limonin, nomilin
Phenolic acids	Whole fruit (pulp, seed and peel)	dihydroferulic acid, p-hydroxybenzoic acid, 3-(2-hydroxy-4-methoxyphenyl)propanoic acid, synapic acid
Carboxylic acids	Whole fruit (pulp, seed and peel)	citric acid, galacturonic acid, glucuronic acid, glutaric acid, homocitric acid, 3-hydroxymethylglutaric acid, isocitric acid, malic acid, quinic acid
Coumarins	Whole fruit (pulp, seed and peel)	citropten (5,7-dimethoxycoumarin), scopoletin
Furanocoumarins	Whole fruit (pulp, seed and peel)	bergamottin
Amino acids	Whole fruit (pulp, seed and peel)	L-alanine, L-arginine, L-asparagine, L-aspartic acid, dimethylglycine, glutamic acid, L-phenylalanine, DL-proline, L-tryptophan, L-tyrosine, L-valine
Carbohydrates	Peel	monosaccharides: arabinose, fructose, B-fructofuranose, B-fructopyranose, galactose, glucose, mannose, myoinositol, rhamnose, scylloinositol, xylose
Vitamins and theirs metabolites	Whole fruit (pulp, seed and peel)	disaccharides: sucrose
	Whole fruit (pulp, seed and peel)	choline, pantothenic acid, trigoneline, vitamin C

3-4- ***Citrus peels*** **inhibitor of deases** [100].

Cancer cells differ from normal cells by their ability to proliferate without control, resistance to apoptosis, ability to form new blood vessels, and metastasis to distant parts of the body. Flavonoids found in citrus peels have been shown to suppress these events through modulation of multiple cellular proteins that inhibit cell proliferation by downregulation of oncoproteins.

Among the anti-carcinogenic(colorectal cancer) activities of quercetin, the most notable described in Colorectal cancer are inhibition of cellular proliferation and growth, cell cycle arrest, induction of apoptosis, reduction in tumor size, decrease in number of tumor nodule, suppression of metastasis, decrease in inflammation , and reduction in multidrug resistance.

III- molecular docking

Insilico techniques have been successful and have become powerful tools in the search to cure diseases [101] **;** reducing the use of animal models in pharmacological research, assisting in

the rational design of novel and safe drug candidates, and repositioning marketed drugs. [102]. They are vital in identifying viable therapeutic candidates at a low cost and time by using sophisticated computers and information technology to speed up drug discovery, lead optimization, drug development, and design [103].

Studying protein-ligand (showing in figure 17) interactions continues to be very important in life science fields [104]. There are variations in protein-ligand complex structures due to different docking methods [105].

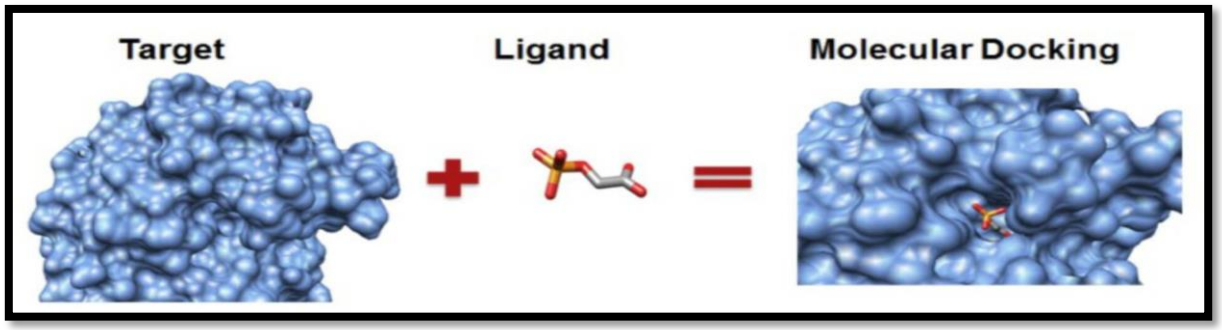


Figure 17: Lock-and-key analogy[105].

Table05: Docking terminology [106].

Term	Meaning
Receptor or target or host or lock	The "receiving" molecule, commonly a protein
Ligand or guest or key	The complementary molecule binding to a receptor, often a small organic molecule

Docking Insilico apps stages are:

1- **PubChem** It is an American database of chemical molecules managed by NCBI (the National Center for Biotechnology Information). It currently contains several million of compounds whose structure and physio-chemical properties are freely accessible [107].

Figure 18: Screenshot of interface of PubChem.

2- **Uniprot** Is a worldwide collection of three-dimensional structure data (3D structure) biological macromolecules: essentially proteins and nucleic acids [108].

Figure 19: Interface of uniprot data.

3- **Gaussian 09w** Locating the minimum energy structure of molecules, typically referred to as geometry optimization, is one of the first steps of any computational chemistry calculation. Earlier research was mostly dedicated to finding convenient sets of molecule-specific coordinates for a suitable representation of the potential energy surface, where a faster convergence toward the minimum structure can be achieved [109].

Figure 20 : Gaussian app.

4- **Gauss view 6-0** is a program that helps you prepare input to Gaussian by using visual guides when simulating molecules and reactions, and also examine the output from Gaussian when the simulation runs. It allows the user to quickly sketch and design complex and large molecules, then rotate and zoom around the entire model**.** [110].

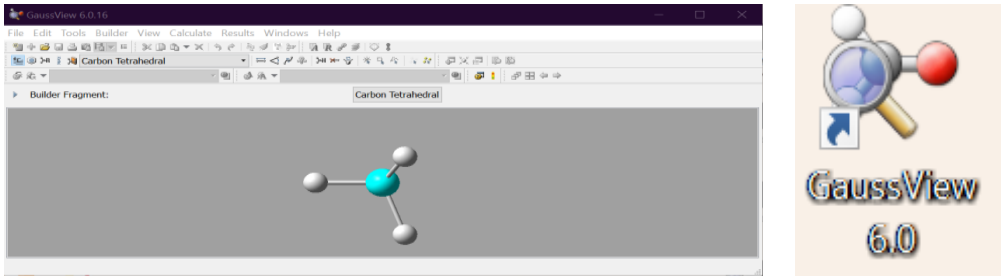


Figure 21: Interface of Gaussview app.

- **<u>The value of using optimization step</u>**

Optimization of the geometry of molecules is an important process to obtain the equilibrium molecular structures in quantum chemistry computations. Since the physical

and chemical properties of molecules are dependent on their specific geometrical structures, elucidation of the optimized structures enables the prediction of properties and identification of chemical products.[131]

A main challenge in drug discovery is finding molecules with a desirable balance of multiple properties. Here, we focus on the task of molecular optimization, where the goal is to optimize a given starting molecule towards desirable properties.[130]

We found some key aspects of Gaussian

- Requires an input file that specifies the molecular system, calculation type, and other parameters. The output provides information about the computed properties.
- Density Functional Theory (DFT): Balances accuracy and computational cost.
- Molecular Mechanics (MM): Employs force fields for large systems1.
- Geometry Optimization: Determines the most stable molecular geometry

5- **AutoDock TOOLS** is a suite of automated docking tools. It is designed to predict how small molecules, such as substrates or drug candidates, bind to a receptor of known 3D structure. AutoDock consist of two generations of software: AutoDock 4 and AutoDock Vina. More recently, we developed AutoDock [111] .

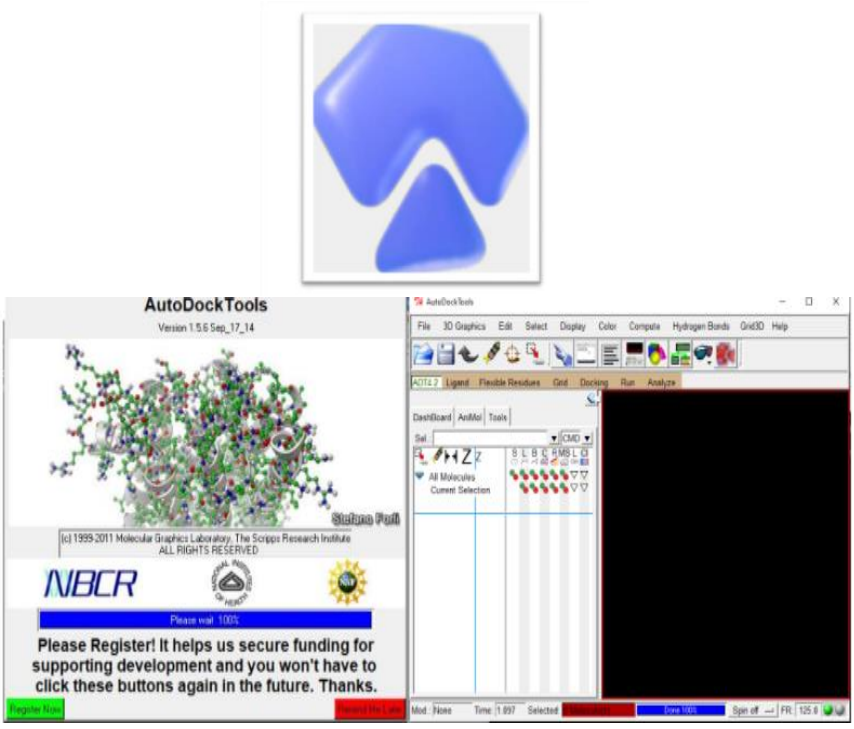


Figure 22: Interface and app of Autodock tools .

6- **AutoDockVina** is the newest member of AutoDock suite introduced in 2010.Only three dimensional structures of molecules (with polar hydrogen's only, as AutoDockVina still uses United Atom model) and a box definition of a search space is required. Partial charges, solvation parameters or pre-calculated interaction energy grids are not necessary for the simulation [112].

7- **BIOVIA Discovery Studio** brings together over 30 years of peer-reviewed research and world- class in silico techniques such as molecular mechanics, free energy calculations, bio therapeutics develop ability; With Discovery Studio you can Investigate and test hypotheses in silico prior to costly experimental implementation, thus reducing the time and expense involved in bringing products to market [113].

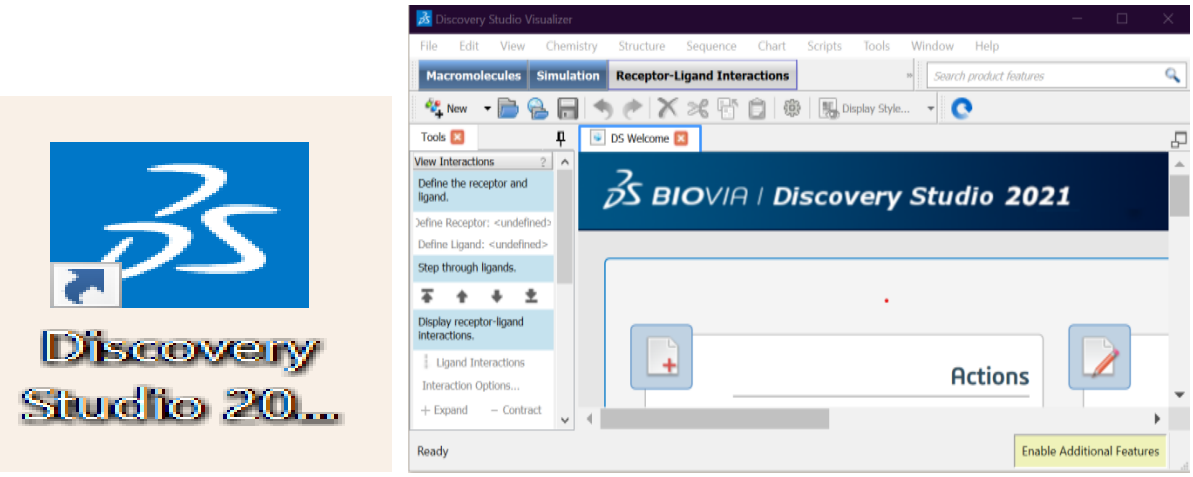


Figure 23: Interface and app of biovia discovery.

Conclusion

Colon cancer and heart failure are considered as one of the diseases of the age, as they are considered as fast spreading diseases in the body, from their current treatment with drugs and radiotherapy the disease is controlled, but with more complications in side effects on the other hand. On this basis, the detailed study of the diseases was carried out using medicinal herbs to minimize the side effects and inhibit the disease.

This Chapter summarizes recent findings of critical role played by molecular docking in the process of drug discovery and development. The application of docking approach will assist to design a dosage form in the most cost effective and time saving manner.

Material and methods

IV-introduction:

This chapter provides an overview of the physical and chemical properties of citrus peels; thymus vulgaris and curcuma longa and their extracts, as well as the development of antibacterial activity against pathogenic bacteria and their antioxidant activities also anti-inflammatory effect .

We are presenting work consists of a comparative study of the antimicrobial power of bark of *lemon peel*, *thyme (thymus vulgaris)* and *curcuma longa* ;the application of these plant extracts to the formulation of a drinkable emulgel.

This work is being carried out jointly at:

- Laboratory of the process engineering department of the HASSIBA BEN BOUALI CHLEF University.
- Laboratory of Microbiology, physico-chemical, complex SAIDAl Algeria Dar el beidha.
- Laboratory of microbiology hospitals in soeur badj EpH .
- The internship took place from February to May 2024.

IV-1.Pharmacological effects

1-1- Colon cancer and heart failure disease

After studying the toxicity of each of the selected plants and determining the small dose necessary and sufficient to give efficacy and effectiveness (LD50) against the disease in patients with gastrointestinal diseases and myocardial dysfunction as a dose over 6 months.

1-2- Anti-inflammatory effect of plants

Anti-inflammatory effects of drugs mainly include: acting on receptors and signaling pathways, regulating the response of target tissues to inflammatory mediators; reversing the effect of the medium on the target tissue; produce anti-inflammatory mediators and so on. Curcumin exerts anti-inflammatory effects by regulating inflammatory signaling pathways and inhibiting the production of inflammatory mediators; We recommend that *citrus limon* be used in individuals to reduce oxidative stress and inflammation caused by physical activity and intense exercise. *T. vulgaris* has been traditionally used for inflammation-related diseases such as rheumatism, muscle swelling, insect bites, pains and cold [114.115.116]

1-3- Antimicrobial activity

The extract from the peel of the fruit of *Citrus Limon* was separated from fruits, shade dried, powdered and extracted using methanol, analyzed for phytochemical constituents using standard methods. [117]

Curcumin blocks bacterial growth owing to its structural characteristics and the generation of anti-oxidation products [118]. *Curcumin* can inhibit bacterial virulence factors, inhibit bacterial biofilm formation. [119]

The antimicrobial activity of *thymus vulgaris* justify the use of *Thymus* plant species in the treatment of minor wounds and disorders of the oral cavity, and as an antibacterial agent in oral hygiene[120]

1-4- Anti-oxidant activity

Curcumin is known to protect bio membranes against per oxidative damage [121] Then *Thymus vulgaris* is volatile extracts possess high antioxidant activity [122]

Citrus flavonoids are the powerful antioxidants and potent free radical scavengers that help in the prevention of diseases that occur due to reactive oxygen species [.123]

V-1-Material

1-1- Biological materiels

The latter are available at the level of Microbiology, physico-chemical, complex Pharmacotoxicology laboratories SAIDAL dar el beida- algeries.and microbiology laboratory of eph soeur badj

- **Gram-negative:** bacterial strains: *Pseudo aeruginosa, Escherichia coli.*
- **Gram-positive:** bacterial strains : *Staphylococcus aureus; streptococcus spp.*

1-2- Equipment used

- Pressurized rotary evaporator (Heidolph).
- Hot plate stirrer (VELP SCIENTIFICA)
- Oven (BINDER, MEMMERT).
- Glassworks (PYREX, ISOLAB).
- Precision balance (OHAUS).
- UV-visible spectrophotometry(PERKIN ELMER)
- pH. Meter (METROHM 913à
- Fridge (IEBHERR).
- Viscosity meters
- Densy meter (ANTON PAAR)
- Conductivity meter (COND 51)
- Agitator (VEJP SCIENTIFICA ATE MAGNETIC STIRRER)
- Rotative agitator
- Centrifuge

1-2- Glassware

- Round-bottomed flask
- Vials
- Funnels
- Beakers
- Test tubes
- Graduated pipettes
- Erlenmeyer flask
- Watch glass
- Magnetic rung
- Micropipette
- Volumetric flasks
- Filter funnel

1-3-Chemical reagents:

The products have been giving kindly by Saidal Groupe and by the engineer of laboratory.

Products	Properties
Ethanol (C2 H6 O)	M= 46 g/mol
DPPH (C18H12N5O6)	M = 394.3g/mol

V-2- Vegetal materiel

2-1-Harvest

The plants chosen for this study: lemon zest (*Citrus lemon*), thyme (*Thymus vulgaris*), curcumin (*curcuma longa*)

- Sample 1: *Citrus limon* species, *lemon peel* from a lemon tree in February 2024.
- Sample 2: The species *Thymus vulgaris* was harvested in January 2024, from the

Zeboudja chlef region. The part taken into consideration for this study is the aerial part (leafy stems).

- Sample 3: *curcuma longa* from a herbal shop

2-2- Drying plants

The aerial part of the plant is dried directly after harvesting on clean, dry absorbent paper in the open air, away from light, for a period of two weeks; in the open air away from light for a period of two weeks like in figure 24. Can also be used to improve the dryness of the cupboard.

(A) (B) (C)

Figure 24: Plants used: *A/ citrus Limon peels; B/ curcuma longa; C/ thymus vulgaris*

2-3- Grinding and preservation

The dried aerial part is ground to a fine powder using an electric grinder; The resulting powder is stored away from air, moisture and light in hermetically sealed glass bottles ; hermetically sealed glass bottles.

2-4-Extraction processes

2-4-1-Maceration extraction method

Extraction was carried out by macerating 10g of plant in 100ml ethanol solvent (60% ethanol + 40% water distillate) in a closed container at room temperature. After 72 h, the solution was filtered through 150 mm diameter filter paper. Finally, the filtrate was stored at 25 degrees Celsius in the dark for subsequent analysis.

Next, the solvent is separated of the solvent from the extract is carried out using a device known as a Rotavapor. In this device, vacuum evaporation is performed using a vacuum pump with a control valve. control valve. During evaporation, the flask is rotated and immersed in a heated liquid bath. liquid bath. The unit is fitted with a cooler and a condensate recovery tank. condensate recovery tank. Rotation of the flask creates a larger exchange surface, enabling rapid evaporation. Lowering the pressure enables the solvent to evaporate at a reduced temperature of 40°C, and the extract is recovered, avoiding thermal degradation. All the steps are in figure 25

A B C D

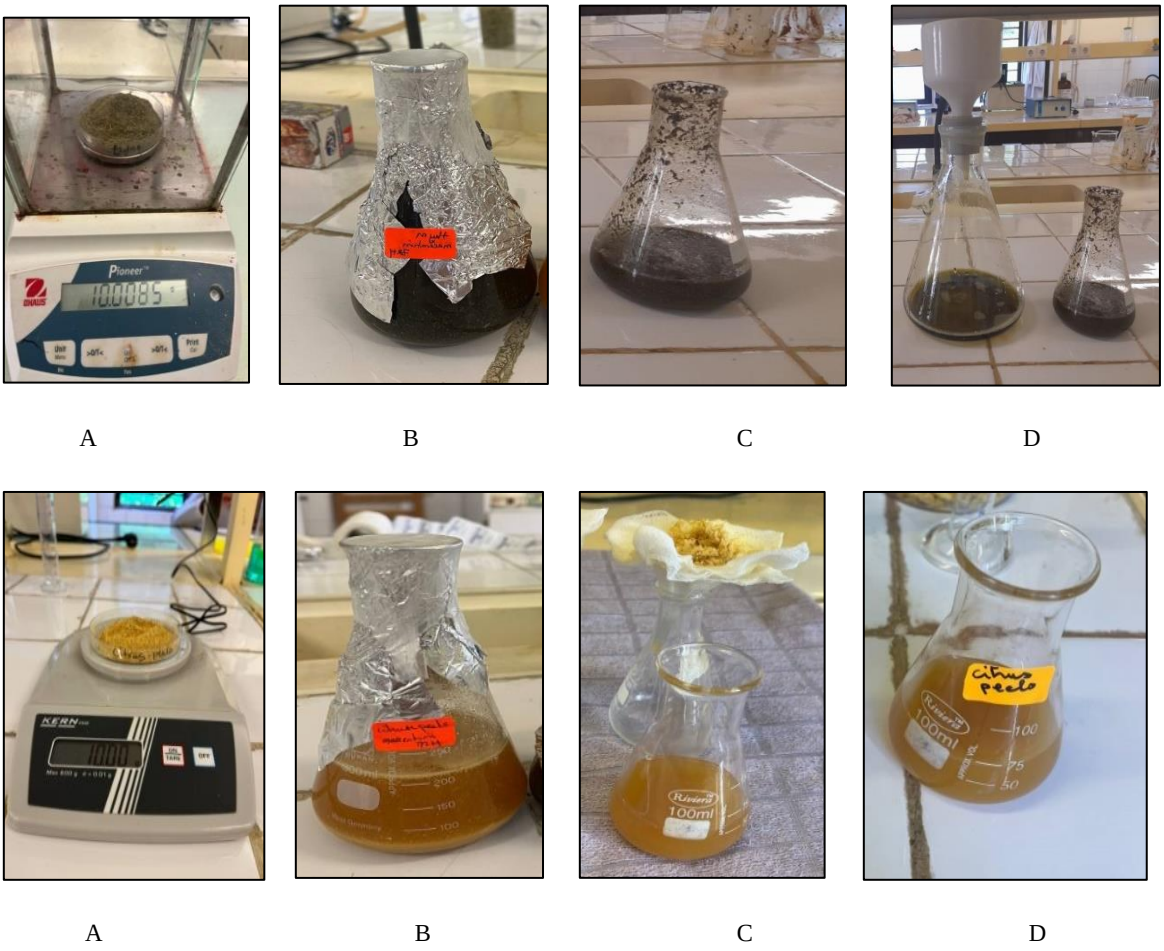


Figure 25: Extraction methods of plants.

The solvent is separated from the extract using a Rotavapor at a temperature of 40°.

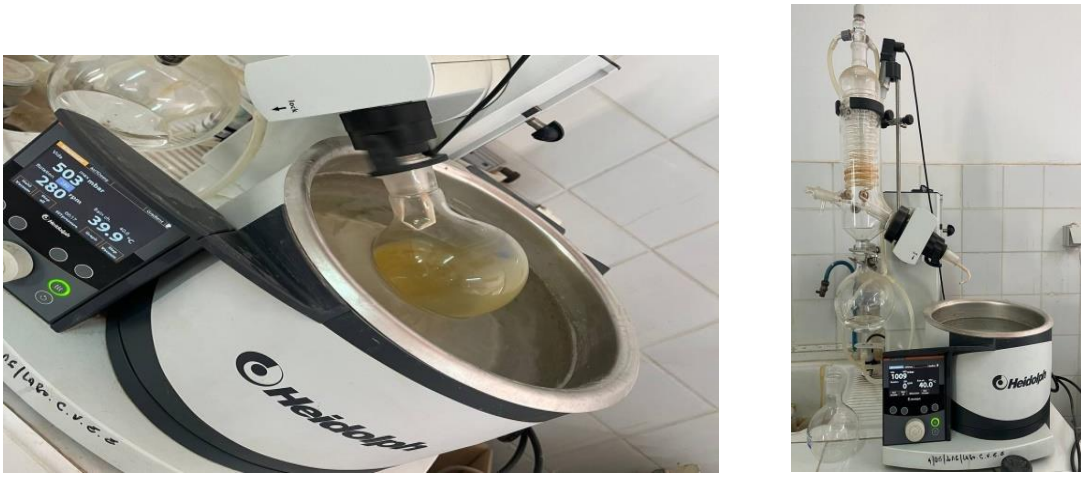


Figure 26 : Rotavapor technique.

In order to exhaust the plant, the filtrate is dried at 40°C and then weighed in order to calculate the extraction yield Y according to the formula below :

$$Y=(Wf/Wi) \times 100 \ldots\ldots..(1)$$

Y: Yield

Wf: terminal weight

Wi : initial weight

VI- Physicochemical study of extracts

1-Chromatographic and spectroscopic methods of separation and identification: 123

As the name implies, GC uses a carrier gas in the separation, this plays the part of the mobile phase. The carrier gas transports the sample molecules through the GC system, ideally without reacting with the sample or damaging the instrument components. Mass spectrometry (MS) is an analytical technique that can be hyphenated to a GC and used instead of the GC detector.[123]

Data from a GC-MS is three dimensional, providing mass spectra that can be used for identity confirmation, to identify unknown analysts and to determine structural and chemical properties of molecules, as well as the chromatogram that can be used for qualitative and quantitative analysis.[123]

1-Méthode CG-MS

The experimental conditions of the GC-MS system were as follows: standard non-polar column TR 5-MS capillary, dimension: 30 Mts, ID: 0.25 mm, film thickness: 0.25 µm. Flow of the mobile phase (carrier gas: He) was adjusted to 1 ml min−1. Temperature program (oven temperature) was raised from 40 °C to 220 °C at 4 °Cmin−1, and the injection volume was 1 µl. Samples were run fully at a range of 20 and 550 m/z, and the results were compared using the Wiley and NIST Spectral Library Search Programme. [124]

2-Visible UV

- **Principle of visible UV**

UV-visible electronic transitions are the most critical chemical energies in a molecule (Approximately from 13000 to 50000 $Cm{-}1$ or 160 to 665 kJ $mol{-}1$). The energies involved are on the same order of magnitude as the molecules' binding energies, and this radiation can occasionally trigger bond breakage. They generate electronic transitions between different levels of energy in molecules in general [125]

Figure 27: Spectrophotometer visibe UV.

3-pH meter Analysis

- **Adjustment:** We make sure that the pH meter is clean and calibrated for precise measurements, we use buffer solutions (pH 4, pH 7 and pH 10).
- **Read the simples :** Put the plant into a clean container and immerse the pH meter electrode in the solution to allow the result to be read.

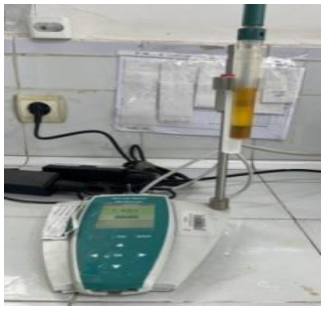


Figure 28: pH meter .

4- Density

The density of a substance is denoted d and corresponds to the ratio of the density of this substance by the density of pure water to define the density of our extract; we assumed a method that depends on the measurement of the density of the extract and the density of the water.

$$d = \frac{\rho extract}{\rho water}$$

$\rho_{extract}$: the density of the extract

ρ_{water}: the density of water =1

Figure 29: Density meter.

5- Conductivity meter

Is an analytical instrument that measures the conductivity of a solution. It works by passing an electrical current through the solution and measuring resistance to the flow of electrons, which is then used to determine the solution's ion concentration.

Figure 30: Conductivity meter.

6-Determination of antioxidant activity using the DPPH test

- **Principle of the DPPH assay:** The anti-free radical activity of the extracts was tested using the DPPH radical (Method of Blois (1958) with some modifications). DPPH, 2.2'- Diphenyl-1-picrylhydrazyl (Sigma, C18H12N506: PM-394.33), is a stable free radical soluble in methanol (or ethanol). Intense in violet color, it has a maximum absorbance at 517 nm. When an antioxidant molecule reduces the DPPH radical, the violet color quickly disappears to give a pale yellow color), according to the following reaction (126).

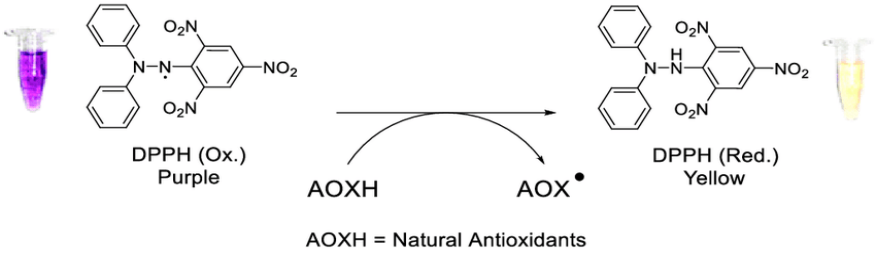


Figure 31: DPPH reaction .

- **Operating mode:** The preparation of the DPPH solution is 0.004% (m/v), obtained by dissolving 0.004g of DPPH in 100ml of ethanol.

Figure 32: DPPH prepared.

- **Preparation of extract concentrations:** Take 2 ml of the extracts of each plant and fill in with ethanol until 4 m

  C0= 0.1 g/l---------- V0=2 ml

  C1=? ---------- V1= 4ml

  C0 V0 = C1 V1 ----------C1= 0.05 g/L

C1 V1 = C2 V2---------- C2 =0.025g/L

C2 V2 = C3 V3 ----------C3 = 0.0125g/L

C3 V3 = C4 V4---------- C4 = 0.00625 g/L

C4 V4 = C5 V5---------- C5 = 0.003125g/L

Figure 33: Preparation of extract concentrations.

- After the 30min incubation, the reading is done by visible UV at 517 nm, the blank is Ethanol.
- **Preparation of ascorbic acid samples**: In order to compare the possible antioxidant effect of our extracts, we have studied the antioxidant effect of ascorbic acid (vitamin C), known for its very strong anti-radical effect. Different dilutions of ascorbic acid at low concentrations expressed in mg /ml (0.004 ; 0.006 ; 0.008 ; 0.01 ; 0.015 ; 0.02 ; 0.04 ; 0.05 ; 0.06 ; 0.08) prepared according to the experimental protocol available from Saidal dar baida .
- 0.1ml of each aqueous solution of ascorbic acid (standard) at different concentrations are added to 3.9ml of the ethanoic solution of DPPH (0.024mg /ml)
- In parallel, a negative control was prepared by mixing 0.1ml distilled water with 3.9ml of DPPH ethanolic solution.
- The absorbance for each concentration was read against a prepared blank at 517nm after 30 minutes incubation at room temperature.

Figure 34: Preparation of different concentration of ascorbic acid .

- The evaluation of antioxidant activity using the DPPH method is expressed as percentage according to the following formula:

% Inhibition = [(*Abs Control*−*Abs Extr*at) /*Abs Control*].100……(02)

Abs Control: absorbance of ethanol Abs Extract: absorbance of plants exact

7- Antimicrobial activity of extracts

- The bacterial strains used: In our study, we tested the sensitivity of nine (4) reference bacterial strains (American Type Culture Collection '*ATCC*') from the microbiology laboratory at the EHP SOEURS BADJ CHLEF. TWO (2) Gram negative and TWO (2) Gram positive): *Staphylococcus aureus ATCC 6538, Streptococcus spp ATCC 12228, Pseudomonas aeruginosa ATCC 9027, Escherichia coli ATCC 8739* indicated in table 07.
- All these micro-organisms are pathogenic, have different potentials depending on the type of infection they cause, cause hospital infections and are resistant to antibiotics. Infection they cause, are the cause of hospital infections, and are resistant to

antibiotics, which is why they have to make choices. The strains' culture media, grams, and incubation temperatures are listed in the table below:

Table06: Microbial strains used for antimicrobial test

NUM	MICROORGANISME	GRAM	culture media	Incubation temperature
1	*Staphylococcus aurous ATCC6538*	positif	chapman	37
2	*Escherichia coli ATCC14028*	negatif	hektoen/slp/mackonki	37
3	*Pseudo aeruginosa ATCC8626*	positif	hektoen/slp/mackonki	37
4	*Streptococcus spp ATCC 12228*	negatif	chocolat gelose or cold gelly	37

7-1- Aromatogramme

The aromatogram allows the qualitative estimation of the effect of the extract. It consists of using Petridishes containing a suitable agar medium, already solidified and inoculated with the microbial strain tested. [127].

Discs of filter paper, blotting paper or Wattman, previously impregnated with known quantities of EO, are then placed on the surface of the agar .after incubation the bacteria grow all over the agar surface except where they encounter a sufficient concentration of HE to inhibit their growth. [128].

A circular zone free of colonies is thus observed around the discs, called a zone of inhibition. The larger the diameter of this zone, the more the strain is sensitive; the smaller it is, the more resistance.[128].

7-2- .Operating mode (129)

- Aseptically pour the agar culture medium (Muller Hinton) in super fusion into Petri dishes at a rate of 10 to 15mL per dish.(consumables).
- The growth medium is left to cool and solidify on the bench (under the hood). - A bacterial suspension corresponding to the standards of 0.5 Mc Ferland was prepared from a pure and young culture (24 hours).
- These standards equivalent to an optical density of 0.08-0.1 at 625 nm, this inoculum is used to inoculate MH poured into Petri dishes.

- Using sterile forceps, place sterile Schleicher & Schuell discs 9 mm in diameter as needed on the surface of the Petri dish containing inoculated agar then impregnated with 15 µl of each extract.
- The inoculated dishes containing the discs of the extracts were put at 4° C. for 2 hours to facilitate the diffusion of the extracts.
- The Petri dishes were closed and left to diffuse at room temperature for 1 hour. They were subsequently incubated at 37° C. for 24 hours.
- After incubation, the absence of bacterial growth expressing an antimicrobial activity results in a translucent halo around the disk, of the same color than sterile agar and whose diameter is measured using a caliper.
- Reading is done by measuring the diameter of the inhibition zone around each disc using a calliper or a ruler in (mm). see it in figure 36
- The results are expressed by diameter of the inhibition zone and can be symbolized by signs according to the sensitivity of the strains versus extracts.

- D≥30mm: Very strongly inhibitory.
- 21mm≤D≤29mm: Strongly inhibitory.
- 16mm≤D≤20mm: Moderately inhibitory.
- 11mm≤D≤16mm: Slightly inhibitory.
- D<10mm: Non-inhibitory.

Figure 35: Preparation of the bacterial suspension.

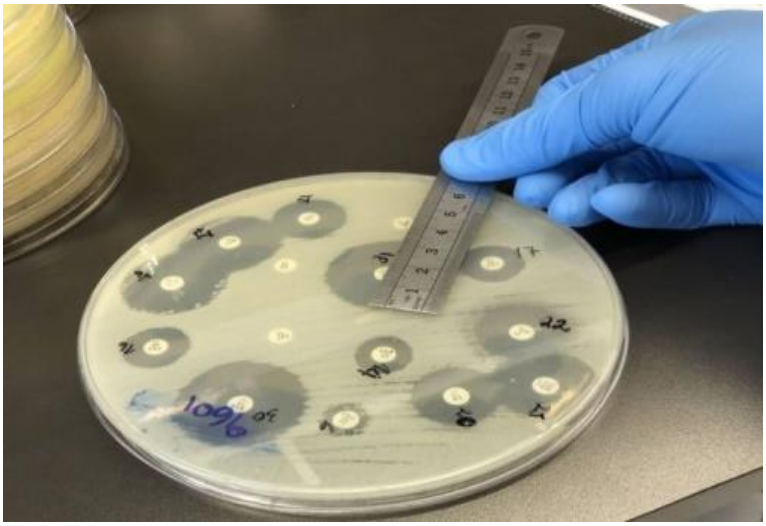


Figure 36: Aromatogramme interpretation results.

7-3- Antimicrobial test of final formulation Using the same prescription of work we test the sensibility of oral gel; using the same microbial strains also.

8–Oral emulgel formulation The preparation of drinkable emulgel is an interesting area of pharmaceutical development. These gels are designed for oral administration and may contain drugs, vitamins or other active substances.

The aim of this section is to optimize the formulation of a dietary supplement for an anti-inflammatory, antioxidant and antimicrobial drinkable emulgel. To do this, we use bio-extracts from the plans available in our region and experiments that allow us to plan the formulation trials, rationalize the number and ensure the quality of the products.

8-1- Sterility test of final oral emulgel To test the sterility of the final product, place the product on absorbent paper and apply to a nutrient agar and incubate in the oven for 24 hours at 37 degrees to determine the viral load.

8-2- Equipment used for preparation

- Common glassware of different sizes
- Hot plate
- Precision balance
- Sterilized bottle for the finished product.

- Blender
- Mixer
- Thermometer
- pH meter
- Conductivity meter

8-3-Table of different doses of formulation ingredients with a sweep

We did a sweep for the formulation of oral emulgel; each time we set the ingredients and we make the exchange on one; table08 below present our scanning:

Table 07: Sweep of ingredients in oral emulgel formulation

Ingredients/ tests	Test 01	Test02	Test 03	Test04	Test05	Used to
Starch	20g	40g	50g	40g	40g	Gelling agent
Clay	10g	15g	5g	10g	5g	Polymer
Soap	5g	6g	7g	8g	9g	Emulsifier
Water	100ml	150ml	150ml	100ml	100ml	Solvent
***Thymus* oil**	10g	10g	10g	10g	10g	PA
***Curcuma longa* oil**	10g	10g	10g	10g	10g	PA
Aqueous extract of *citrus lemon peels*	5g	5g	5g	5g	5g	PA
Honey	5g	5g	10g	20g	10g	Sweetness
Flavor	1 drop	1drop	3 drops	3drops	4 drops	Smell

8-4- Manufacturing of Oral Emulgel

- We heated the water to 70°C and the oils to 40°C in a 250ml Beecher.
- We weighed the clay and the rice starch.
- Using a mixer, dissolve the clay in the hot water, add the rice starch and mix for 15 minutes.
- Reduce the temperature to 40°C, add the oils, mix for 5 minutes and add the lemon extract.
- Using a blender, we mixed the mixture well to get the right consistency with the addition of soap.

- Finally, we added honey for sweetness and natural lemon fragrance to enhance the taste

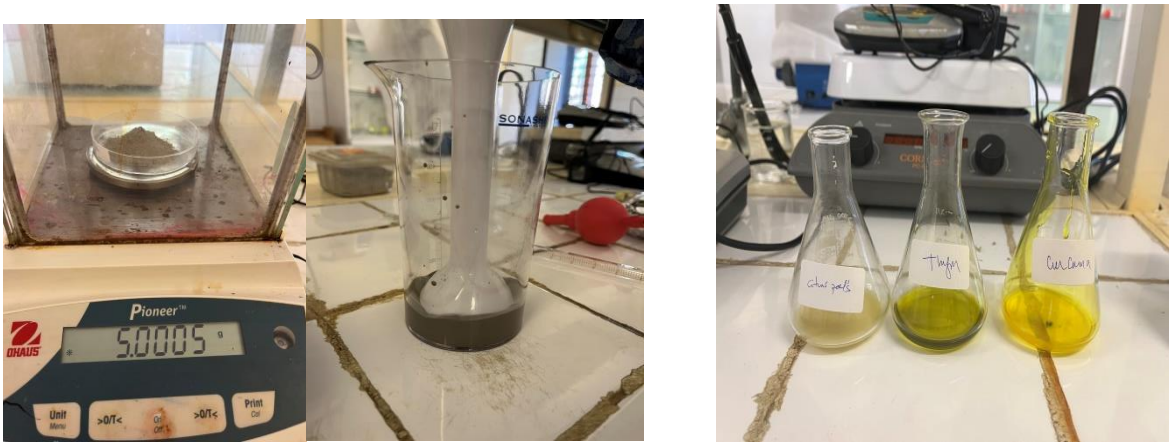


Figure 37: Varrious steps of preparation of oral emulgel.

8-5- Caracterization of oral gel

- **pH test:** pH measurement is an important parameter given that the product is designed to be consumed directly by humans.

Figure 38: pH meter measure of oral emulgel final formed.

- **Conductivity meter :** measure of conductivity to be able been given that the product is designed to be consumed as the concentration of ions natural in oral way

Figure 39: Conductivity of oral emulgel formed.

- **Cap locking:** The bottles are inverted and kept that way for a week. The cap locking would be confirmed if the cap cannot be easily opened.
- **Stability test** : we put the finished product in a test tube and center it every 5 min and 10 min at speed 2000 tour/ min and 4000 tour/ min for each test (see the figure 40 below) .

Figure 40: centrifuge material.

VII-In silico study

In our study, we used a microcomputer in laboratory of doctorate student of matter science department in Ouled Fares University; having a memory of 4.00 GO and an AMD E2- 7110APU WITH AMD Radeon R2 Graphics 1.80 GHz, under system 32-bit operating system, x64 processors versions 1511. All the programs used are installed under the Windows 10 operating system.

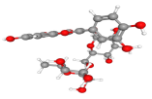
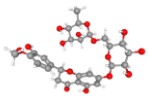
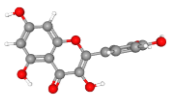
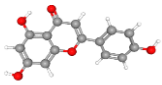
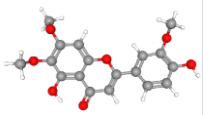
- **Insilico work steps**

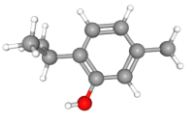
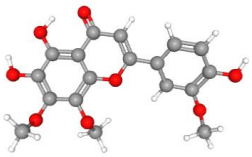
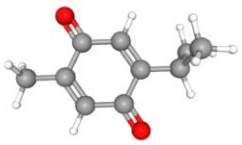
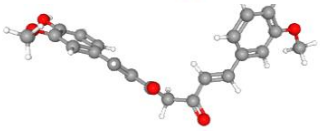
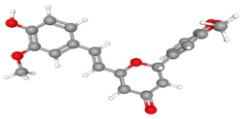
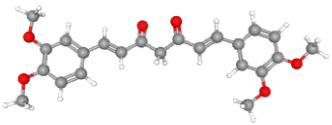
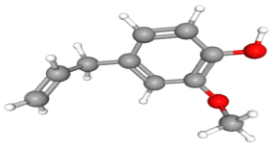
1. **Pubcham :** select the molecule with SDF format

The data on chemical constituents in *citrus peels; curcuma longa* and *thymus vulgaris* was acquired ; compounds were collected ; 25 compounds collected ; 07 in *citrus peels* ; 07 in *curcuma longa* and 11 on *thymus vulgaris* .

We classified them with the best molecule receptor interaction to: 03 in *citrus peels* ; 04 in *thymus vulgaris* and 04 in *curcuma longa* .

Table 08 : the table present the 11 molecule choosing as the best molecule-receptor interaction and there format identified in pubcham data base

Plant	**Molecule**	**Pubcham format**
Citrus peels	rutin	
	hesperidin	
	quercetin	
Thymus vulgaris	apegenine	
	cirsilineol	

	thymol	
	thymonine	
	thymoquinone	
Curcuma longa	curcumin	
	Cyclo curcumin	
	Didmethyl curcumin	
	eugenol	

- **Preparation of molecule in gauss view / gaussian**

2. **Gaussview :**

- Open the app then choose your molecule study (molecule in enter on figure 41)

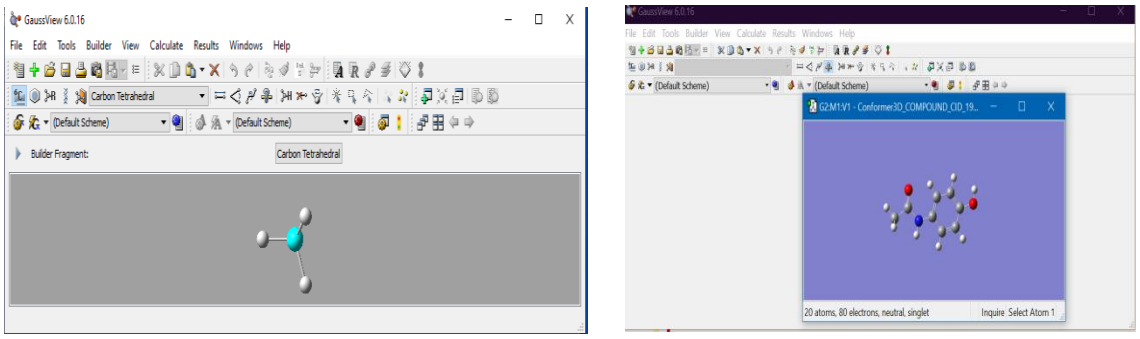


Figure 41: opening of gaussview app and enter of molecule

- Open calculate tools and choose Gaussian calculation setup

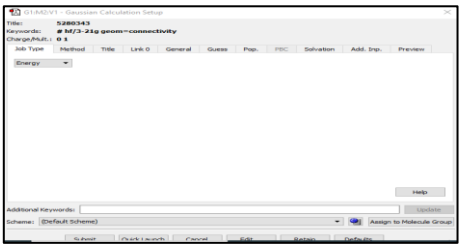
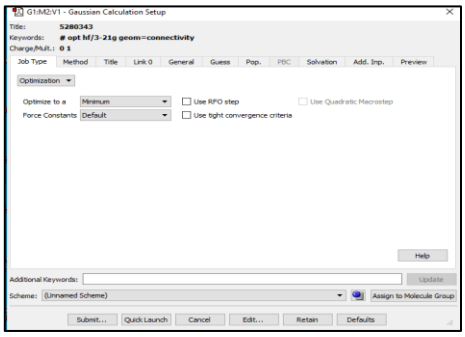
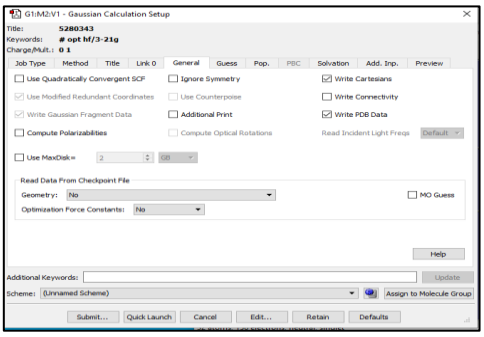


Figure 42: step of calculation program tools

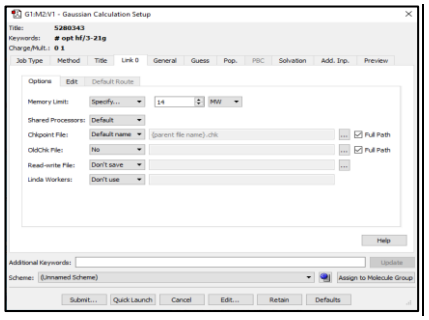
- Get some parameters notes changes



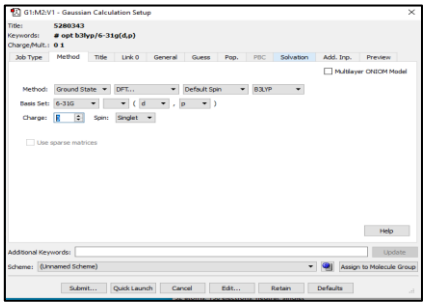
-A-Choose job type and change energy to optimization



-B-go to general and choose Cartesians with pdb data



-C-select link 0 and get the hight data base repear to each program



-D-in methode choose dft program with 6-31g; d/p; and cherge 1

Figure 43: A, B, C, D, steps of activation of optimization in Gaussview

- Save the changes with gjf form

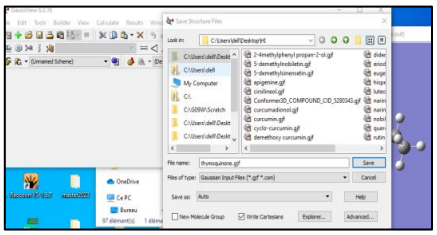


Figure 44: GJF forme

3. Gaussian

- Open gjf molecule in gaussian and start the calculate ; example of calculation is showing on figure 45

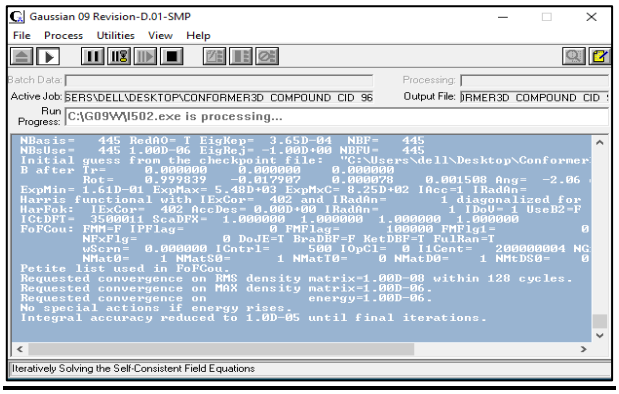


Figure 45: calculate work of Gaussian

- The calculate can take more than 3 days calculating till it finished

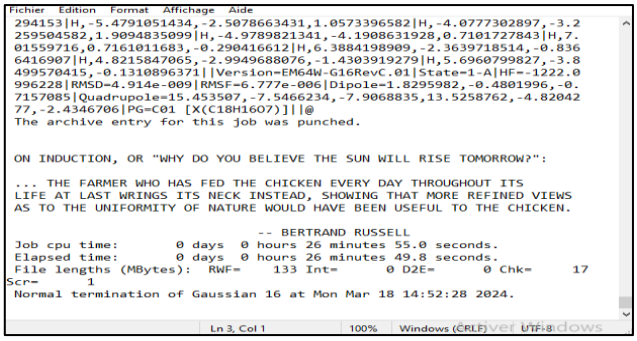
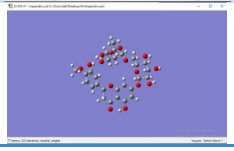
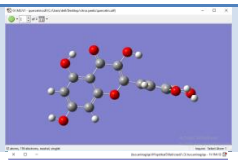
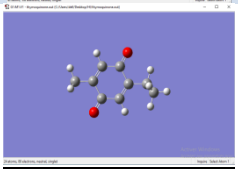
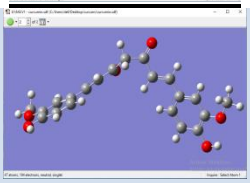
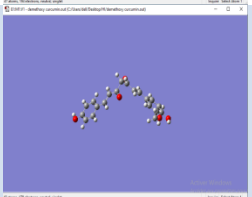
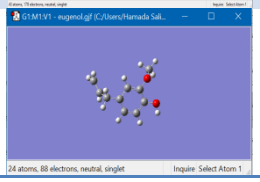


Figure 46: finish calculate of Gaussian

- the molecules are ready to get into Interactions after they was finish present on table 09

Table 09: forme final molecule after optimization

Plant	**Molecule**	**Molecule optimized**
Citrus peels	rutin	
	hesperidin	

	quercetin	
Thymus vulgaris	apegenine	
	cirsilineol	
	thymol	
	thymonine	
	thymoquinone	
Curcuma longa	curcumin	
	Cyclo curcumin	
	Didmethyl curcumin	
	eugenol	

- change molecule from GJF to PDB and save it

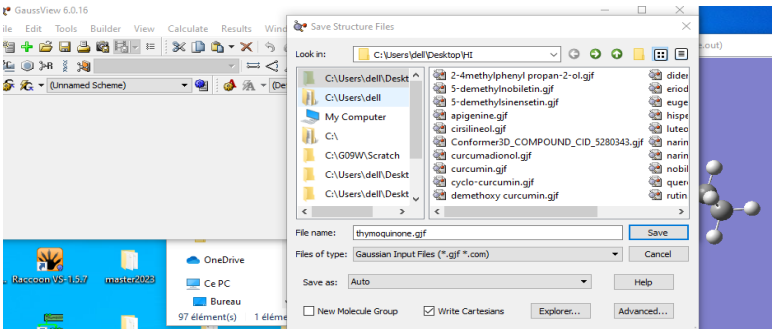
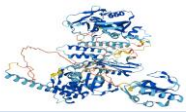
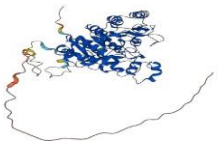
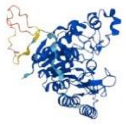
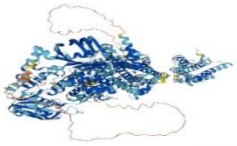


Figure 47: Transform GJF to PDB molecule

4. **UNIPROT site:** in this web site we select our receptors in pdb forma (all receptors are organized on table 10)

Table 10: format of receptors obtain from Uniprot

Receptor	Receptor type	Pdb format
PARP-1	PARP-1	
HDACs	HDAC1	
	HDAC3	
MDR	ABCB1	
	ABCC1	

5. **AUTODOCK:** interactions are determinate on Autodock tools app to see the site of fixation receptor- ligand; and defined the grid box of interaction.
   - **Lipinski's rule:** Lipinski's Rule of 5, formulated by Christopher A. Lipinski, describes a set of physicochemical properties that influence the absorption and

permeation of a compound in the gastrointestinal tract. The rule helps researchers quickly evaluate the potential of a compound to be absorbed orally.

- **Molecular Weight:** The molecular weight should be less than 500 Daltons. Larger molecules tend to have difficulties crossing cell membranes and can have lower absorption rates.
- **Lipophilicity (LogP):** The logarithm of the octanol-water partition coefficient (LogP) should be less than 5. This property measures the compound's hydrophobicity or lipophilicity. Excessive lipophilicity can lead to poor solubility and hinder absorption.
- **Hydrogen Bond Donors:** The number of hydrogen bond donor groups (e.g., OH or NH groups) should be less than 5. Compounds with many hydrogen bond donors may form strong interactions with water, reducing their ability to cross lipid membranes.
- **Hydrogen Bond Acceptors:** The number of hydrogen bond acceptor groups (e.g., oxygen or nitrogen atoms) should be less than 10. Compounds with numerous hydrogen bond acceptors may be more water-soluble but can have lower membrane permeability.[140]

- **Preparation of receptor**

- Add the molecule to the app and respect previous orders
- [Edit, Delete, Delete All Molecule, CONTINUE]. Now we bring a protein from a file to a program and prepare it with the following operations.
- Edit Add Delete water if present in the molecule [Edit->Delete Water] and Add charges [Add Kollman Charges, ok]
- Add Hydrogen to protein [Edit , hydrogens , Add , polar Only , ok]
- Then save the changes made [grid, macromolecule, choose] and a window appears .we save the changes in the previous file with the name protein pdbqt.as in figure 48

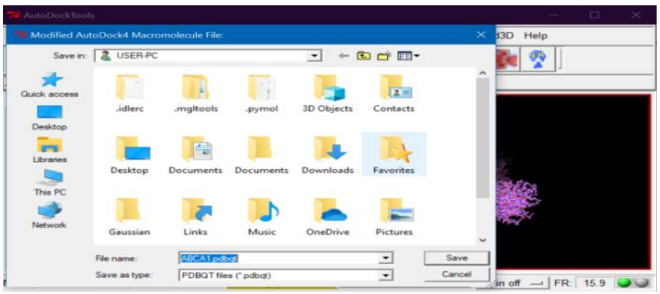


Figure 48: present the final presentation of molecule as PDBQT

- **Ligand preparation**

- Add the ligand In box of work: molecule optimized in pdb form
- copy the molecule in the form of pdb in a program AutoDock Tools and it is after that we return to the program A0[Edit , Delete ,Delete All Molecule , CONTINUE].
- Then we click on [ligand , input ,choose, select Molecule for AutoDock, ok
- The next step is to save the changes by clicking [ligand, Output, Save As PDBQT…] we Call ligand.pdbqt and click save to Previous File. we can see the save on figure 49

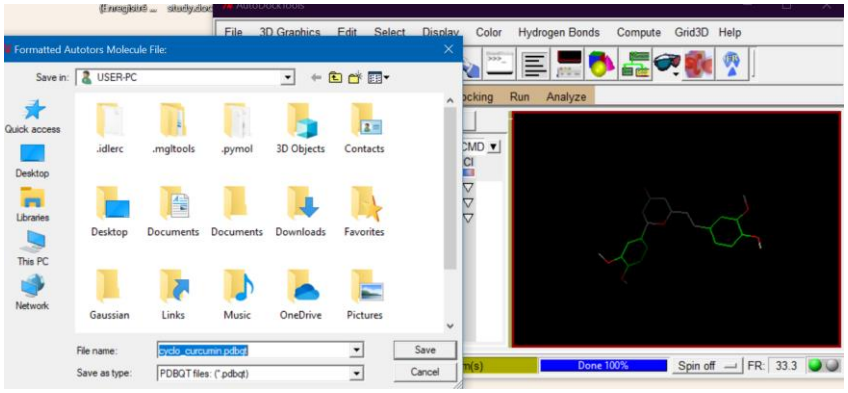


Figure 49: in this figure we present the last step of saving ligand as PDBQT

- **Interaction ligand- receptor**

- The first step is to go back autodoock tools to A0
- The second step is to copy the protein.pdbqt into a program autodoock tools and we add the ligand.pdbqt
- Now we click on [Grid, Macromolecule , choose, ok] copy a protein and click on Select Molecule and we click on No.(see the select on figure 50)

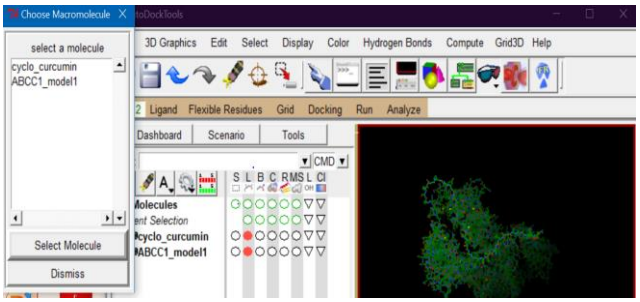


Figure 50: present the interaction between ligand receptor

- we click [Grid , Grid Box]

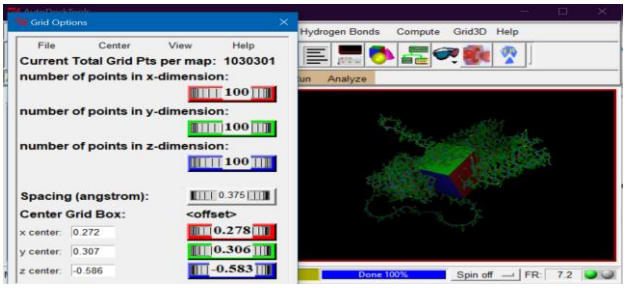


Figure 51: present the grid box ; a cub defined the interaction place

- We click on [File , Output grid dimensions file] and save it as grid.txt in the previous file.
- We open the previous file and we find the changes we made, the information in file grid.txt(see figure 52)

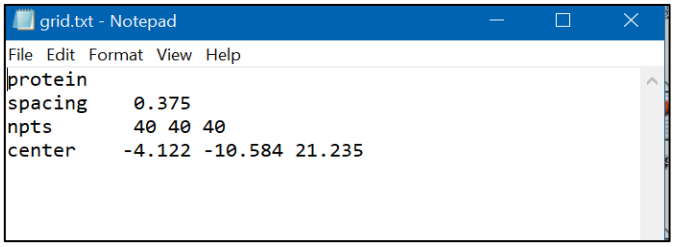
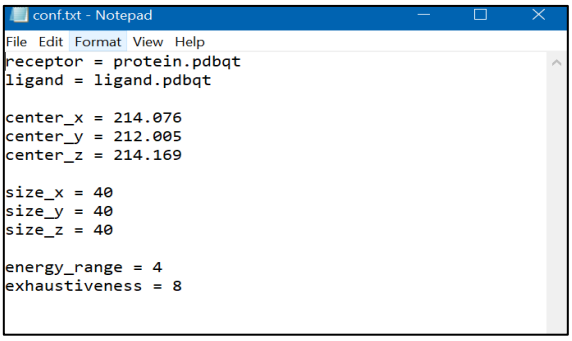


Figure 52: changes of center distance in grid.txt

- We open the fishier txt and call it config and put the information and dimensions Which we will work with a marge



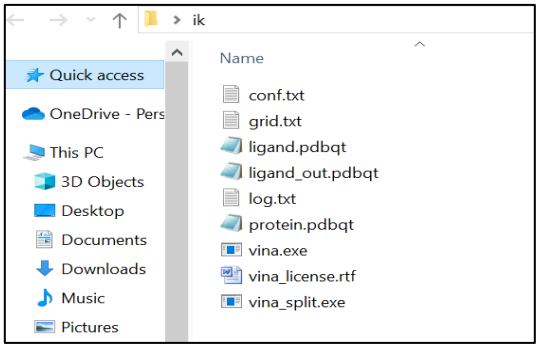


Figure 53: present the conf test and all dock base in the work if vina autodock

6. **AutoDockVina**

- In the previous file, we add 3 files from vina as follows[vina , vina-licence , vina-split] , After collecting in one file the pdbqt protein, ligand.pdbqt, grid and config and 3 files from vina, we are now looking for Command Prompt (cmd) on the computer and open it
- We write cd and then copy the link in the previous file containing the information we changed earlier, then paste it in cmd and press Enter and type [vina.exe - - configconfig .txt - - log.txt] and press the enter button and wait for it to appear In the file two fichier log.txt (figure53) and ligand-out.pdbqt.(show result figure 54)

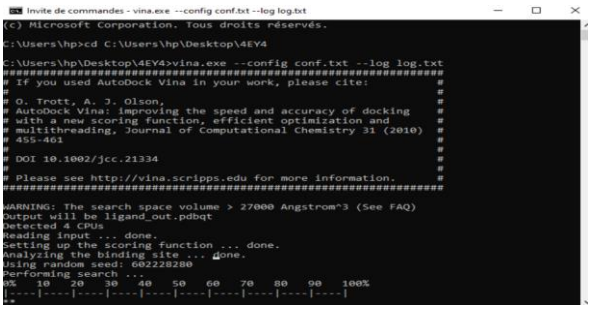


Figure 54: Autodock Vina docking in command window; the dock can take more time to be finished

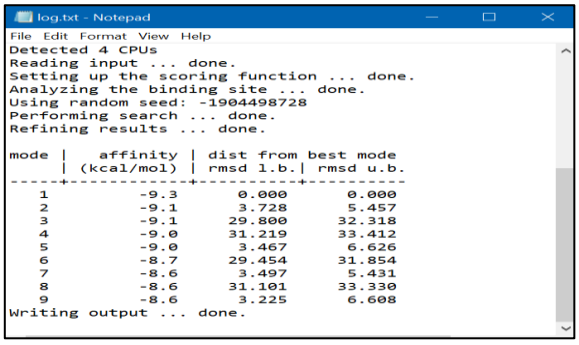
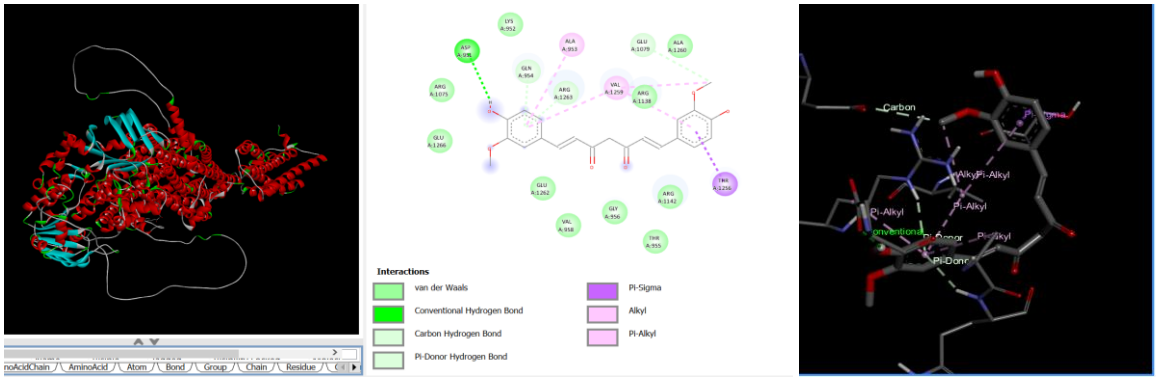


Figure 55: log txt ; the note present the finished work of Autodock Vina an results of distance interaction ligand- receptor

7. **BIOVIA Discovery Studio**

- At the last stage, we open a program BIOVIA Discovery Studio, We click on a file and then open it and then display the open dialog box and choose the previous file to open it and click on all files and then choose ligand-out.pdbqt and click on open and it
- appears on BIOVIA Discovery Studio and then click on the first box ligand-out-model-0, We repeat the same process and get protein.pdbqt, then we remove the

ligand groups by copying it and clicking on Delete we copyligand-out-model-0and paste it at protein. pdbqt ,and click on ligand Interaction and convert it to show 2D Diagram(see figure 56/ B)



A B C

Figure 56 : different interaction views in biovia discovry:A/ the view of ligand into chain of receptor , B/ 2D diagram interaction , C/ interaction grid box and defeind of amine acid interaction

- **Databases**

1- **SwissADME:** SwissADME is a web-based tool that predicts various pharmacokinetic properties and drug-likeness rules for small molecules. It is developed and maintained by the Swiss Institute of Bioinformatics (SIB) and provides valuable insights into the absorption, distribution, metabolism, and excretion (ADME) properties of chemical compounds. [141]

➢ To work with it we have to paste the smile of molecule or here name; the case is identified in figure 57

Figure 57: SwissADME window in site web and the way to present molecule with their smiles

2- **Pass on ligne :** predicts over 4000 kinds of biological activity, including pharmacological effects, mechanisms of action, toxic and adverse effects, interaction with metabolic enzymes and transporters, influence on gene expression, etc. To obtain the predicted biological activity profile for your compound, only structural formula is necessary; thus, prediction is possible even for virtual structure designed in computer but not synthesized yet, works with smile of molecule also .[142]

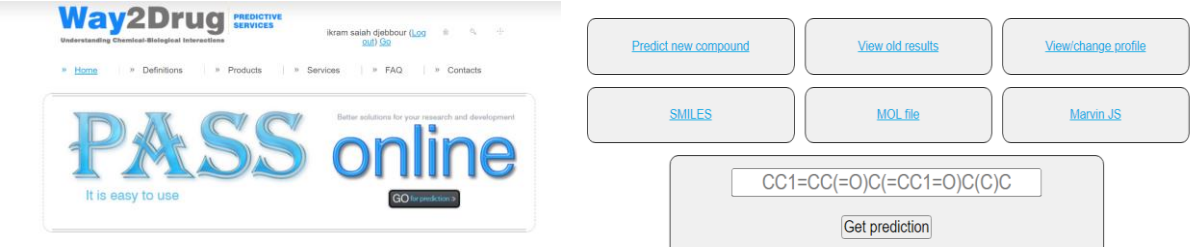


Figure 58: Pass online window in site web and the way to present molecule with their smiles

3- **PLIP(protein ligand interaction profiler):** The Protein Data Bank (PDB) hosts nearly 100 000 deposited protein structures, with over 75% of them solved in complex with a small molecule ligand. Binding of a ligand to its host protein requires a specific arrangement of attractive, typically non-covalent contacts between both molecules. With such rich data at hand, we can gain deep insights into how ligands interact with their protein targets.[143]

- PLIP is complementary to other state-of-the-art web tools such as SwissDock and can thus be applied in evaluation of docking results , drug design , binding site similarity assessment and drug repositioning . The PLIP web service allows for comprehensive detection and visualization of protein–ligand interaction patterns from 3D structures, either directly from the PDB or in user-provided structures.[144]
- We use the PLIP to identified hydrophobic bonds in interaction; hydrophobic interactions describe the relations between water and hydrophobic **(**low water-soluble molecules). Hydrophobes are nonpolar molecules and usually have a long chain of carbons that do not interact with water molecules. The mixing of fat and water is a good example of this particular interaction. The common misconception is that water and fat doesn't mix because the Van der Waals forces that are acting upon both water and fat molecules are too weak.[144]

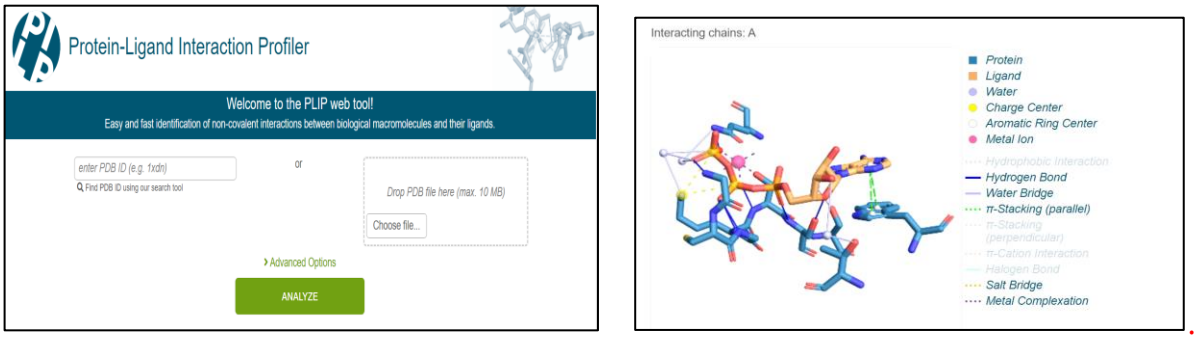


Figure 59: Plip data base window screen shot and example of interaction presentation

4- **CB dock:** is protein-ligand docking methods which automatically identifies the binding sites, calculates the center and size, customizes the docking box size according to the query ligands and then perform the molecular docking with AutoDock Vina. Large-scale benchmarks show that the cavity-focused docking can enhance the hit ratio and accuracy of blind docking. Accordingly, CB-Dock can facilitate the docking procedure and improve the accuracy by predicting the binding sites of target proteins using our curvature-based cavity detection approach (**CurPocket**) and the binding poses of query ligands.[145]

- The CB dock work by the enter of each ligand and receptor in PDB format
- Then start the interaction till it finished
- The CB dock choses all position of best ligand-receptor interaction

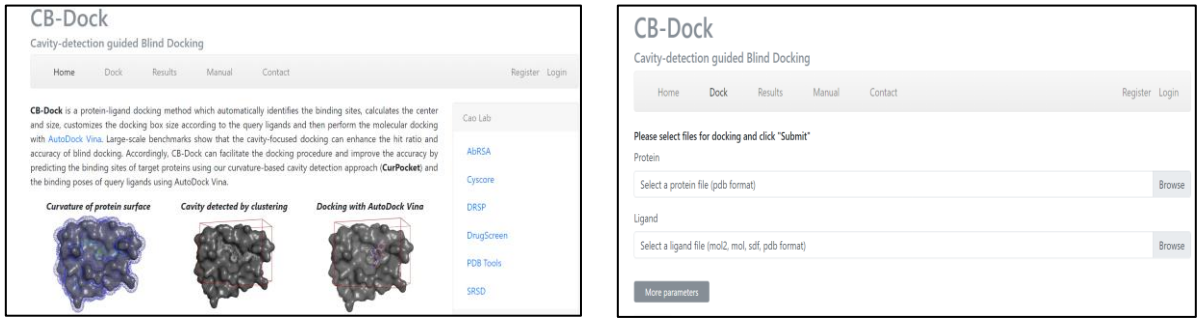


Figure 60: the screen shoot of CB dock home page with the enter of each ligand – receptor

5- **SwissTargetPrediction:** is a web-based tool, on-line since 2014, to perform ligand-based target prediction for any bioactive small molecule. The user-friendly graphical interface shields non-experts from methodological pitfalls and specialists from tedious technical

efforts. The site present different probabilities defended interactions between ligand and deferent receptors.[146]

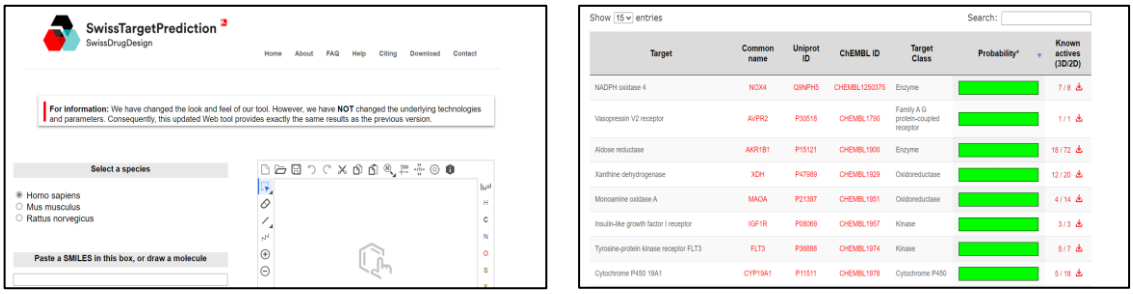


Figure 61: Swiss Target screen shoot page and example of deferent probabilities of ligand receptors interaction

6- **Drulito**: Drug-likeness tools rules are set of guidelines for the structural properties of compounds, used for fast calculation of drug-like properties of a molecule. These guidelines are not absolute, nor are they intended to form strict cutoff values for which property values are drug-like and which are not drug-like. Nevertheless, they can be quite effective and efficient.DruLiTo is an open source virtual screening tool. It's calculation is based on the various drug likeness rules like Lipinski's rule, MDDR-like rule, Veber rule, Ghose filter, BBB rule, CMC-50 like rule and Quantitative Estimate of Drug-likeness (QED). DruLiTo uses the Chemistry Development Kit (CDK), a Java library for descriptor calculation.[147]

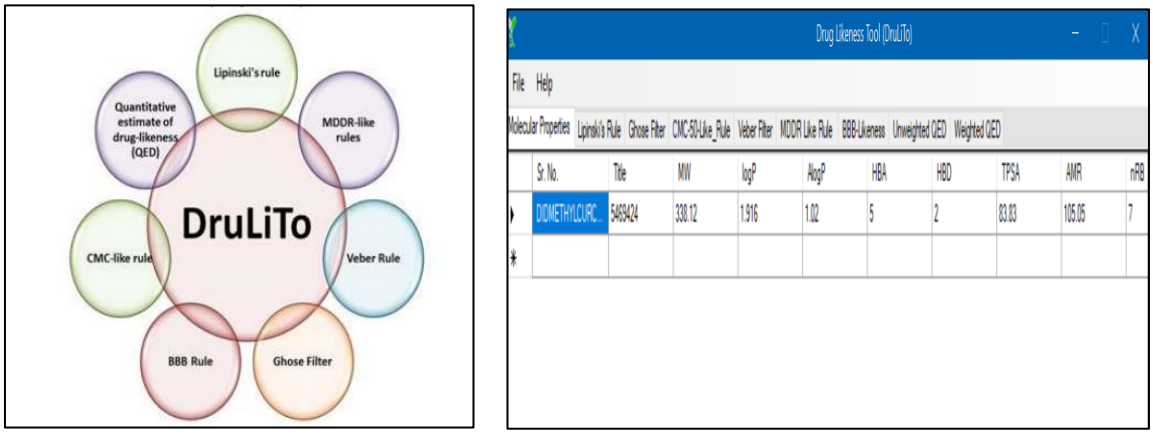


Figure 62: present the DruLiTo molicular docking benefits and app home screen shoot

7- **Protox-3:** provides a freely available webserver for in silico toxicity prediction for toxicologists; he webserver takes a two-dimensional chemical structure as an input and

reports the possible toxicity profile of the chemical for 33 models with confidence scores, and an overall toxicity radar chart along with three most similar compounds with known acute toxicity. See the window of Protox prediction in figure below

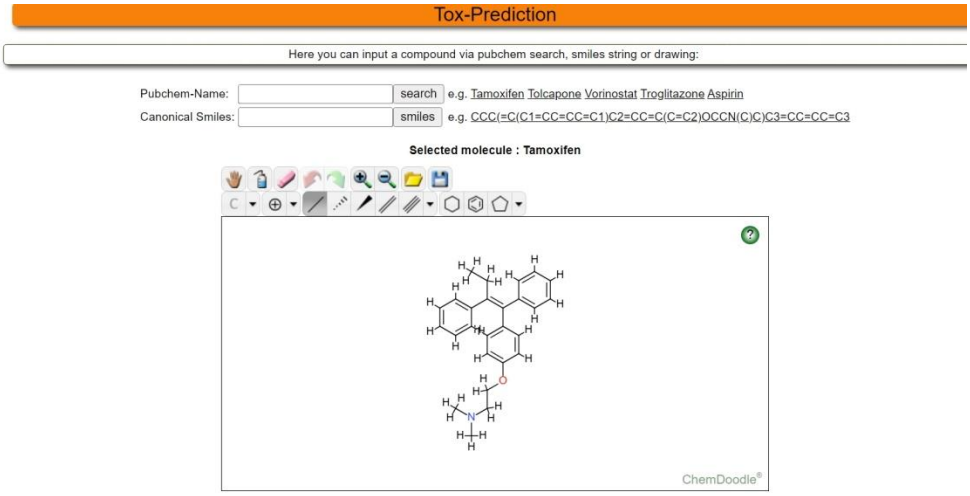


Figure 63: Present screen shot of protox data bases.

Results and Discussions

VIII-Introduction

This chapter presents the results collected from the in vitro and in silico studies that we have done in the previous chapter and their interpretations.

VIII- 1 -Results of physio-chemical analysis

1-1- Extraction yield

- Yield calculation: The average yields of the prepared extracts were calculated on the basis of the dry plant matter of the plant. The following histogram illustrates these results.

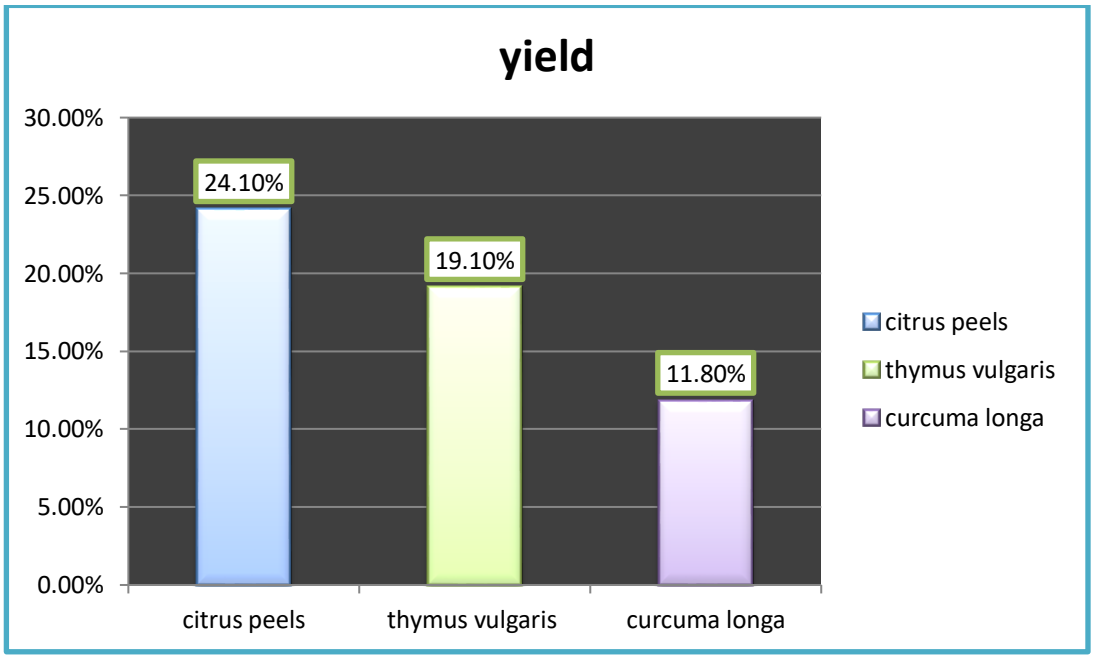


Figure 64: Histogram of the yield of ethanol/water extracts 60%/40%.

1-2- The species were extracted by maceration (for 72 h) using 60%+40% ethanol/water solvents. The yield of our *citrus peels* plant (24.10%) is much higher than that obtained from *Thymus vulgaris* (19.10%) and finally *curcuma longa* with the lowest yield (11.80%). Results present in figure 6.

1-3- Analysis of chemical composition: (GCMS)

Chemical analysis of ethanoic extracts of *curcuma longa* and *thymus vulgaris* using phase-gas chromatography-mass spectrometry (GC-MS) yielded the following results

The GC-MS mass spectra reveal that our extract contains different molecules, with a retention time and molecular weight characteristic of each molecule.

Optimal conditions used for this study

- Equipment: GCMS model CLARUS 500 from Perkin-Elmer

GC method:

- Injected volume: 1ul
- Injector temperature: 250 deg
- Column: Elite series 5-MS, 30 m, 0.25 mmID, 0.25 um stationary phase thickness

Temperature programming

- Initial temperature: 70 deg for 4 min
- Ramp: 4deg/min to 220 deg for 15 min
- Analysis time 56.5 min

MS method

- Ionisation mode: Electron impact
- Energy: 70 ev
- Temperature: source: 250 deg
- Transfer line temperature: 250 deg
- Solvent delay: 5.9 min
- Software: TURBOMASS
- Component identification by searching spectral libraries: NIST, PFLEGER, NBS,WILEY

1 – Curcuma longa Chromatogram of ethanoic *curcuma longa* obtain with maceration; see figure (65)below :

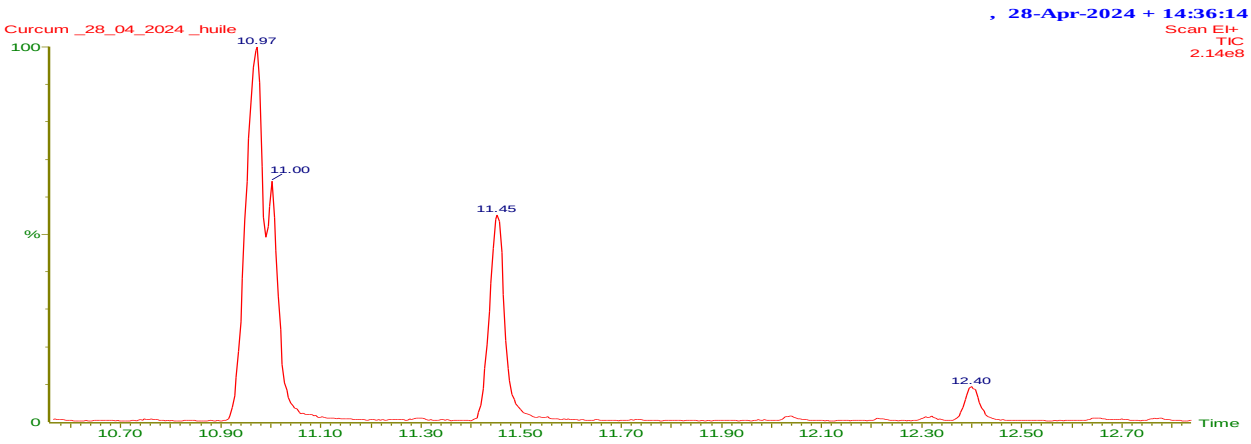


Figure 65: Chromatogram of ethanolic *curcuma longa*.

Mass spectrum

a)- AR-TUMERONE: t= 10.97 min

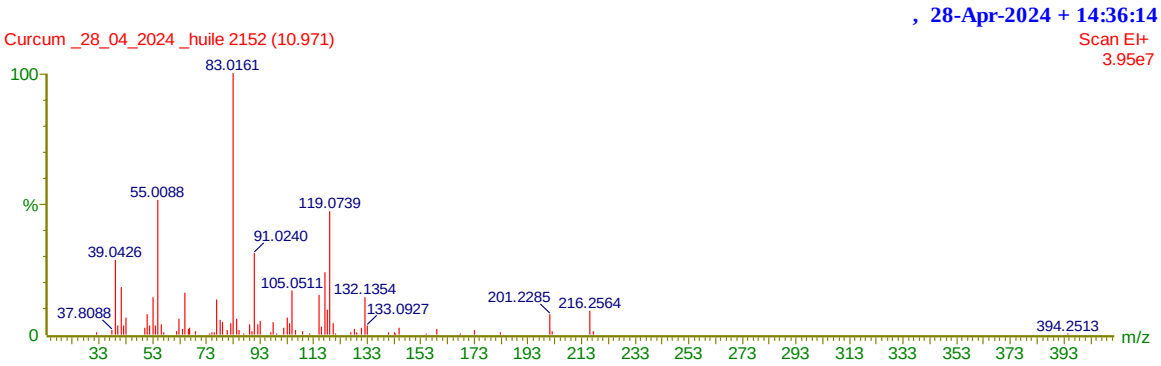


Figure 66: Mass spectrum of AR-TUMERONE with a retention time of 10.97 min.

b)- 1(7),3,8-o-menthatriene: t=7.74min

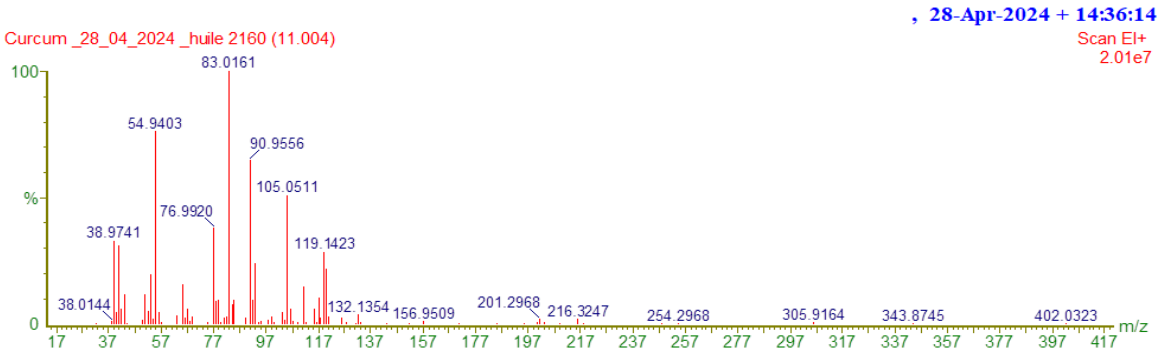


Figure 67: Mass spectrum of 1(7),3,8-o-menthatriene with a retention time of 7.74 min.

c) cyclohexene ,1-(1-propynyl): t=11.45min

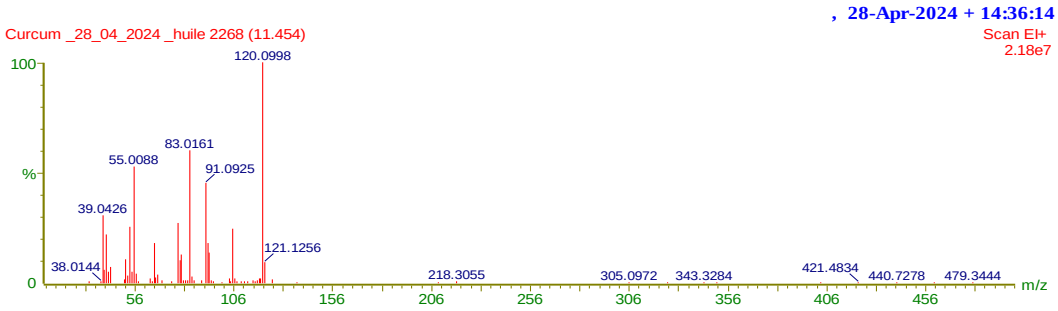


Figure 68: Mass spectrum of cyclohexene ,1-(1-propynyl) with a retention time of 7.74 min.

d)- (cis-cis)-2-bromo-1-methyl-1-3-cyclononadiene,1,3-cyclononadiene,2-bromo: t=12.40min

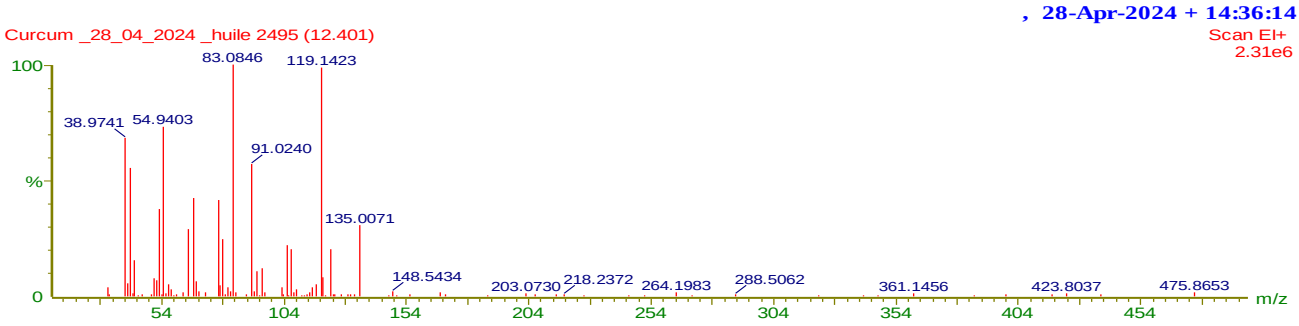


Figure 69: Mass spectrum of(cis-cis)-2-bromo-1-methyl-1-3-cyclononadiene,1,3 cyclononadiene,2-bromo with a retention time of 7.74 min.

All the results of *curcuma longa* mass spectrum are identified in one table 11 below ; we chose hors molecules form selecting molecules with same molecular weight and retention time

Table 11: Molecule compounds of extract *curcuma longa* obtained by CG-MS analysis spectrum is presented in table below

Molecules	**Retention time /min**	**Molecular weight g/mol**
AR-TUMERONE	10.97	216
1(7),3,8-o-Menthatriene	7.74	134
Cyclo Hexene,1-(1-propynyl)	11.45	120
(cis,cis)-2- Bromo-1 methyl-1,3Cyclononadiene,2-bromo	12.4	214

2- ***Thymus vulgaris*** Chromatogram of ethanoic *thymus vulgaris* obtain with maceration ; see figure (70) below :

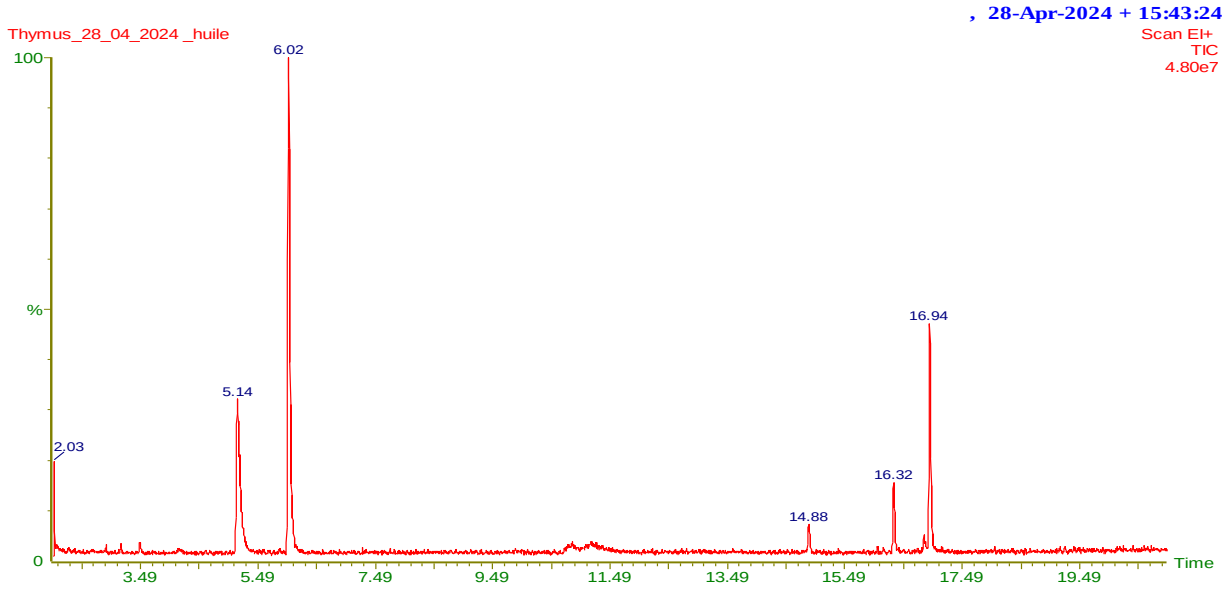


Figure70: Chromatogram of ethanolic *thymus vulgaris*.

a)- phenol,2-(1,1-dimethyl ethyl) –o- tert- butylphenol2-T-BUTYLPHENO: t=5.14min

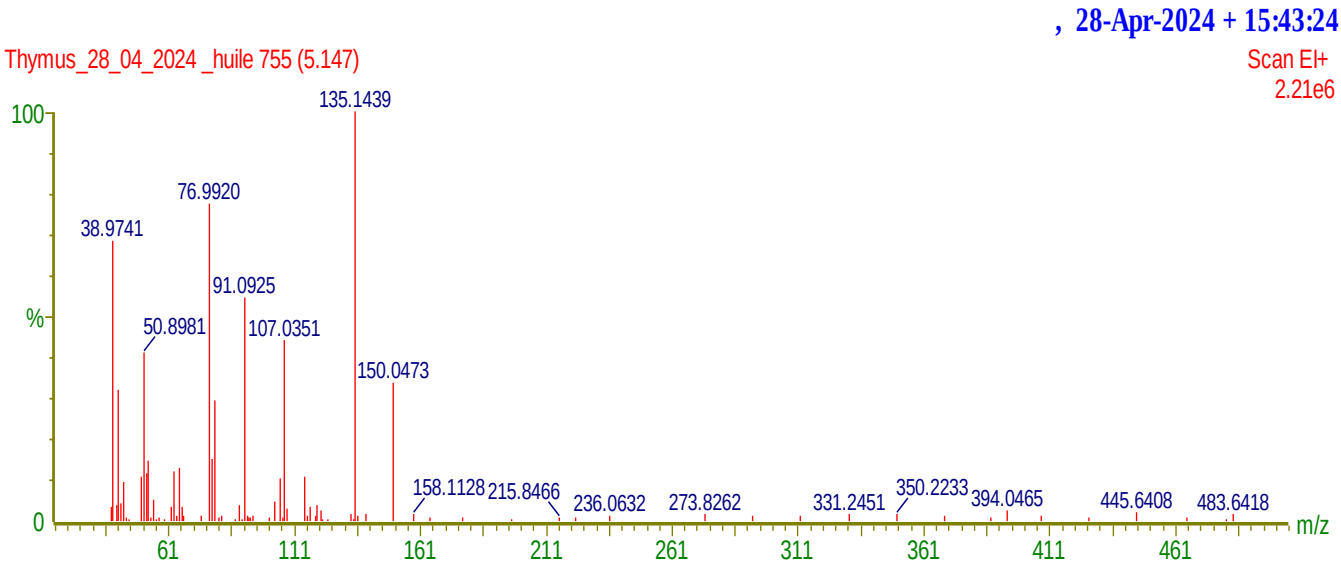


Figure 71: Mass spectrum of phenol,2-(1,1-dimethyl ethyl) –o- tert- butylphenol2-T-BUTYLPHENO with a retention time of 5.14 min.

b)- phenol,5-methyl-2-(1methylethyl)-thymol m-thymol: t= 6.02min

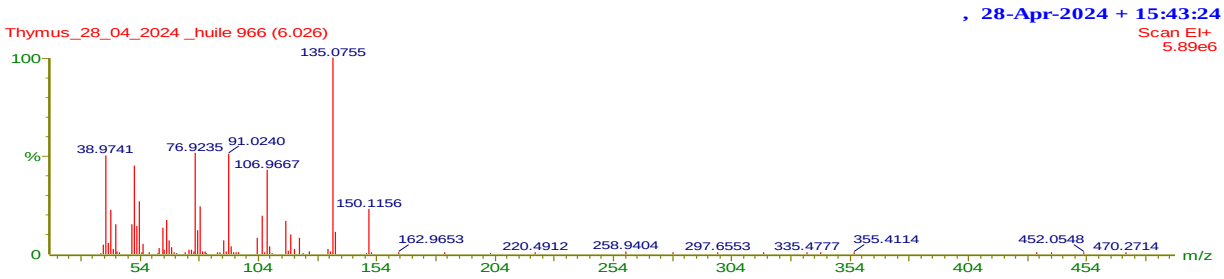


Figure 72: Mass spectrum phenol,5-methyl-2-(1methylethyl)-thymol m-thymol with a retention time of 6.02 min.

c)- pentadecanoic acid4,6,10,14-tetramethyl , methyl ester –methyl 4,6,1: t= 14.88min

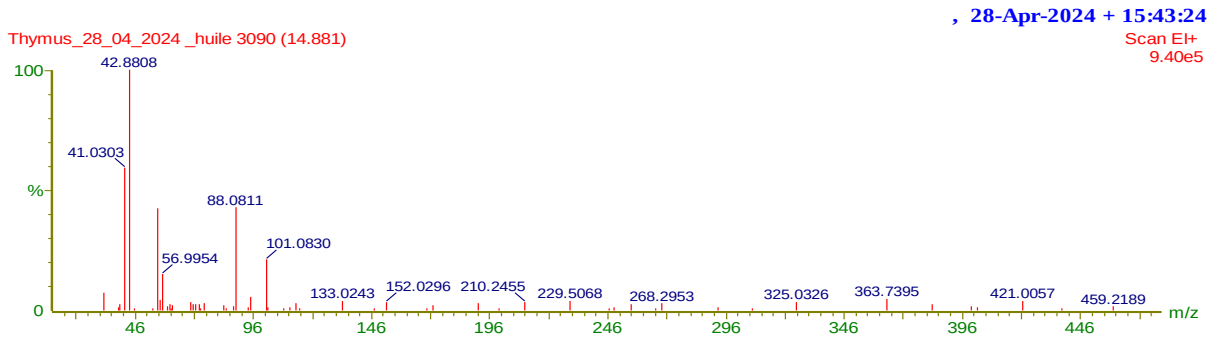


Figure 73: Mass spectrum of pentadecanoic acid4,6,10,14-tetramethyl , methyl ester –methyl 4,6,1 with a retention time of 14.88 min.

d)- Hebtadecane,2-methyl -2methylheptane ,16-methylheptadecan: t=16.32min

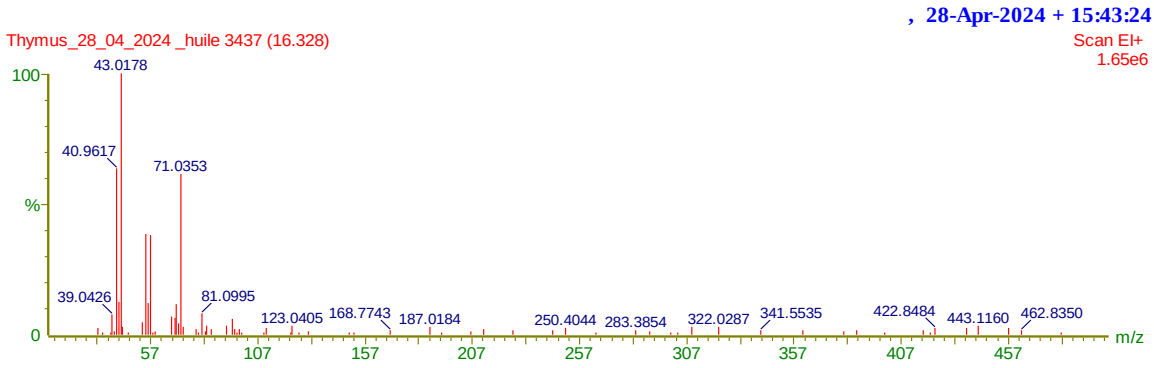


Figure 74: Mass spectrum of Hebtadecane,2-methyl -2methylheptane ,16-methylheptadecan with a retention time of 16.32 min.

e)3- TETRADECEN-5-YNE,(z): **t=5.42min**

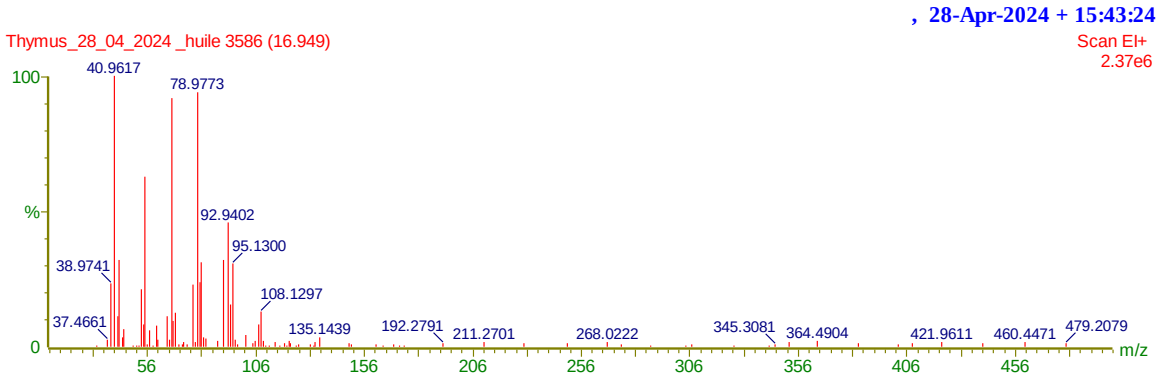


Figure 75: Mass spectrum of 3- TETRADECEN-5-YNE,(z) with a retention time of 5.42 min.

All the results of *thymus vulgaris* mass spectrum is presented in table12 below

Table 12: Molecule compounds of *Thymus vulgaris* obtained by CG-MS analysis

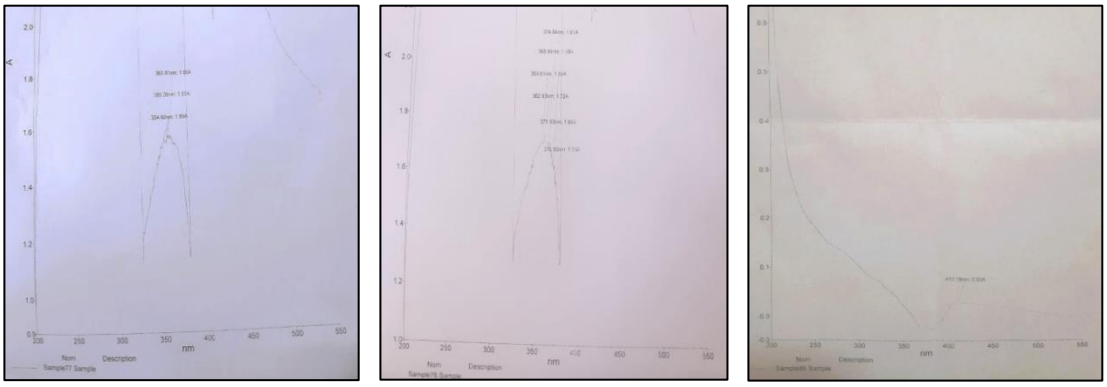
Molecules	**Retention time /min**	**Molecular weight g/mol**
phyenol,2-(1,1-dimethylethyl)-o-tert-Butylphenol2-T-BUTYLPHENO	5.14	150
phenol,5-methyl-2-(1methylethyl)-Thymol-Thymol	6.02	150
pentadecanoic acid 4,6,10,14-tetramethyl,methylester-methyl 4,6,1	14.88	312
Heptadecan,2-methyl-2methylheptane,16-methylheptadecan	16.32	254
3-TETRADECEN-5-YNE,(z)	5.42	192

2-1- visible Uv

To determine the Wavelength $\lambda max$of our extract in fact a scan in the interval [$400nm$ − $800nm$] we find two wavelengths:

Table 13: λmax of our extract

PLANTS	**Wavelength λmax (nm)**
Citrus lemon peels	361
Thymus vulgaris	347.84
Curcuma longa	412.18



A B C

Figure 76: Determine the Wavelength *λmax* of*: A/ citrus peels; B/ thymus vulgaris; C/curcuma longa.*

The table presents the different absorbance of 7 diluted samples

Table14: Absorption of plants.

Absorption / Plants	***Citrus peels***	***Curcuma longa***	***Thymus vulgaris***
A1	0.0045	0.524	0.0308
A2	0.0444	0.625	0.0405
A3	0.2121	0.670	0.1231
A4	0.6653	0.709	0.2256
A5	1.3042	0.730	0.4470
A6	1.6823	1.090	1.5224
A7	2.0899	1.807	2.0610

2-2- <u>Results of : pH / density / conductivity</u> table below present the deferent results

Table 15: Results of pH ; density and conductivity of our plants.

Plants	pH	Conductivity µs/ cm	Density g/cm³
Citrus peels	5.155	56.8	0.9243
Curcuma longa	7.124	88.1	0.9080
Thymus vuegaris	6.770	65.0	0.9239

IX- Antioxidant activity

The antioxidant activity of *citrus peels; thymus vulgaris* and *curcuma longa* extract was determined by reducing the free radical DPPH.

1- **Determination of anti-oxidant activity**

To evaluate the antioxidant effect of plants extracts by 2,2'-diphenyl-1- picrylhdazyl (DPPH) radical technique was used.

The DPPH radical is one of the most commonly used substrates for the rapid and direct assessment of antioxidant activity due to its stability in radical form and its simplicity of analysis. This spectrophotometric method uses the violet-colored DPPH (2,2'-diphenyl 1picrylhydrazyl) radical as a reagent, which turns yellow in the presence of RLS sensors, and is reduced to 2,2'-diphenyl-1-icrydrazine.

After 30 min of incubation of the DPPH-extract solution (at different concentrations), the violet color turns to yellow. This color change is due to the reduction of DPPH, which shows that the sample has a DPPH radical scavenging effect; see the results on figure 65.

The values obtained allowed to draw exponential curves with the presence of a stationary phase which signifies the almost total reduction of DPPH in its non-radical form. From these curves, it is possible to determine the percentage of inhibition obtained as a function of the concentrations used as well as the value of IC50 of each extract.

The results of DPPH are summarized in and , which indicates the uptake of DPPH as a function of extract concentration, with increasing uptake shows in figure 77.

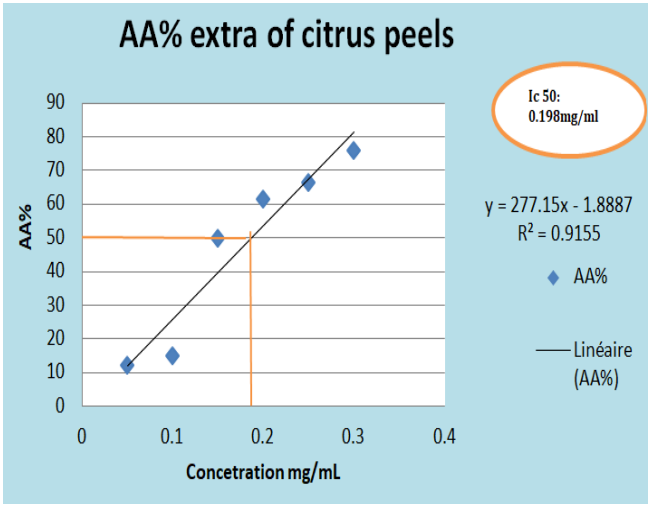
(A)

(B)

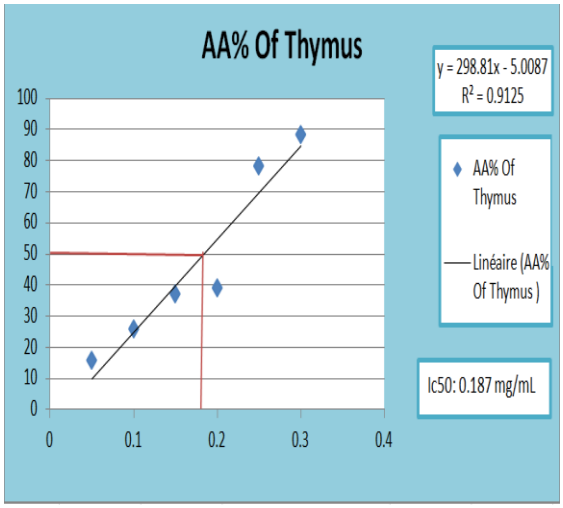
(C)

Figure 77 : DPPH incubation of plants extract at different concentrations *A/ citrus peels ; B/thymus vulgaris ;C/ curcuma longa.*

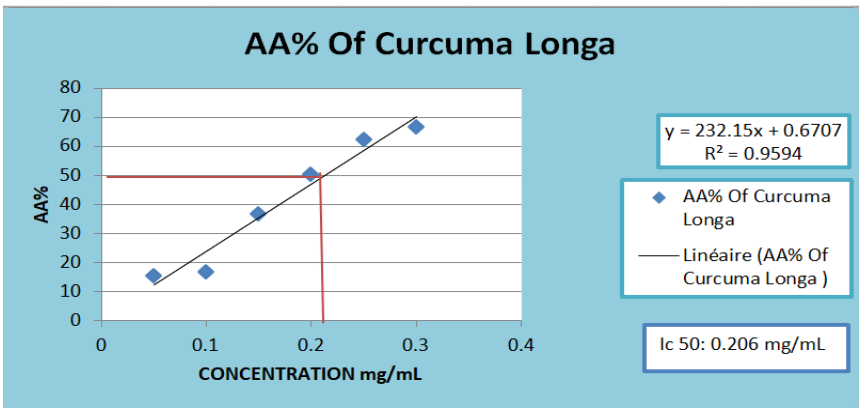
The values presented in the figures show the reducing power of the extracts at different concentrations.



A



B



C

Figure 78: Anti-radical activity of extracts: *A/citrus peels ; B/ Thymus vulgaris ; C/curcuma longa*.

- **Figure 78** shows an increase in the percentage inhibition (PI) of the absorbance of the DPPH of the extracts tested. solution as a function of the concentrations of the extracts tested. At the lowest concentration (0.05 mg/ml), PI=15.63% for *Thymus*, PI=11.98% for *Citrus peel* and PI=15.29% for *Curcuma longa*, while at the highest concentration (0.3 mg/ml), PI=88.36% is highest for *Thymus vulgaris.* See figure 79.
- **This shows that the species *Thymus vulgaris* has a high antioxidant potential.**

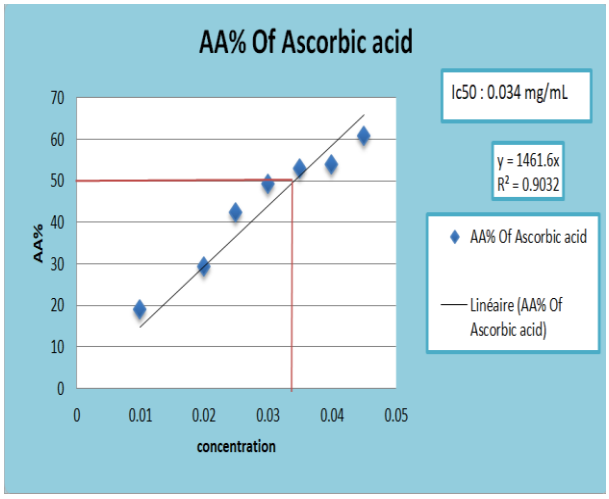


Figure 79: Reductive power of ascorbic acid.

Figure80: Antioxidant activity of ascorbic acid.

- The results in figure 80 showed that the increase in extract concentration (OD at 517nm) was proportional to the increase in ascorbic acid concentration.

- All our extracts showed antioxidant activities that were significantly lower than those of the reference (ascorbic acid), with the latter showing an almost total reduction from a concentration of 0.034 mg/ml. It should be noted that our extract shows acceptable antioxidant activity.
- The antioxidant activity assessed for the different citrus extracts that the standard used is expressed in IC50 (inhibitory concentration 50); this is the concentration of extract that neutralises (reduces) 50% of the free radical (DPPH), the lower the IC50, the more powerful the antioxidant potential of the extract. All the antioxidant activity results expressed as in IC50 is shown in the table below :

Table 16: Ic50 determinate of our plants

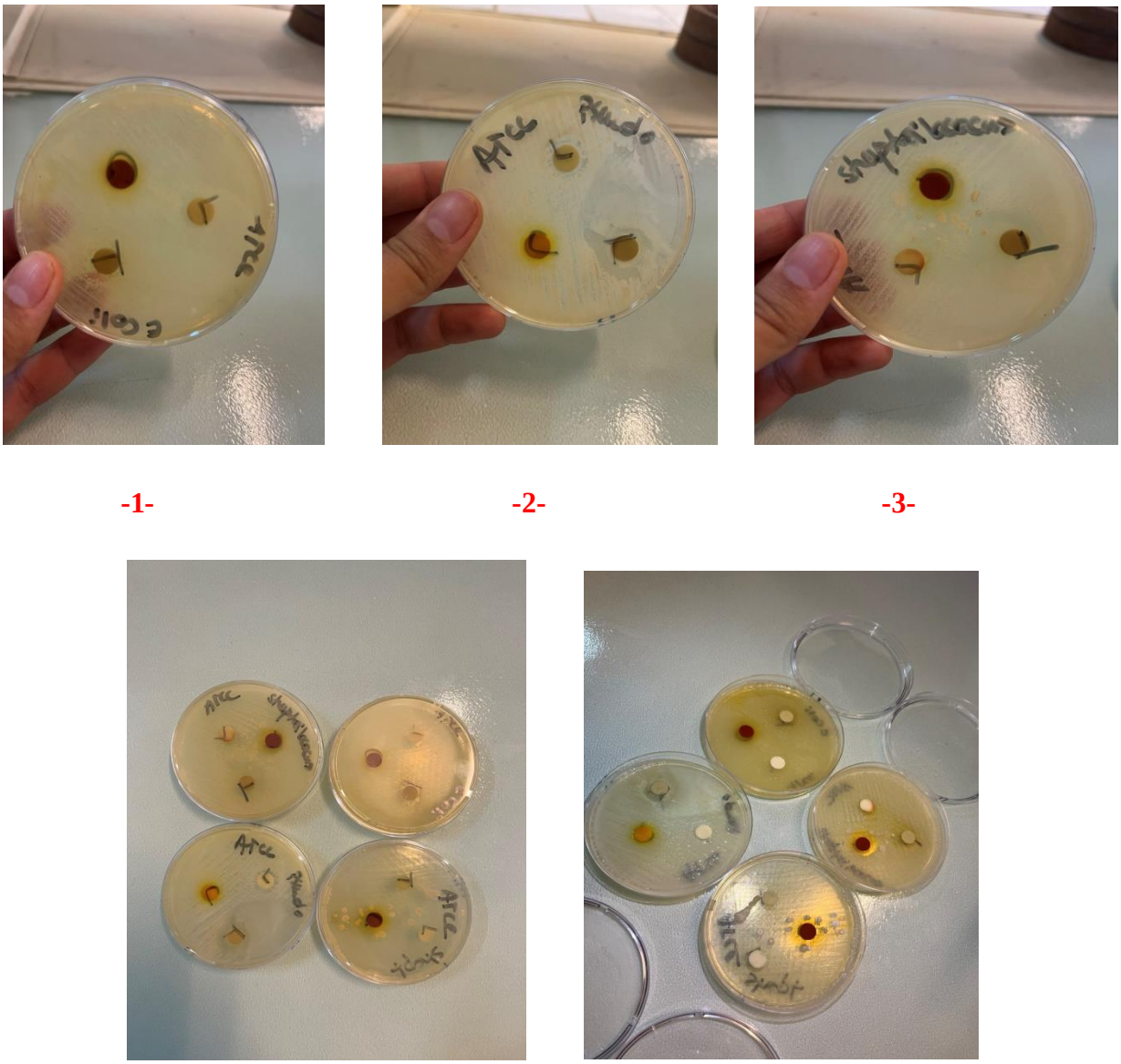
Plants	**Ic50 mg/mL**
Citrus peels	0.198
Thymus vulgaris	0.187
Curcuma longa	0.206
Ascorbic acid	0.034

- The difference in DPPH free radical scavenging activity between the three extracts analyzed is probably due to their composition of different phenolic compounds. The reduction of DPPH is generally not due to the action of a single compound but to the interaction between several compounds, and these interactions may exist in one extract but not in another, leading to this difference in activity between extracts.
- Our results are in line with those already published, which showed that curcuma longa represented the fraction richest in polyphenols, which revealed a powerful antioxidant potential, followed by citrus peels and, lastly, thymus vulgaris.

X- Anti bacterial activity

1- For extracts

The observations made showing the effect of citrus peels ; curcuma longa and thymus vulgaris extracts on growth bacterial strains tested*: Esherichia coli* (ATCC 8739*), Pseudomons aeruginosa* (ATCC27853), *Staphylococci aureus* (ATCC 6538),and *streptococcus spp* (ATCC 12228). The results are represented in the figures below:



-1- **-2-** **-3-**

-4- **-5-**

Figure 81: The antibacterial effect of plants extracts.

2- For oral emulgel

The observations made showing the effect of our oral emulgel formulated on growth bacterial strains tested*: Esherichia coli (ATCC 8739), Pseudomons aeruginosa (ATCC27853), Staphylococci aureus (ATCC 6538),and streptococcus spp (ATCC 12228).* The results are represented in the figures below:

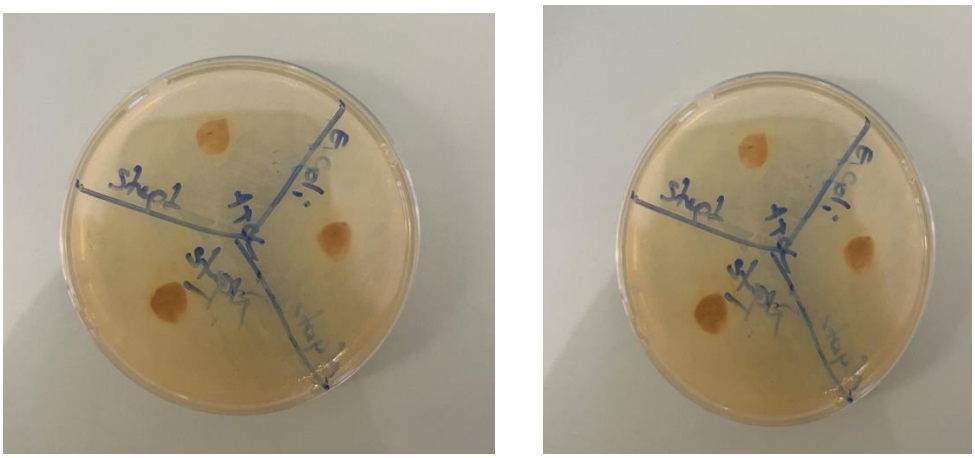


Figure 82: Antibacterial activity of oral emulgel.

3- Evaluation of the antibacterial activity

a/ Extracts : the results of antibacterial test present in table17 below

Table 17: Sensitivity antibacterial for extract

Gram	ATCC	*curcuma longa*	*thymus vulgaris*	*limon citrus peels*
Negatif	*pseudomenas airogenosa*	(08mm) resistant	(12mm) sensitive	(20mm) sensitive
Negatif	*E coli*	(06mm) resistant	(12mm) sensitive	(12mm) sensitive
Positif	*staphylococcus aureus*	(14mm) sensitive	(06mm) resistant	(14mm) sensitive
Positif	*streptococcus spp*	(19mm) sensitive	(25mm) sensitive	(08mm) resistance

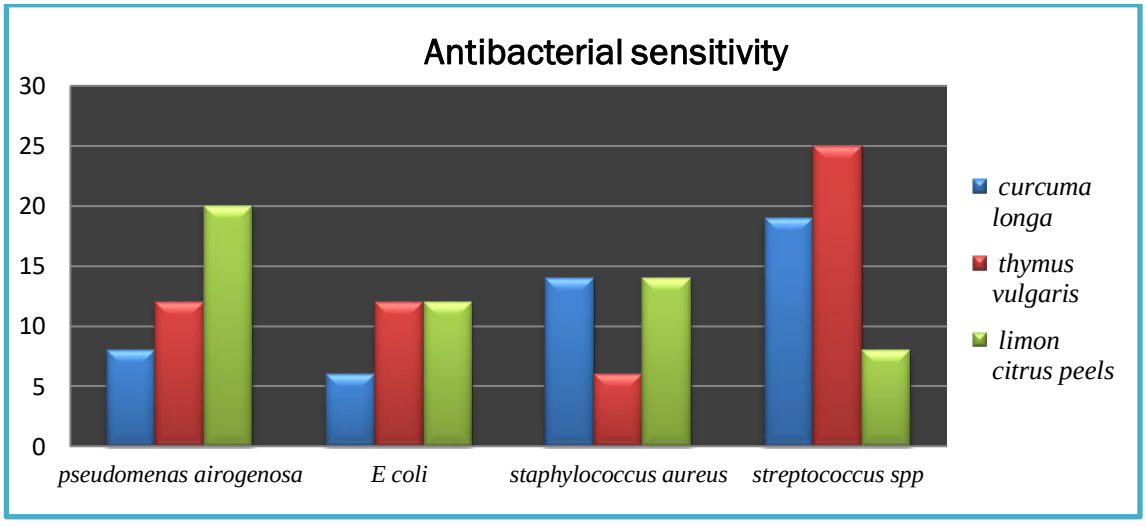


Figure 83: Diagram showing the antibacterial activity of extracts.

- **Table 17** shows the antimicrobial effect of the different extracts of *Citrus peels, thymus and curcuma longa* on the microorganisms tested. The best diameters of the inhibition zones were 25 mm with *Streptococcus spp ATCC 12228*, which is considered a very strong inhibitory diameter considered to be a very highly inhibitory diameter. See diagram on figure 83
- The strains differed according to the zone of inhibition; the extracts show a variation between gram-positive and gram-negative bacteria; *citrus peels and thymus vulgaris* were the best inhibitors for the different strains studied.
- The results obtained are shown in table 17.

b/ Oral emulgel : Results of antibacterial test of final form presented in table below

Table 18: Sensitivity antibacterial for final product

gram	ATCC	Oral emulgel
negatif	*pseudomenas airogenosa*	14 (sensitive)
negatif	*E coli*	28 (sensitive)
positif	*staphylococcus aureus*	18 (sensitive)
positif	*streptococcus spp*	11 (sensitive)

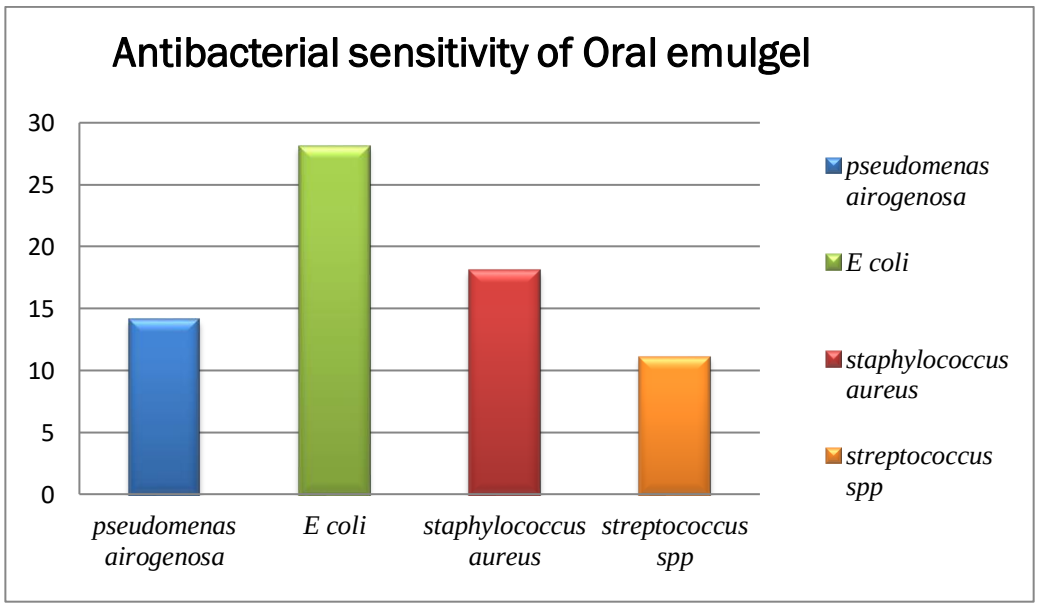


Figure 84: Diagram showing the antibacterial activity of oral emulgel.

- Figure84 shows the sensitivity of the final form of oral emulgel to gram-positive and gram-negative bacteria. It can be seen that our oral emulgel has a high sensitivity to both strains, but is particularly sensitive to *E.coli* with a diameter of 28 mm.
- Our final form can be considered as an antibacterial for both types of bacteria.

C/ Oral gel formulation: **Table 19:** Result organoleptic and physicochemical control of final oral emulgel

Parameters	**Test 01**	**Test 02**	**Test 03**	**Test04**	**Test 05**
Color	green	dark green	green	clear green	green
Smell	Nice fresh	Nice fresh	Nice fresh	Nice fresh	Nice fresh
Taste	not really sweet	less sweet	less sweet	normal sweet	less sweet
pH	6.30	6.80	7.66	7.32	6.78
Conductiviy (µs/ cm)	65.7	63.1	65.9	61.6	62.4
Thickness	Slightly thick	Extremely thick	Moderately thick	perfectly thick	Moderately thick
Dephasing	yes	yes	no	no	no

- As we had just finished our training course at the Saïdal group and the laboratory at our university had limited capacity, all we had to do was measure pH (acidity) and conductivity and observe by eye in case of color change and macrological test for the final form of our product, see figure 85.

Figure 85: Final product.

- **Sterility test :** the results shows that our product have few charge(≤06 cell) of benefit bacteria present in final product as *Staphylococcus spp* , see figure 86.

Figure 86: Sterility test of final product.

- **Stability test of oral emulgel :** the results of centrefuge the oral emulgel shows that the oral emulgel can be separate after long time of centrefugation as 10 min in 4000 tour

(A) (B) (C) (D)

Figure 87: Centrefugation of final pruduct :A/ 5min in 2000 tour ; B/10min in 2000tour; C/5min in 4000 tour ; D/ 10min in 4000 tour.

- As we can see in figure 87 as much we active more speed with more time the separation is taking . the stabilite of our product is taking in midium indicat.

XI - *In silico* (molecular docking)

Introduction

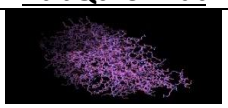
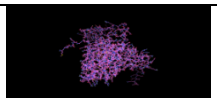
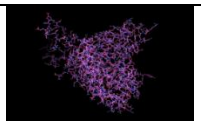
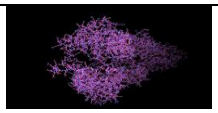
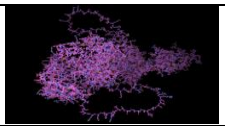
In the field of molecular modeling, docking is a method for determining the preferred docking site. The interaction between the orientation of a molecule and a second, when bonded together, ensures its stability. The formation of the complex and the triggering of a responsible signal therapeutic effect. The molecular docking technique was swiftly introduced in the domain of biological research. The Docking Program has been designed to facilitate the creation of molecules possessing therapeutic potential.

1- Presentation of protein and ligand

1-1- RECEPTORS (THE PROTEINS)

The proteins used are HDACs; PARP-1 and MDR which we obtained from the UNIPROT data bank in the form of 3D, which we prepared with the previously mentioned steps into protein pdbqt, and the (table 20) below represents this .

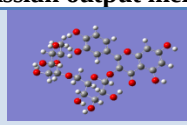
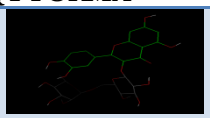
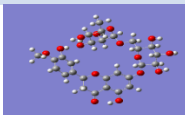
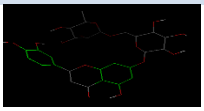
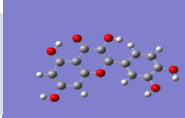
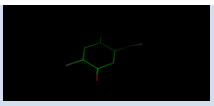
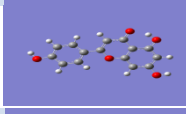
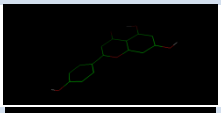
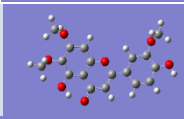
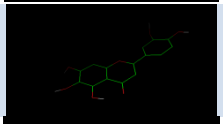
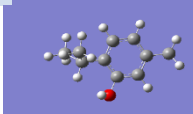
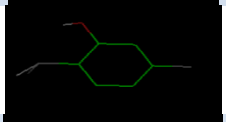
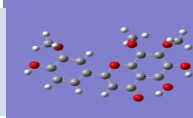
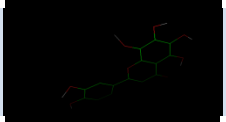
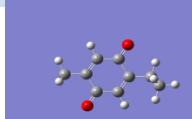
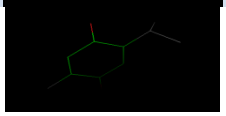
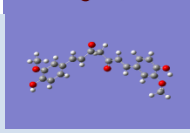
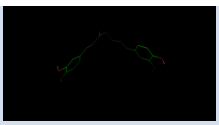
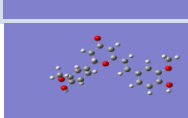
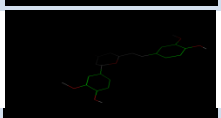
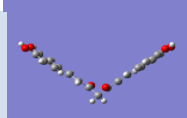
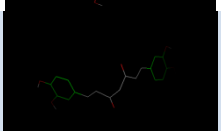
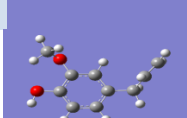
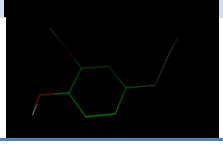
Table 20: PDBQt form of receptors prepared.

Receptor	Receptor type	PdbQt format
PARP-1	PARP-1	
HDACs	HDAC1	
	HDAC3	
MDR	ABCB1	
	ABCC1	

1-2- Ligand preparation We prepared the compounds *(quercetin ; hesperidin; rutin* from Citrus peels */ apigenin; cirdilineol;thymol; thymonine* and *thymoquinone* from *Thymus*

vulgaris / *curcumin ; cyclocurcumine ; didmethyl-curcumin;* and *eugenol* from *Curcuma longa*) in Gaussian / Gaussview and Autodock tools by converting them from pdb-out to pdbqt as previously described, and got the results in the table 21 below :

Table 21: PDBQt and Gaussian output forma of ligand prepared.

Plant	Molecule	Gaussian output melecule	PdbQT FORMA
Citrus peels	*Rutin*		
	Hesperidin		
	Quercetin		
Thymus vulgaris	*Apegenine*		
	Cirsilineol		
	Thymol		
	Thymonine		
	Thymoquinone		
Curcuma longa	*Curcumin*		
	Cyclo curcumin		
	Didmethyl curcumin		
	Eugenol		

1-3- **Applying Lipinski's rule**: for the four molecules was hypothetical and it was necessary to complete the study by application the Lipinski's rule of 5 to the four compositions. Using DruLiTO app we can made the application of Lipinski's rules easier. The results of this test are shown in Table 22.

Table 22: Results of molecules proprieties.

Name	MW(g/mol)	Log P	HA	HD	PA	PI	TPSA(A²)	nRB	GI abs
Rutin	610.52	-0.735	16	10	0.728	0.013	269.43	06	LOW
Hesperidin	610.56	-1.11	15	08	0.846	0.003	234.29	07	LOW
Quercetin	302.24	1.834	07	05	0.689	0.017	131.36	01	HIGH
Apegenine	270.24	1.89	05	03	0.732	0.004	90.90	01	HIGH
Cirsilineol	344.32	2.57	07	02	0.645	0.009	28.36	04	HIGH
Thymol	150.22	2.32	01	01	0.549	0.044	20.23	01	HIGH
Thymonine	360.31	3.07	08	03	0.775	0.004	118.59	04	HIGH
Thymoquinone	164.20	1.99	02	00	0.601	0.003	34.14	01	HIGH
Curcumin	368.38	3.27	06	02	0.677	0.019	93.06	08	HIGH
Cyclo curcumin	368.38	3.12	06	02	0.521	0.051	85.22	05	HIGH
Didmethyl curcumin	340.33	1.54	06	04	0.332	0.041	115.06	06	HIGH
Eugenol	164.20	2.37	02	01	0.491	0.060	29.46	03	HIGH

- **MW:** Molecular weight
- **LogP:** water/octanol partition coefficient ($-2 \leq \log P \leq 5$)
- **HA :** Hydrogen acceptor (nO,N)(≤ 10).
- **HD:** Hydrogen Donor(nOH,NH)(≤ 5).
- **Pa:** refers to the partition coefficient (also known as octanol-water or logP) pa>0.7 and pa>pi
- **Pi:** refers to the polar surface area (PSA) of a compound
- **TPSA:** rate of polar surface area (PSA) (A^2)
- **nRB**: number of Rotatable Bond Count
- **GI abs :** gastrointestinal absorption

Interpretation

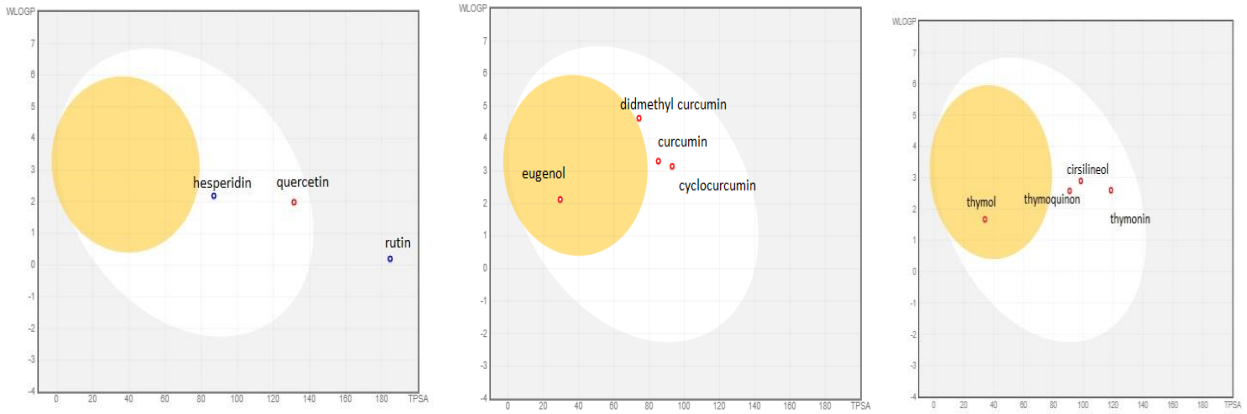
The table 22 is result of working with SwissADME and pass online data bases and according to DruLiTo app work. From this table we can result:

- LogP (Partition Coefficient): The Log P interval between **-1.11 ≤log p≤3.28**. The value is indicator of lipophilicity. As we see Lipinski's rule suggests that compounds with a logP value between -0.4 and 5.6 are more likely to have good oral bioavailability.
- Hydrogen Donors: we can class it on interval of (01-10). Lipinski's rule does not specify a maximum limit for hydrogen donors, but a higher number of hydrogen donors might indicate the potential for forming hydrogen bonds .for example rutin and quercetin .
- Hydrogen Acceptors: we can class them in interval of (01-16) . Similarly, Lipinski's rule does not have a specific limit for hydrogen acceptors, but a higher number may indicate the potential for forming hydrogen bonds like rutin and hesperidin.
- Polar Surface Area (Pi): The polar surface area (presumably in units of square angstroms, $Å^2$) suggests a relatively small polar surface area. Lipinski's rule indicates that compounds with a polar surface area greater than 140 $Å^2$ might have lower oral bioavailability. Example thymol and curcumin has great oral bioavailability.
- Partition Coefficient (Pa): The partition coefficient value (presumably octanolwater partition coefficient or logP) indicates moderate lipophilicity. As mentioned earlier, Lipinski's rule suggests that compounds with a log value between -0.4 and 5.6 are more likely to have good oral bioavailability.
- Polar surface area (PSA) or topological polar surface area (TPSA): The polar surface area (PSA) or topological polar surface area (TPSA) of a molecule is defined as the surface sum of overall polar atoms or molecules, primarily oxygen and nitrogen, also including their attached hydrogen atoms. TPSA was calculated from a 2D chemical structure by means of the summation of tabulated surface area values for 43 polar fragments. Must be PSA ≤ 140 to say a good oral bioavailability; as we see in the table eugenol and thymol are a good oral bioavailability products.

We present the low The Veber filter is a rule of thumb filter for orally active drugs described in Veber. The default parameters used here are: • Rotatable bonds <= 10. • Topological polar

surface area <= 140. They concluded that molecular flexibility, polar surface area (PSA), and hydrogen bond count are important determinants of oral bioavailability.as an example of our molecules: all molecules are good except rutin and hesperidin.

- It's important to note that while Lipinski's rule provides guidelines for assessing the drug-likeness of compounds based on specific criteria, it is not an absolute rule and does not guarantee a compound's efficacy or safety.
- We can use SwissADME also as reference to class our molecule in terms of their nature lipophilic or hydrophilic ;molecule using boiled eff diagram as we can see in figure88.



(A)- citrus peels *(B) -curcuma longa* *(C)-thymus vulgaris*

Figure 88: Diagram of boiled egg shows in SwissADME of our molecules.

- From this diagram figure 88,we can classified molecules: molecules setting in the yellow part are predictive to be passed through blood brain barrier but molecule classified in white part are predictive to be absorbed in gastrointestinal tract so we classified molecule as *hesperidin; rutin ; quercetin;curcumin;cyclocurcumin;didmethyl curcumin; cirseliniol ; thymoquinon* and *thymoni*n as hydrophilic molecules also *eugenol* and *thymol* are lipophilic molecules.
- There is another class that could be added SwissADME show in the boiled egg: a bleu point present the molecule that can be affected to nervous system with P-glycoprotein also the red point present the molecule that cannot be affected to nervous system with P-glycoprotein. All molecules are affected to nervous system except *rutin* and *hespiridin.*

1-4- Molecular docking study

The results of the interaction details of the compounds (ligand) studied; with three target proteins (PARP-1; HDACs and ABC) are summarized in the following tables:

Tableau 23: The results of all studied interaction

Receptor	Ligand	Ligand type	Receptor pocket	Interaction Category	Distance (Å)
Parp-1	Rutin	C==O	A:GLU763	Hydrogen Bond	1.95335
		C==O	A:TYR907	Hydrogen Bond	2.22624
		C==O	A:HIS909	Hydrogen Bond	2.32011
		C==O	A:TYR896	Hydrogen Bond	1.7306
		C==O	A:ASN868	Hydrogen Bond	2.21339
	Hesperidin	N==O	A:ASP756	Hydrogen Bond	1.8882
		C==O	A:GLY894	Hydrogen Bond	3.04671
		N==O	A:TYR907	Hydrogen Bond	2.22624
		C==O	A:HIS909	Hydrogen Bond	2.32011
		C==O	A:ASN868	Hydrogen Bond	1.92798
	Quercetin	C==O	A:ASP766	Hydrogen Bond	2.09811
		C==O	A:SER864	Hydrogen Bond	2.22925
		N==O	A:TYR907	Hydrogen Bond	2.14424
		C==O	B:SER864	Hydrogen Bond	2.49447
	Apeginine	C==O	B:ASP766	Hydrogen Bond	2.06104
		C==O	B:ASP766	Hydrogen Bond	2.72653
		C==O	B:GLY780	Hydrogen Bond	2.39982
		C==O	B:GLY894	Hydrogen Bond	2.40192
HDAC1	Thymol	C==O	A:GLU398	Hydrogen Bond	2.21719
		C==O	A:VAL122	Hydrogen Bond	1.94795
		C==O	A:ALA119	Hydrogen Bond	1.86839
		C==O	A:TYR14	Hydrogen Bond	1.84127
	Thymonine	C==O	A:LYS412	Hydrogen Bond	2.33463
		C==O	A:LYS412	Hydrogen Bond	2.57908
		C==O	A:TYR14	Hydrogen Bond	1.84127
		C==O	A:ASP395:	Hydrogen Bond	2.97912

	Thymoquinone	C==O	A:TYR14	Hydrogen Bond	1.8678
		C==O	A:TYR14	Hydrogen Bond	1.84127
		C==O	A:ALA119	Hydrogen Bond	1.86839
		C==O	A:ILE362	Hydrogen Bond	1.92412
	Didmethyl	C==O	A:ALA119	Hydrogen Bond	1.86839
	curcumin	C==O	A:VAL122	Hydrogen Bond	1.94795
		C==O	A:LYS123	Hydrogen Bond	1.99732
		C==O	A:LYS123	Hydrogen Bond	2.87866
	Eugenol	C==O	A:LEU317	Hydrogen Bond	1.95367
		C==O	A:LEU317	Hydrogen Bond	2.93984
		C==O	A:TYR318	Hydrogen Bond	1.93732
		C==O	A:ILE441	Hydrogen Bond	2.05915
	Quercetin	C==O	A:SER312	Hydrogen Bond	2.07491
		C==O	A:SER312	Hydrogen Bond	2.56229
		C==O	A:GLU317	Hydrogen Bond	2.0402
HDAC3	Thymoquinone	C==O	A:HIS172	Hydrogen Bond	1.83969
		C==O	A:PHE144	Hydrogen Bond	2.76941
		C==O	A:HIS172	Hydrogen Bond	1.97958
	Cyclocurcumin	C==O	A:SER74	Hydrogen Bond	1.86924
		C==O	A:ALA30	Hydrogen Bond	2.34414
		C==O	A:SER405	Hydrogen Bond	2.18834
	Rutin	C==O	A:ARG1263	Hydrogen Bond	1.95019
		C==O	A:ARG1263	Hydrogen Bond	2.88132
		C==O	A:GLU1144	Hydrogen Bond	1.92777
	Quercetin	C==O	A:VAL1259	Hydrogen Bond	2.73557
		C==O	A:ARG1142	Hydrogen Bond	2.70669
ABCC1		C==O	A:GLN1139	Hydrogen Bond	2.67531
	Curcumin	C==O	A:GLU1079	Hydrogen Bond	2.24282
		C==O	A:VAL1259	Hydrogen Bond	2.73557
		C==O	A:THR1256	Hydrogen Bond	2.02841
		C==O	A:ARG1263	Hydrogen Bond	1.95019
ABCB1	Cirsilineol	C==O	A:HIS145	Hydrogen Bond	2.15623
		C==O	A:LEU890	Hydrogen Bond	2.05574
		C==O	A:LEU924	Hydrogen Bond	2.92658

- In this study, we tested the interaction of the three receptors with several molecules from three different extracts*: citrus peels, curcuma longa and thymus vulgaris,* each extract took

several molecules each molecule was hooked up to different amino acids; different types of bond and different distances from one amino acid to another.

- The table 23 shows that :
- parp-1 is interacted by two extracts; citrus peels: rutin by amino acids in hook (A:GLU763; A:TYR907;A:HIS909;A:TYR896 ;A:ASN868) in hydrogen bond interactions with distance (1.95335;2.22624 ;2.32011;1.7306;2.21339). ,hesperidin by amino acids in hooking (A:ASP756;A:GLY894;A:TYR907;A:HIS909;A:ASN868) in hydrogen bond interactions with distance (1.8882;3.04671;2.22624;2.32011;1.92798). also with quercetin ; *thymus vulgaris*: apigenine by amino acids in hydrogen bond interactions with distance (2.06104;2.72653;2.39982;2.40192).
- From table 23; hdacs family: hdac1 is inhibited by *thymus vulgaris* (thymol;thymoquinone;thymonine) and with *curcuma longa* (didmethyl curcumin and eugenol); another half HDAC3 is inhibited by the three extracts cyclocurcumin from *curcuma longa* ; quercetin from *citrus peels* and thymoquinone from *thymus vulgaris*.
- MDR family : ABCC1 is inhibited by *citrus peels* (rutin and quercetin) and with *curcuma longa* (curcumin); another half ABCB1 is inhibited by *thymus vulgaris* (cirsilineol)
- This shows that several extracts can inhibit the same receptor at the same time, which validates the work of several inhibitors.
- The results are more understanding in discovery 2D and 3D diagram shows in figures below :

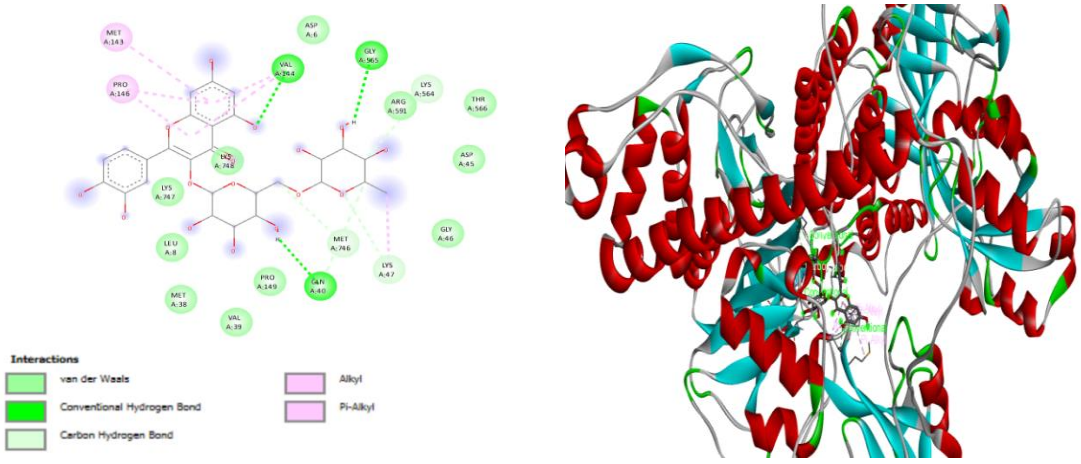


Figure 89: 2D diagram and 3D diagram of interaction PARP-1 with Rutin.

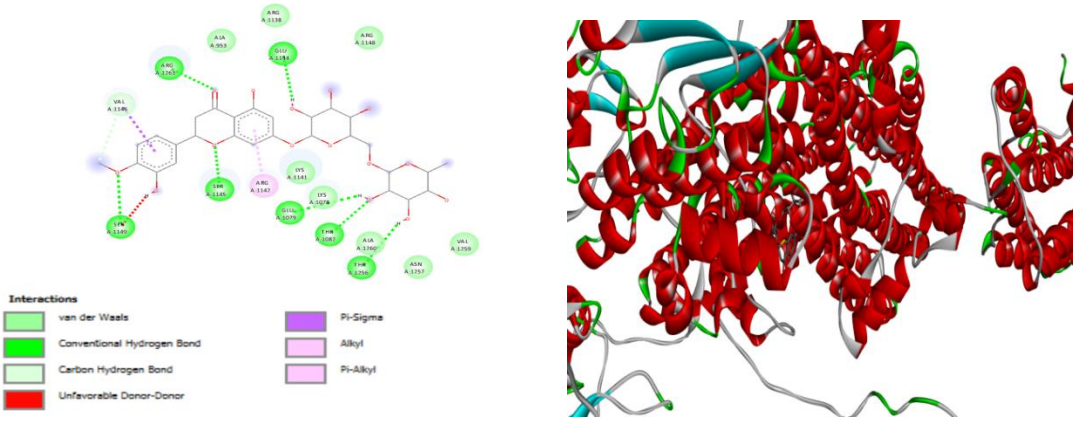


Figure90: 2D diagram and 3D diagram of interaction ABCC1 with Rutin.

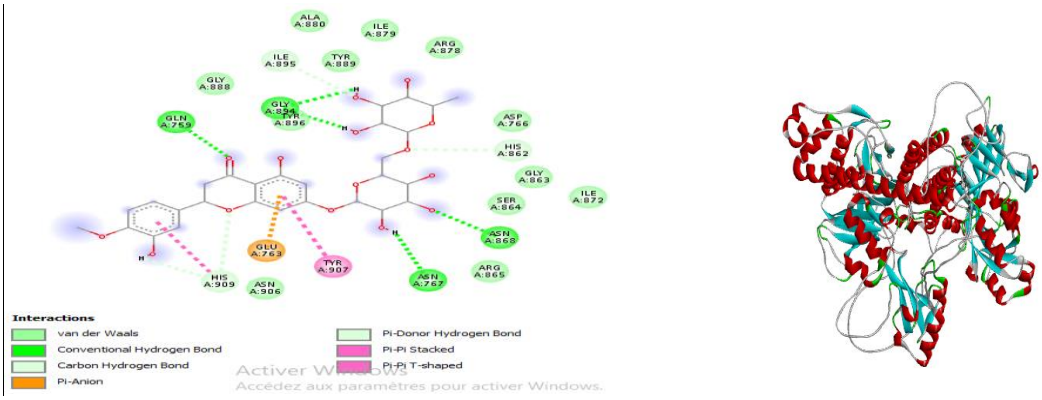


Figure 91: 2D diagram and 3D diagram of interaction PARP-1 with Hesperidin.

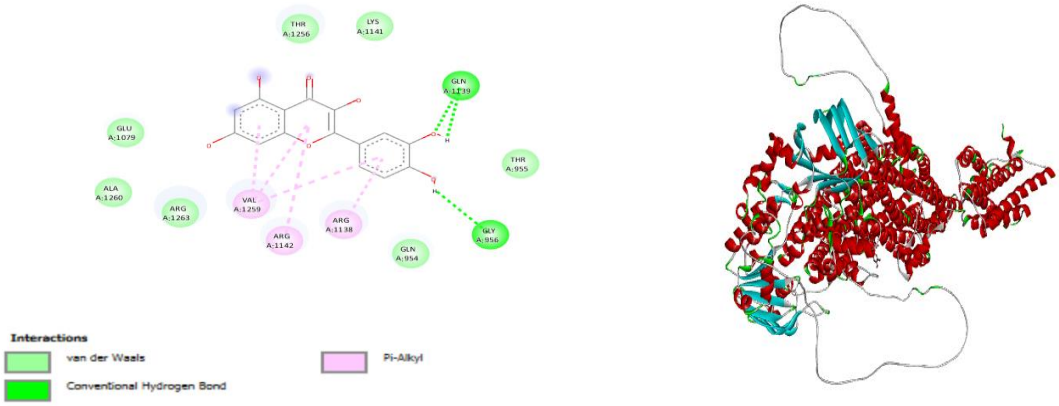


Figure92: 2D diagram and 3D diagram of interaction ABCC1 with Quercetin.

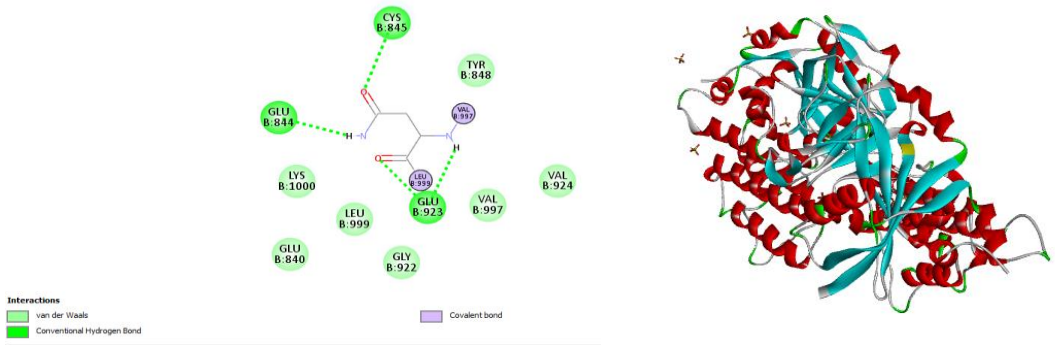


Figure 93: 2D diagram and 3D diagram of interaction PARP-1 with Quercetin.

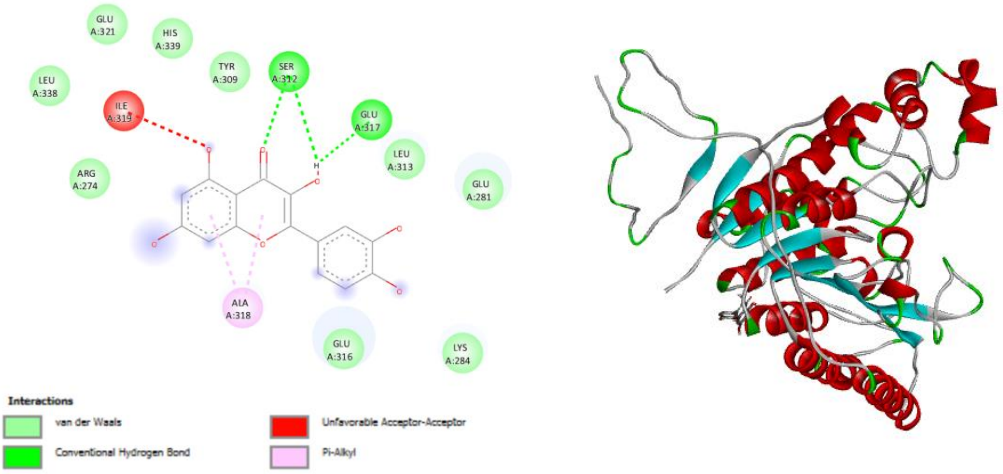


Figure 94: 2D diagram and 3D diagram of interaction HDAC3 with Quercetin.

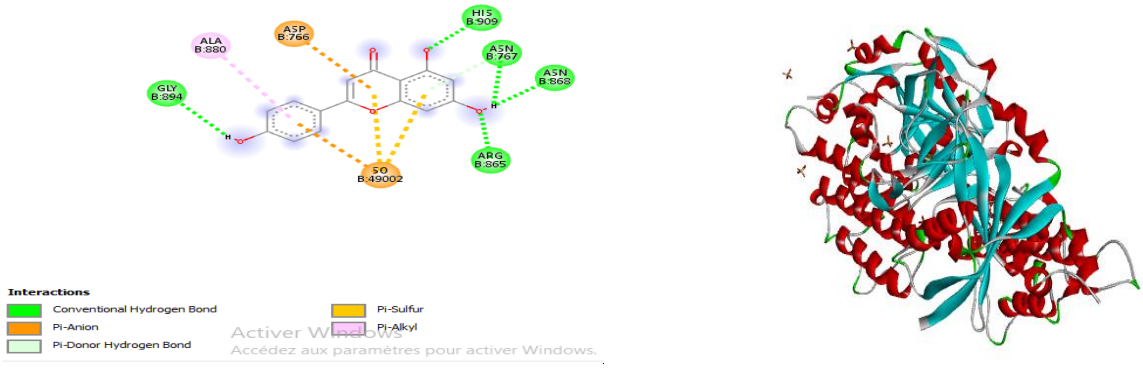


Figure 95: 2D diagram and 3D diagram of interaction PARP-1with Apigenine.

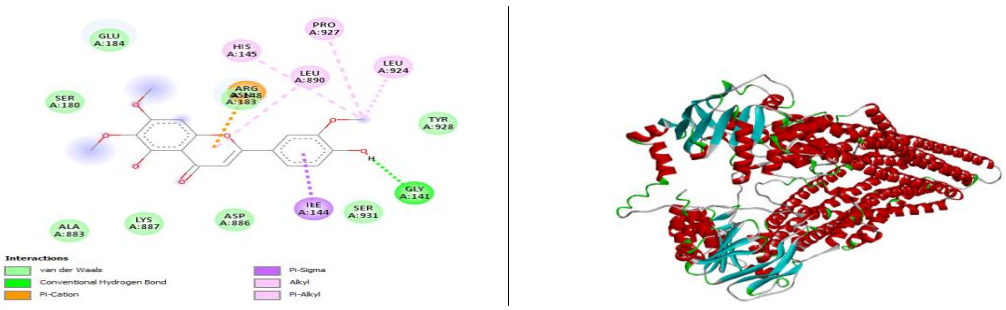


Figure96: 2D diagram and 3D diagram of interaction ABCB1with Cirsilineol.

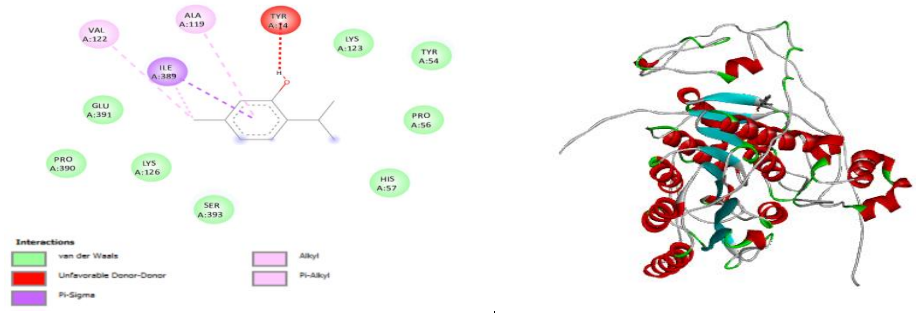


Figure 97: 2D diagram and 3D diagram of interaction HDAC1with Thymol.

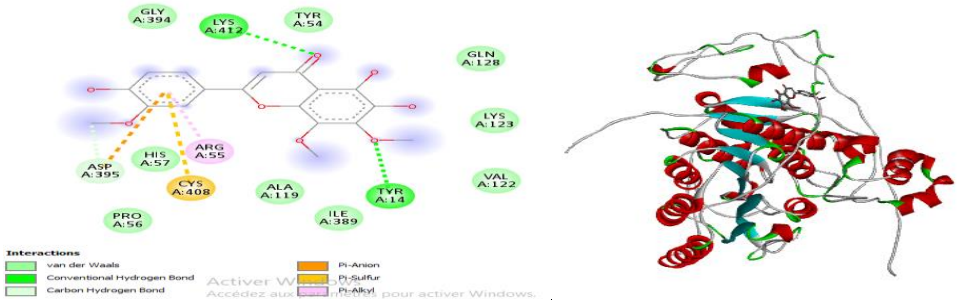


Figure 98: 2D diagram and 3D diagram of interaction HDAC1with Thymonine.

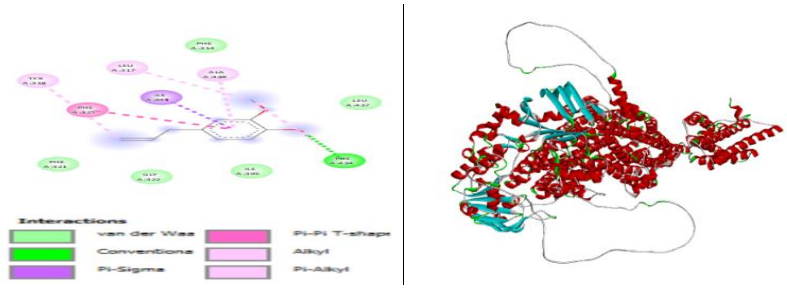


Figure 99: 2D diagram and 3D diagram of interaction HDAC1with Eugenol.

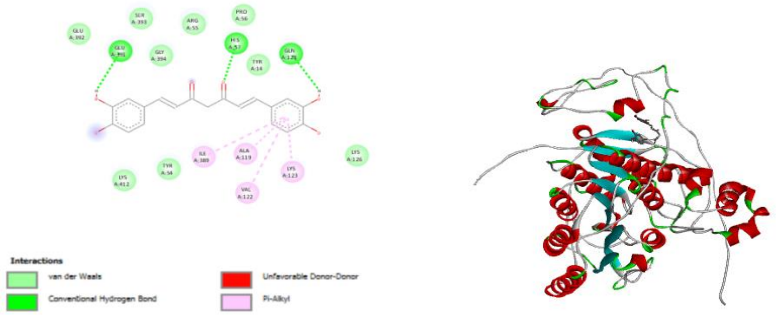


Figure 100: 2D diagram and 3D diagram of interaction HDAC1with Didmethyl Curcumin.

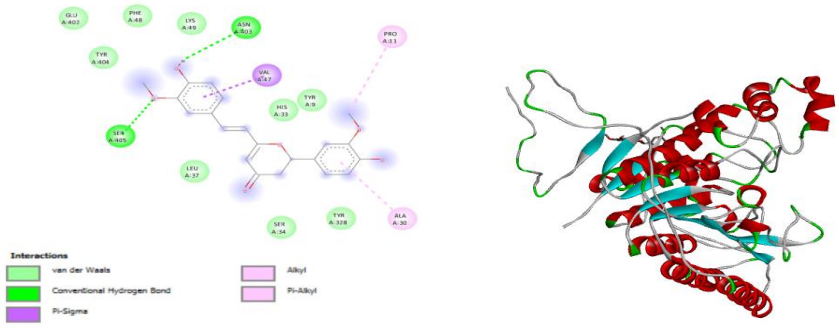


Figure101: 2D diagram and 3D diagram of interaction HDAC3with Cyclocurcumin.

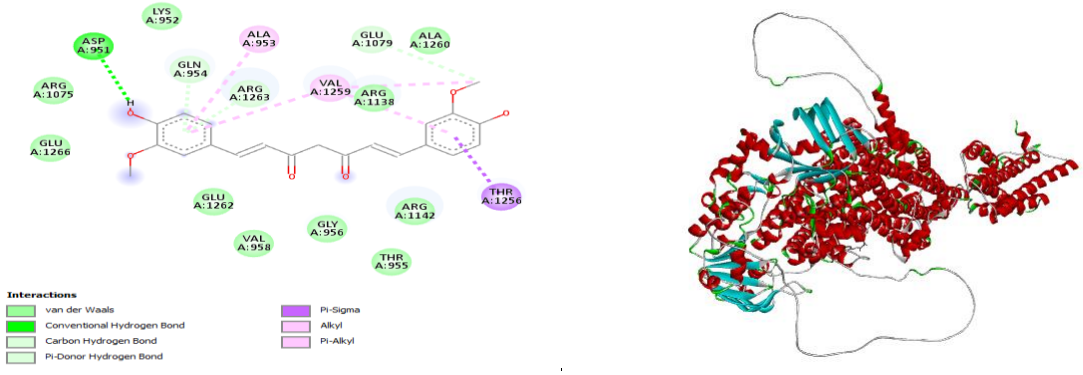


Figure 102: 2D diagram and 3D diagram of interaction ABCC1with Curcumin.

- We are searching in this study to get the best affinity between ligand and receptors with minimization of energy consumption in order to maintain the overall shape of the molecule throughout the docking process.

- The size of atoms is completely fixed, and molecules can only interact by adding more atoms, and this process creates new, different molecules.
- Changes in state occur by adding or removing energy from a substance, which affects the way the molecules interact with each other. Molecularly altered molecular switching compounds can be defined as molecular systems that can reversibly change their molecular structure in response to one or more external stimuli, such as radiation, magnetic or electric field, chemicals, gases, light, heat, mechanical effects, or Ph.
- A single molecule can take on many different configurations, each with a different energy. Given a starting configuration, geometric optimization changes the atomic coordinates to reduce the energy. In practice, this usually means finding the nearest local minimum; Energy minimization is a trade-off between the energy gained by forming new local bonds (to get rid of dangling bonds) with the energy lost due to bond stress caused by the new configuration.
- the BIOVIA DISCOVRY app work on showing the different bond binding in the interaction shows in previous figures ; bonds been Several : hydrogen bonds (H-H) ; Vander Waals (σ-)-(σ+); alkyl ; carbon hydrogen bond (C-H) and hydrophobic bond (H-O).
- **Energy of Bonding Orbital and Antibonding Orbital (HOMO and LUMO)**
- In both physical and organic chemistry, it's important to understand the state of atoms. We learn how atoms bond with each other to form molecules. Knowing the state of these bonds allows us to understand how the synthetic reactions of organic compounds proceed.
- The bonding of molecules is known as HOMO and LUMO. These are also called bonding orbital and anti-bonding orbital: the HOMO is involved in bonding orbitals, and the LUMO is involved in anti-bonding orbitals.
- Example of study : **Hesperidin – PARP-1**

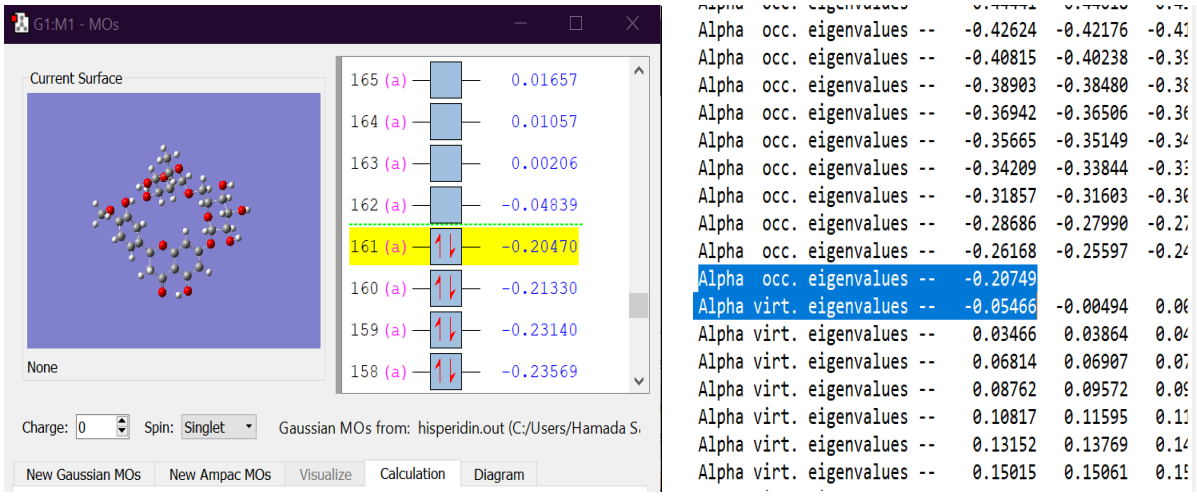


Figure 103: HOMO and LUMO energy orbital.

- After calculating for energy with excel Microsoft we have as results the figure below:

Alpha occ. eigenvalues	-0.20749
Alpha virt. eigenvalues	-0.05466

HOMO	LUMO	ENERGY OF GAP= LUMO-HOMO	(ev)
-0.20749	-0.05466	0.15283	4.1585043

Figure 104: Energy calculation of molecule Hesperidin.

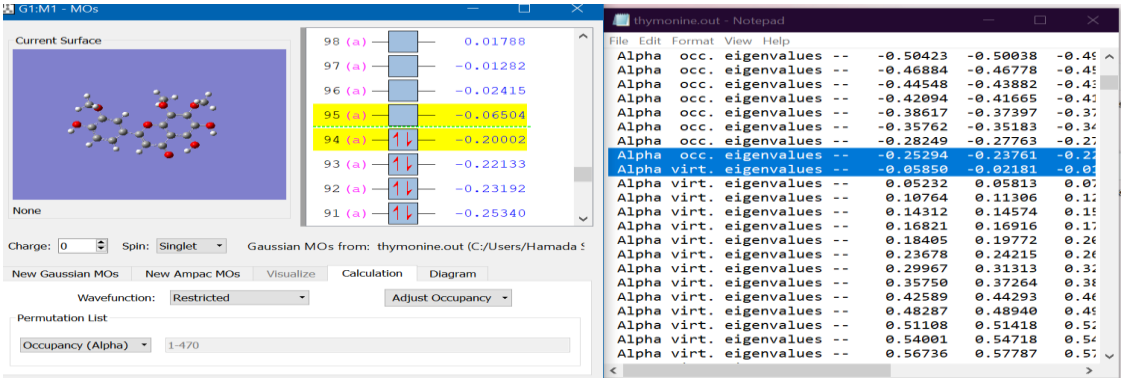


Figure 105: Energy calculation of molecule Thymonene.

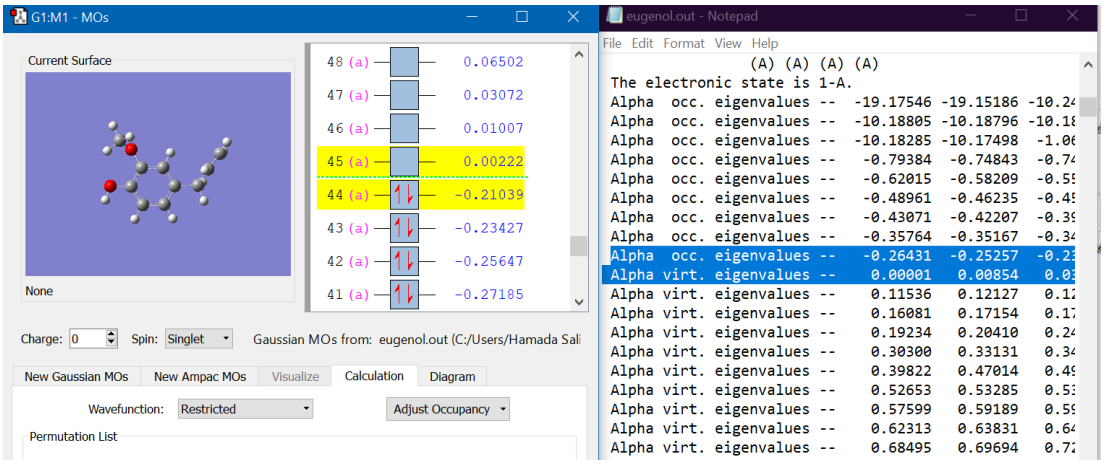


Figure 106: Energy calculation of molecule Eugenol.

- HOMO is the orbital that acts mainly as an electron donor and LUMO is the orbital that acts largely as an electron acceptor, and the gap between HOMO and LUMO, known as the HOMO-LUMO gap, characterizes molecular chemical stability.
- The HOMO is the highest energy molecular orbital that consists of electrons while the LUMO is the next highest energy orbital that is empty. The LUMO is the lowest energy place to excite an electron[151].
- As Prabhat Gautam in 2014 [148] said the energy difference between the HOMO and LUMO or HOMO-LUMO gap is the lowest energy excitation of electrons that exists in a molecule. The smaller a compound's HOMO–LUMO gap, the more stable the compound.so can attract the receptor for maximum time can take. [148]

Data bases

1) **–ProTox3:** from Protox we defined our LD50 and class of toxicity of our molecules also determinate the percentage of probability of safety in human cells showing in table24 below: see annex 01 and annex 03

Table24: The results of using ProTox3 databases.

MOLECULE	LD50 (mg/kg)	Class of toxicity	Probability of safety in human cells
Rutin	**5000**	**5**	**Neurotoxicity: :89% Hepatotoxicity:80%**
Hesperidin	**12000**	**6**	**BBB-barrier: 99% Carcinogenicity:93%**
Quercetin	**159**	**3**	**Neurotoxicity:89% Cardiotoxicity: 99%**
Apigenine	**3919**	**5**	**Neurotoxicity:86% Nutritional toxicity:56%**
Cirsilineol	**5000**	**5**	**Hepatotoxicity:83% Mutagenicity:93%**
Thymol	**640**	**4**	**Cardiotoxicity:99% Immunotoxicity:93%**
Thymonine	**5000**	**5**	**Neurotoxicity:83%**
Thymoquinone	**2400**	**5**	**Cardiotoxicity:75% Respiratory toxicity:78%**
Curcumin	**2000**	**4**	**Cytotoxicity:88% GABA receptor (GABAR):99%**
Cyclocurcumin	**1500**	**4**	**Immunotoxicity:98% Neurotoxicity:79%**
Didmethyl curcumin	**4000**	**5**	**Immunotoxicity:85%**
Eugenol	**1930**	**4**	**Respiratory toxicity: 98% Cardiotoxicity:89%**

2) **<u>Protein Ligand Interaction Profiler : PLIP</u>**

<u>Dashboard:</u> to read the deferent bonds showing with the PLIP

a-HDAC3:

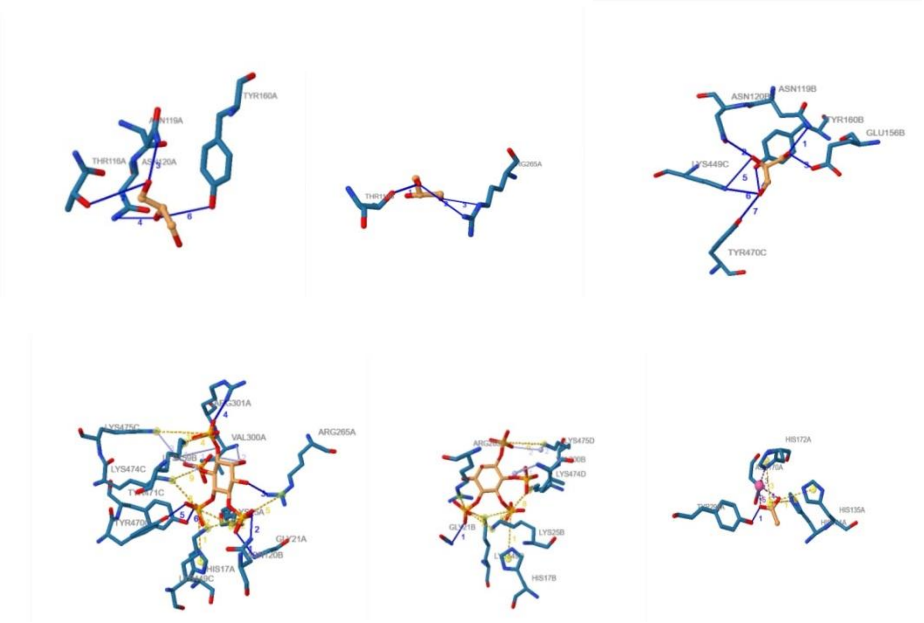


Figure 107: Molecular interaction of the best compounds selected from the database HDAC3, resulting after an ADME study.

b)- PARP-1:

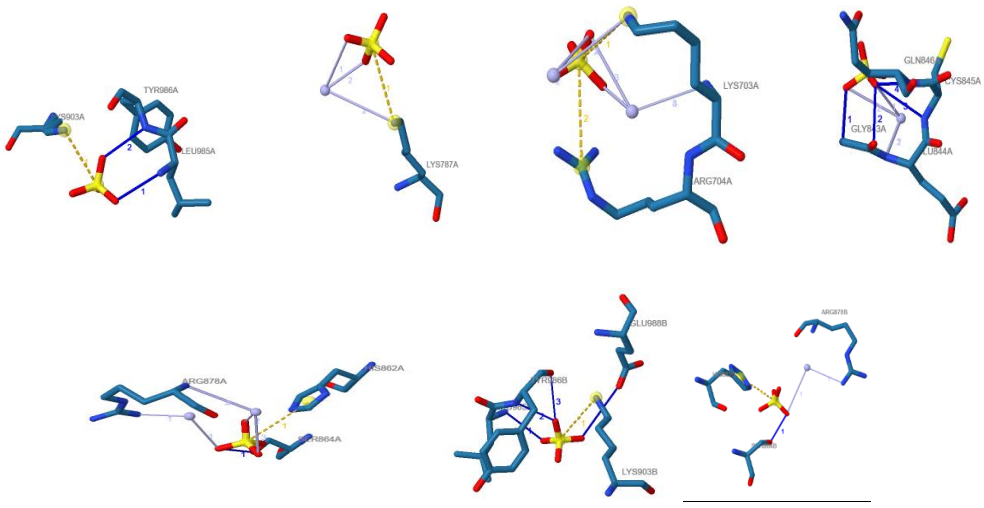


Figure 108: Molecular interaction of the best compounds selected from the database PARP-1, resulting after an ADME study

c)-ABCC1:

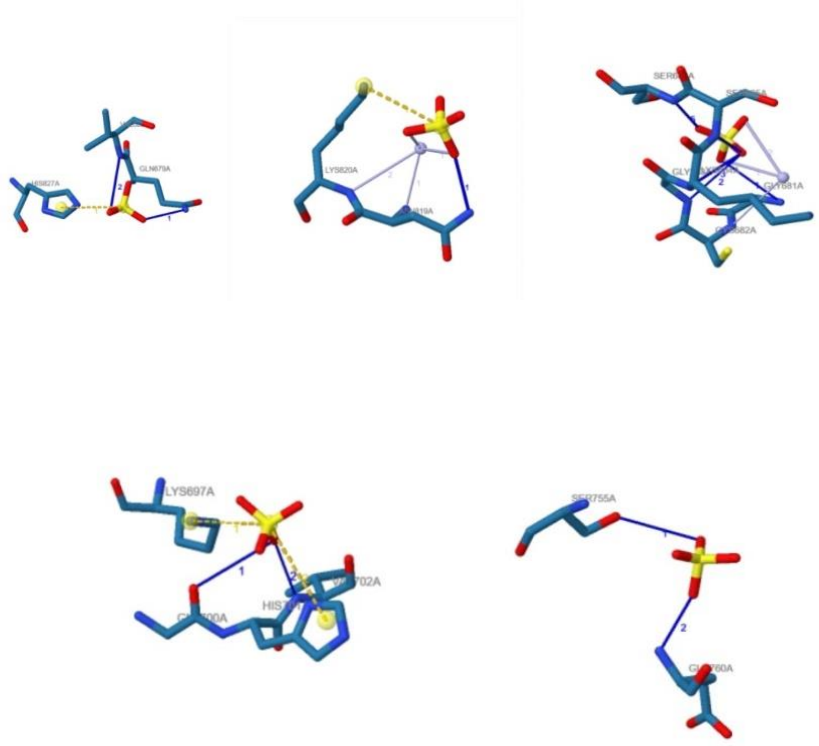


Figure 109: Molecular interaction of the best compounds selected from the databaseABCC1, resulting after an ADME study

d)-ABCB1:

Figure 110: Molecular interaction of the best compounds selected from the databaseABCB1, resulting after an ADME study

e)-HDAC1:

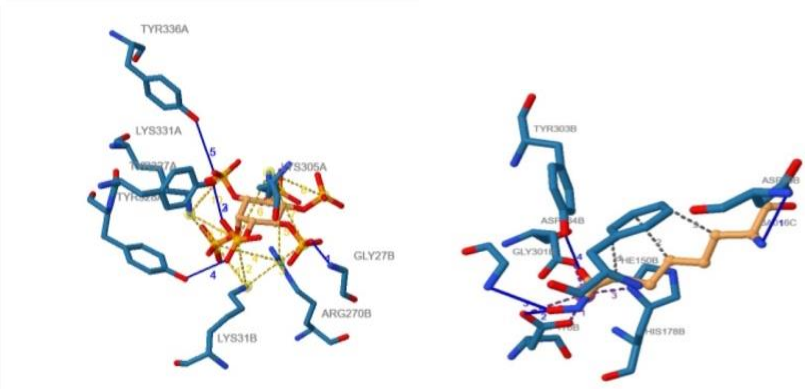


Figure 111: Molecular interaction of the best compounds selected from the database HDAC1, resulting after an Swiss ADME study.

- PLIP, the protein–ligand interaction profiler, detects and visualizes these interactions and provides data in formats suitable for further processing. In our study PLIP has proven very successful in applications ranging from the characterization of docking experiments to the assessment of novel ligand–protein complexes and it can give the proposition of the best interaction position in receptors. From the figure above, we can deduce the following :

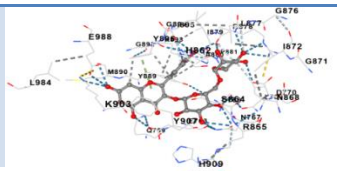
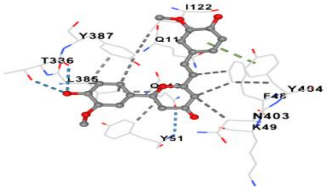
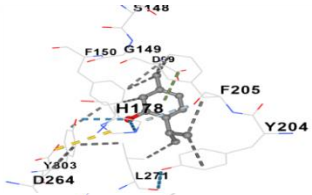
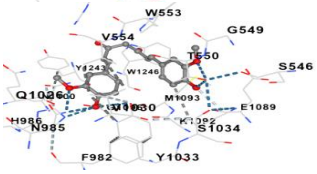
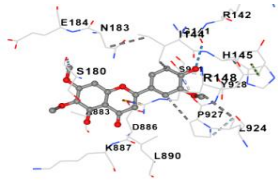
1. Hydrophobic Atoms: An atom is classified as hydrophobic if it is a carbon and has only carbon or hydrogen atoms as neighbors. This atoms are defended as the stable bonds that can change the form of interaction sites to inhibit it.

2. Charged Groups: The detection of charged groups is only exhaustive for the binding site, not the ligands. For proteins, positive charges are attributed to the side chain nitrogen's of Arginine, Histidine and Lysine.

3. Bonds donors and acceptors: Assuming that halogen atoms are not present in proteins (unless they are artificially modified), halogen bond donors are searched for only in ligands. Halogen bond acceptors in proteins are all carbon, phosphor or Sulphur atoms connected to oxygen, phosphor, nitrogen or sulfur.[151]

3) CB dock : The table show some of interaction between ligand and receptor ;table show the simulative amino acid at Biovia Discovery interaction

Table25 : Examples of interactions using CBdock.

Ligand+receptors	CBdock interaction view	simulative acid amine at biovia descovry interaction
Rutin-PARP-1		MET764; VAL762; LYS893;
Cyclocurmin-HDAC3		PHE48;LYS49; TYR404 ;THR386
Tymol-HDAC1		ASN95; VAL96; PHE150 ;HIS178
Curcumin-ABCC1		TYR1190; LEU244 ;ASN245
Cirsilineol-ABCB1		GLY141; HIS145; SER931; TYR928; SER180

- The advantage of CBdock is facility to establish an interaction without the need of app and time; see the structure in 3D form; give an overview of the different positions that the ligand takes to make the best effect.
- CBdock, on the other hand, does not allow you to see the 2D structure of the interaction and the nature of the bonds constructed for the stability of the interaction.

4- Protein-Protein interaction

- Protein interactions are fundamentally characterized as stable or transient, and both types of interactions can be either strong or weak. Stable interactions are those associated with proteins that are purified as multi-subunit complexes, and the subunits of these complexes can be identical or different.
- We constructed a PPI network as you see in the figure 122 as bitam also work on [152],using these common targets to study their interactions .We found that Protein-protein interactions (PPIs) are the physical contacts between two or more proteins and they represent complex biological functions like the activation systematic of groups of receptor interact each other [149].
- this result can show as that even in inhibition of the main receptor we can inhibit much of other receptor in same time so detect the best treatment can been taking
- The figure112 below present the result of receptor-receptor study and the effect of receptor each other during interaction: the five receptors of our study are indicated as activating receptor to each other .

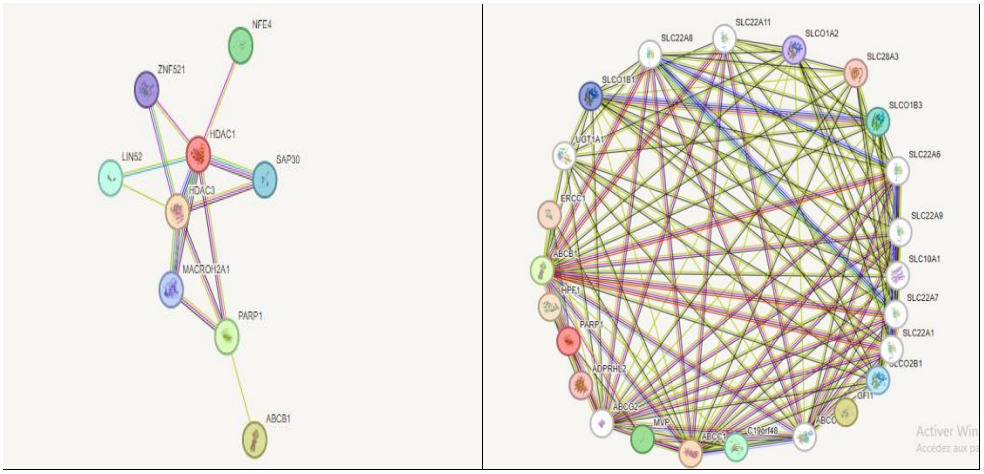


Figure 112: Protein-protein interaction between the studied receptors (PARP-1;HDAC1;HDAC3;ABCB1; ABCC1).

5)- Comparison between the comercial treatment (Olaparib) and our molecule (Apeginine)

a)- Molecule optimization

We use in this step Gaussian and Gaussview . we present results in the figure below:

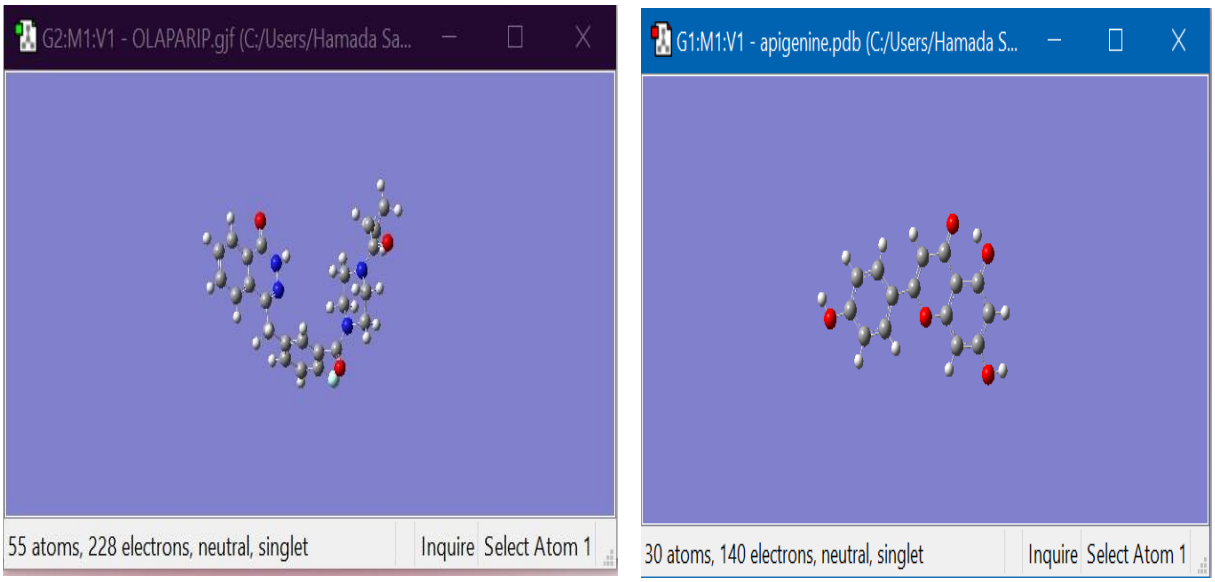
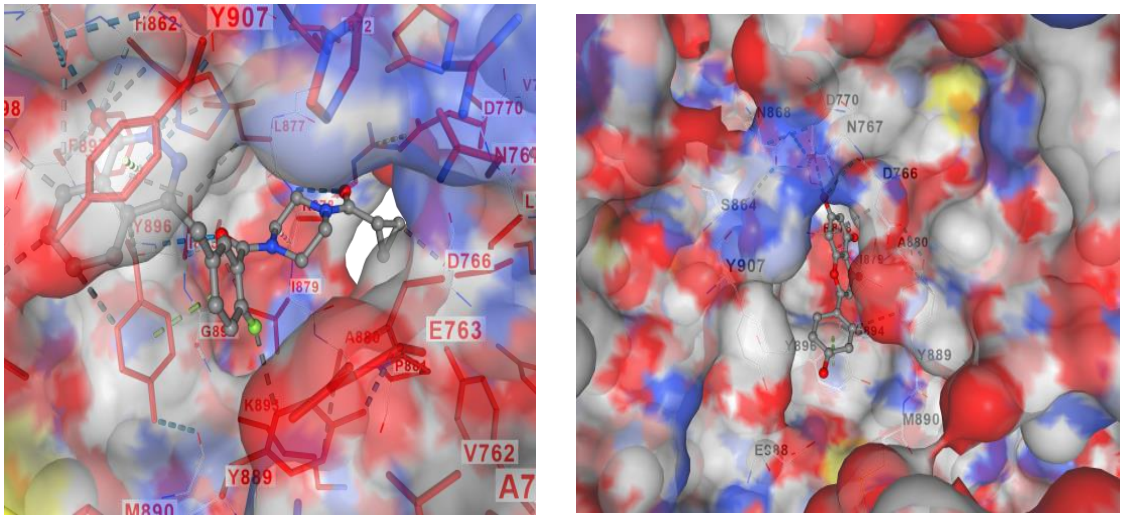


Figure113: Optimization of Olaparib and Apigenine melecules.

b)- CB dock interaction



(A) **(B)**

Figure 114: CB dock interaction: A/ OLAPARIB; B/ Apigenine.

c) – Amino acides chaines

Figure 115: Amino acid chain of CB dock interaction: A/ Olaparib ; B/ Apigenine.

- According to the figure 115 figures; we see that the commercial treatment (Olaparib) and our molecule (apigenine) takes the same interaction site.
- The interaction site take a similar 13 amino acid (ASP766. LEU769. ASP770. HIS862. GLU863. SER864. GLY876. LEU877. ARG878. ILE895. TYR896. TYR907. GLY988).
- We can see from the two chain of amino acid that the number of our molecules amino acid is bigger than Olaparib amino acid ; so we can say that our molecule apigenine inhibit the same site with more interaction stability.

Conclusion

In summary, after screening of more than 21 plants original from chlef State, Algeria. we have screened 25 phytochemicals from the three plants by molecular docking, in silico SwissADME and ProTox III .We selected the *citrus peels ; Thymus vulgaris ; and Curcuma longa*.

The best ethanolic extracts yield is *Citrus lemon peels* (24.10%) and *Thymus Vulgaris* have the best inhibitory activity against *Staphylococcus aureus ATCC6538* (Gram positive) with D=25mm. All the plants have antioxidant activity:IC50(*Citrus*)=0.198mg/ml,IC50 (*Curcuma*)=0.206mg/ml, IC50(*Thymus*)=0.187mg/ml GC-MS analysis indicated several components in extracts.

The study conducted a pharmacoinformatics analysis "*in silico*" of 12compounds from 25 bioactive compounds. *In silico* studies reveal that the inhibitor ligand apigenine with PARP-1, forming 8 amino acid bonds with ΔG = -8.8 kcal/mol and thymonine interacts with HDAC1 forming 7 amino acid bonds with ΔG = -7.6 kcal/mol .

Further studies should focus on evaluating the safety, pharmacokinetics and efficacy of oral emulgel formula. In a protein- protein interaction study showed that there receptors of our study have relationship between them.

General conclusion

In conclusion, the present study focused on the preparation of oral gel using bio molecules of *citrus peels; thymus vulgaris* and *curcuma longa* and its potential therapeutic application for colon disease and hearty failure . Targeting PARP-1; HDACs; and MDR receptors.

Through an in silico study, we have gained valuable insights into the molecular interactions and potential mechanisms of molecules compounds in targeting the pathological colon disease, such as rutin of *citrus peels* with PARP-1 receptor ; curcumin of *curcuma longa* with HDACS receptors and cirsilineol from *thymus vulgaris* with ABC MDR receptors.

During this work, we were interested in the extraction bio made of three plants for the formulation and their associations by maceration ethanoic to physio-chemical analysis , to determine their chemical compositions, and study of antibacterial activity of extracts and final product .

Obtaining extracts by maceration using ethanol 60% solvent remains a simple and effective method, and gives interesting yield of citrus peels as the heights value of 24.10%.

The evaluation of the antibacterial activity on gets the different sensibility of extract on different bacterial strains. This technique has shown that the extracts studied have an inhibitory effect against some strains tested; the best inhibitory activity was *Staphylococcus aureus (ATCC6538)* and *E.coli(ATCC 8739)* with significant zones of inhibition respectively more than 15 mm. *Thymus Vulgaris* have the best inhibitory activity against *Staphylococcus aureus ATCC6538* (Gram positive) with D=25mm. These strains showed significant sensitivity to the action of the extracts.

The anti-radical potential of the extract was determined by the DPPH method, the results of which show that the extract has good activity IC50 value was determined with 95.94% as the heights value to *curcuma longa* oxidant activity, so this plant contains molecules which are considered to be antioxidant agents. All the plants have antioxidant activity: IC50(Citrus)=0.198mg/ml , IC50(Curcuma)=0.206mg/ml, IC50(Thymus)=0.187mg/ml.

Our findings indicate that extracts possesses promising pharmacological properties that may contribute to the prevention and treatment of gastro-intestinal diseases.

The *in silico* study demonstrated that *citrus peels ; thymus vulgaris* and *curcuma longa* exhibits significant interactions with key molecular targets involved in colon pathology , including inhibition of cardiovascular receptors diseases.

In this processes. The interactions suggest that multi molecules of plants can make interactions with same receptor of disease so the interaction can regret the inhibition to the max.

The study conducted a pharmacoinformatics analysis *"in silico"* of 12 compounds from 25 bioactive compounds. We study more than 50 deferent molecule of plants to choose the molecule that gives the best interaction using *Insilco* study ; for there we get as molecules (rutin ; hesperidin; quercetin) from *citrus peels* with more than 8 amino acid in interaction(GLU763;TYR907;HIS909;TYR896;ASN868;ASP756;GLY894; HIS909);(curcumin; cyclocurcumin ; didmethylcurcumin , eugenol) *from curcuma longa* with more than 09 amino acid(LEU317;LEU317;TYR318;SER74;ALA30;SER405; GLN1139:GLU1079;VAL1259) and (thymol; thymoquinon;thymonine ; apeginin; cirsilineol) from *thymus vulgaris* with more than 6 amino acid(HIS172;PHE144;HIS172; HIS145;LEU890;LEU924). In silico studies reveal that the inhibitor ligand apigenine with PARP-1, forming 8 amino acid bonds with ΔG = -8.8 kcal/mol and thymonine interacts with HDAC1 forming 7 amino acid bonds with ΔG = -7.6 kcal/mol .

Optimization part of *Insilco* was the new step in this study of *Insilco*; the reason of this part was to fix the molecule from vibrant and moving to reduce energy of gap how get the best form of molecule so we get every time the best interaction of the best effect using of database can save more time and vast the study, like knowing LD50 from protox; getting fast view 3d of interaction in CB dock; the best form of interaction in PLIP view.

The comparison between the commercial product and our product in hot spot site shows the same site of interaction and the same amino acid, some of the same effects.

It is important to recognize that further preclinical and clinical studies are required to validate the therapeutic potential of our oral emulgel.

Further studies should focus on evaluating the safety, pharmacokinetics and efficacy of this formula. In a protein- protein interaction study showed that there receptors of our study have relationship between them.

Perspective:

- Stability tests to note the expiration date of the product.
- In vivo tests in big population.
- Accomplish this work by other studies to commercialize our product.

In conclusion, our work represents a novel approach to the development of an oral gel formulation of a bio extract product for the treatment of gastrointestinal diseases and heart failure.

In silico study supports the notion that we can study the dynamics of molecules in future investigations and arrived at details of the effect of molecules on diseases directly common.

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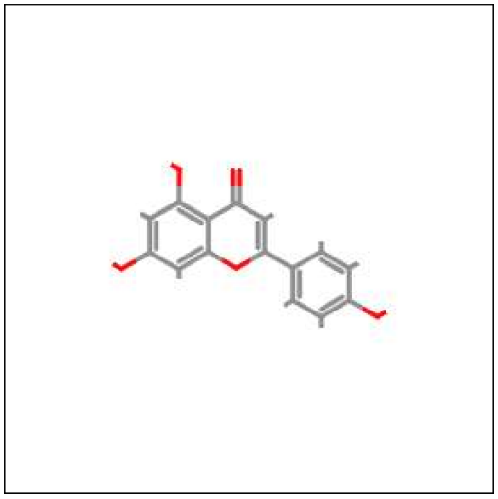
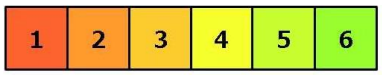
Annex 01: protox3 data bases



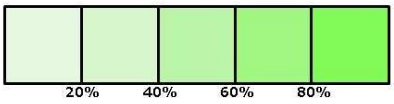
Predicted LD50: 2500mg/kg

Predicted Toxicity Class: 5



Average similarity: 81.24%

Prediction accuracy: 70.97%



Name	apigenin
Molweight	270.24
Number of hydrog bond acceptors	4
Number of hydrog bond donors	3
Number of atoms	20
Number of bonds	22
Number of rotable	1
Molecular refractiv	73.99
Topological Polar Surface Area	90.9
octanol/water part coefficient(logP)	2.58

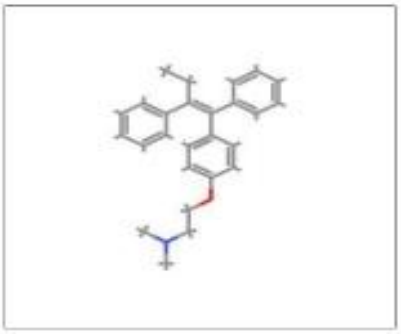
Toxicity Model Report

Classification	Target	Shorthand	Prediction	Probability
Organ toxicity	Hepatotoxicity	dili	Inactive	0.68
Organ toxicity	Neurotoxicity	neuro	Inactive	0.86
Organ toxicity	Nephrotoxicity	nephro	**Active**	0.60
Organ toxicity	Respiratory toxicity	respi	**Active**	0.75
Organ toxicity	Cardiotoxicity	cardio	Inactive	0.63
Toxicity end points	Carcinogenicity	carcino	Inactive	0.62
Toxicity end points	Immunotoxicity	immuno	Inactive	0.99
Toxicity end points	Mutagenicity	mutagen	Inactive	0.57

Classification	Target	Shorthand	Prediction	Probability
Toxicity end points	Cytotoxicity	cyto	Inactive	0.87
Toxicity end points	BBB-barrier	bbb	Inactive	0.51
Toxicity end points	Ecotoxicity	eco	**Active**	0.51
Toxicity end points	Clinical toxicity	clinical	Inactive	0.54
Toxicity end points	Nutritional toxicity	nutri	Inactive	0.55
Tox21-Nuclear receptor signalling pathways	Aryl hydrocarbon Receptor (AhR)	nr_ahr	**Active**	1.0
Tox21-Nuclear receptor signalling pathways	Androgen Receptor (AR)	nr_ar	Inactive	0.99
Tox21-Nuclear receptor signalling pathways	Androgen Receptor Ligand Bindin	nr_ar_lbd	Inactive	1.0
Tox21-Nuclear receptor signalling pathways	Aromatase	nr_aromatase	**Active**	0.61
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Alpha (ER)	nr_er	**Active**	1.0
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Ligand Bindinc	nr_er_lbd	**Active**	1.0
Tox21-Nuclear receptor signalling pathways	Peroxisome Proliferator Activated R Gamma)	nr_ppar_gamma	**Active**	1.0
Tox21-Stress response pathways	Nuclear factor (erythroid-derived 2 responsive element (nrf2/ARE)	sr_are	Inactive	0.99
Tox21-Stress response pathways	Heat shock factor response eleme	sr_hse	Inactive	0.99
Tox21-Stress response pathways	Mitochondrial Membrane Potential	sr_mmp	**Active**	1.0
Tox21-Stress response pathways	Phosphoprotein (Tumor Supresso	sr_p53	**Active**	1.0
Tox21-Stress response pathways	ATPase family AAA domain-contai	sr_atad5	**Active**	0.96
Molecular Initiating Events	Thyroid hormone receptor alpha (	mie_thr_alpha	Inactive	0.90
Molecular Initiating Events	Thyroid hormone receptor beta (T	mie_thr_beta	Inactive	0.78
Molecular Initiating Events	Transtyretrin (TTR)	mie_ttr	Inactive	0.97
Molecular Initiating Events	Ryanodine receptor (RYR)	mie_ryr	Inactive	0.98
Molecular Initiating Events	GABA receptor (GABAR)	mie_gabar	Inactive	0.96
Molecular Initiating Events	Glutamate N-methyl-D-aspartate r	mie_nmdar	Inactive	0.92
Molecular Initiating Events	alpha-amino-3-hydroxy-5-methyl- receptor (AMPAR)	mie_ampar	Inactive	0.97
Molecular Initiating Events	Kainate receptor (KAR)	mie_kar	Inactive	0.99

6/12/24, 1:47 PM ProTox-3.0 - Prediction of TOXicity of chemicals

Oral toxicity prediction results for input compound



Predicted LD50: 1190mg/kg

Predicted Toxicity Class: 4

1 2 3 4 5 6

Average similarity: 100%

Prediction accuracy: 100%

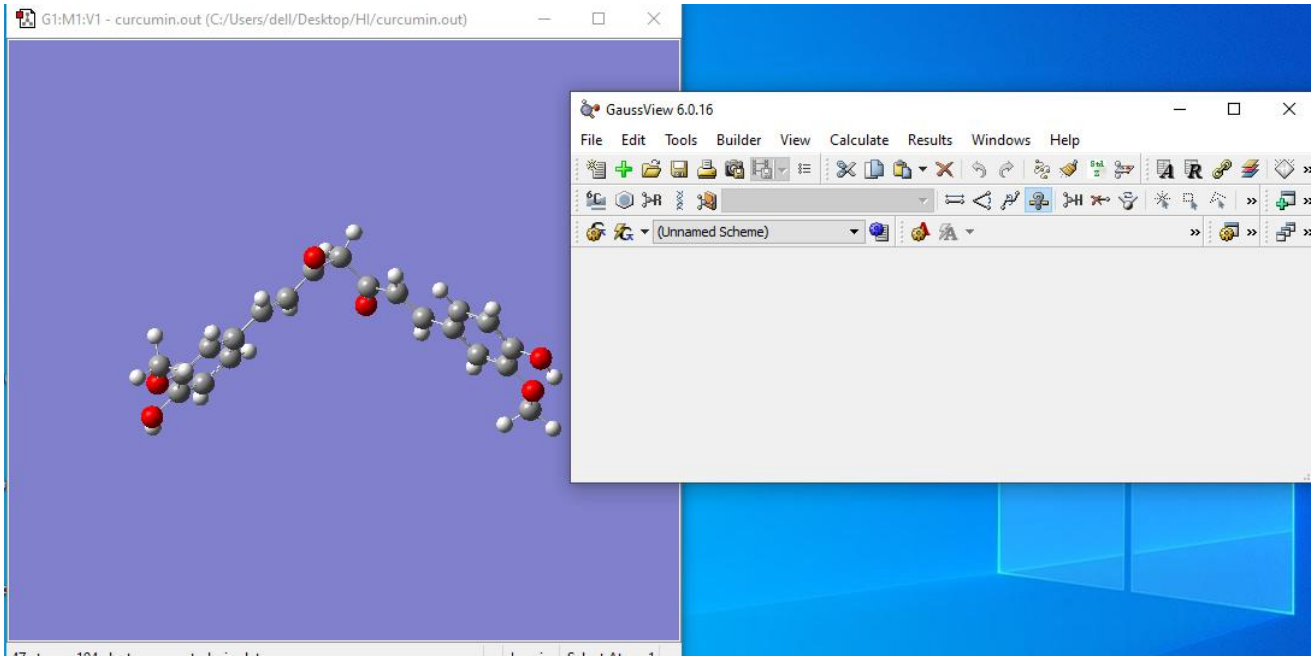
Name	hesperdin
Molweight	371.52
Number of hydrogen bond acceptors	2
Number of hydrogen bond donors	0
Number of atoms	28
Number of bonds	30
Number of rotable bonds	6
Molecular refractivity	119.72
Topological Polar Surface Area	12.47
octanol/water partition coefficient(logP)	6

Toxicity Model Report

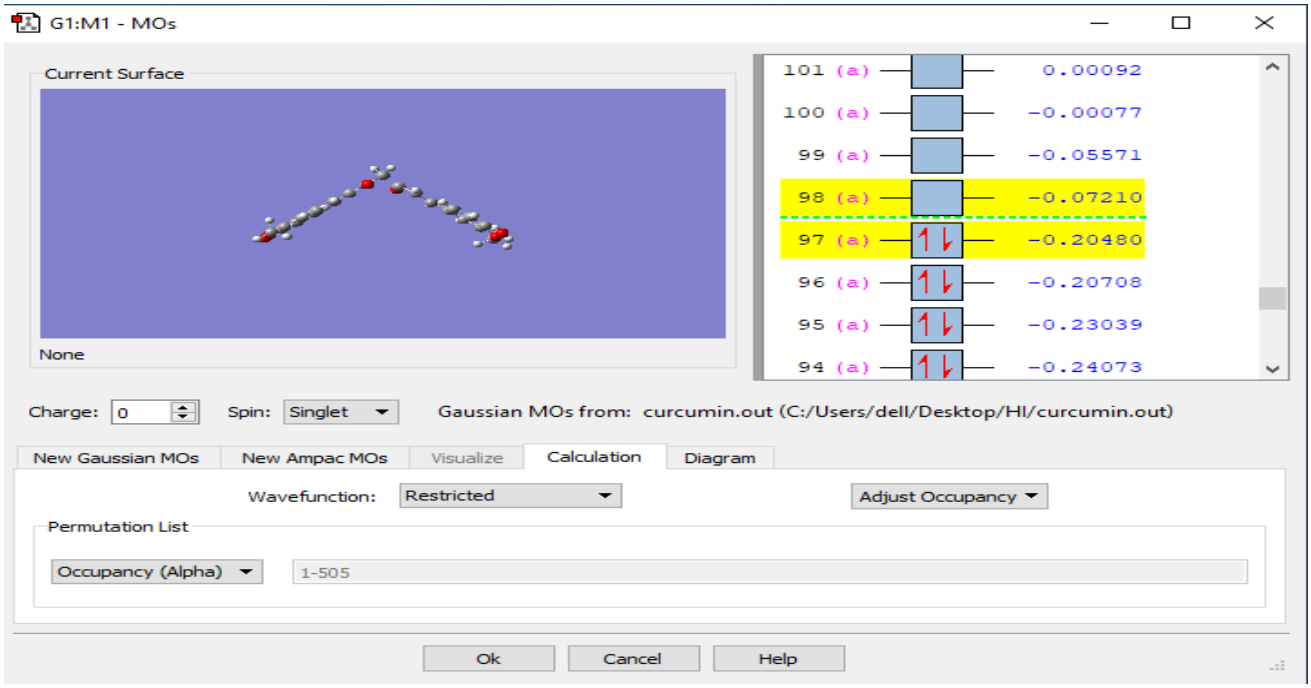
Copy Excel CSV PDF

Classification	Target	Shorthand	Prediction	Probability
Organ toxicity	Hepatotoxicity	dil	Active	0.69
Organ toxicity	Neurotoxicity	neuro	Active	0.87
Organ toxicity	Nephrotoxicity	nephro	Inactive	0.90
Organ toxicity	Respiratory toxicity	respi	Active	0.98
Organ toxicity	Cardiotoxicity	cardio	Inactive	0.77
Toxicity end points	Carcinogenicity	carcino	Inactive	0.62
Toxicity end points	Immunotoxicity	immuno	Active	0.96
Toxicity end points	Mutagenicity	mutagen	Inactive	0.97
Toxicity end points	Cytotoxicity	cyto	Inactive	0.93
Toxicity end points	BBB-barrier	bbb	Inactive	1.0
Toxicity end points	Ecotoxicity	eco	Active	0.73
Toxicity end points	Clinical toxicity	clinical	Inactive	0.56
Toxicity end points	Nutritional toxicity	nutri	Inactive	0.74
Tox21-Nuclear receptor signalling pathways	Aryl hydrocarbon Receptor (AhR)	nr_ahr	Inactive	0.97
Tox21-Nuclear receptor signalling pathways	Androgen Receptor (AR)	nr_ar	Inactive	0.99
Tox21-Nuclear receptor signalling pathways	Androgen Receptor Ligand Binding Domain (AR-LBD)	nr_ar_lbd	Inactive	0.99
Tox21-Nuclear receptor signalling pathways	Aromatase	nr_aromatase	Active	1.0
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Alpha (ER)	nr_er	Active	0.99
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Ligand Binding Domain (ER-LBD)	nr_er_lbd	Active	1.0
Tox21-Nuclear receptor signalling pathways	Peroxisome Proliferator Activated Receptor Gamma (PPAR-Gamma)	nr_ppar_gamma	Inactive	0.99
Tox21-Stress response pathways	Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE)	sr_are	Inactive	0.88
Tox21-Stress response pathways	Heat shock factor response element (HSE)	sr_hse	Inactive	0.88
Tox21-Stress response pathways	Mitochondrial Membrane Potential (MMP)	sr_mmp	Inactive	0.70
Tox21-Stress response pathways	Phosphoprotein (Tumor Suppressor) p53	sr_p53	Inactive	0.96
Tox21-Stress response pathways	ATPase family AAA domain-containing protein 5 (ATAD5)	sr_atad5	Inactive	0.99
Molecular Initiating Events	Thyroid hormone receptor alpha (THRα)	mie_thr_alpha	Inactive	0.90
Molecular Initiating Events	Thyroid hormone receptor beta (THRß)	mie_thr_beta	Inactive	0.78
Molecular Initiating Events	Transthyretin (TTR)	mie_ttr	Inactive	0.97
Molecular Initiating Events	Ryanodine receptor (RyR)	mie_ryr	Inactive	0.98
Molecular Initiating Events	GABA receptor (GABAR)	mie_gabar	Inactive	0.96
Molecular Initiating Events	Glutamate N-methyl-D-aspartate receptor (NMDAR)	mie_nmdar	Inactive	0.92
Molecular Initiating Events	alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptor (AMPAR)	mie_ampar	Inactive	0.97
Molecular Initiating Events	Kainate receptor (KAR)	mie_kar	Inactive	0.99
Molecular Initiating Events	Acetylcholinesterase (AChE)	mie_ache	Active	0.69
Molecular Initiating Events	Constitutive androstane receptor (CAR)	mie_car	Inactive	0.98
Molecular Initiating Events	Pregnane X receptor (PXR)	mie_pxr	Inactive	0.92

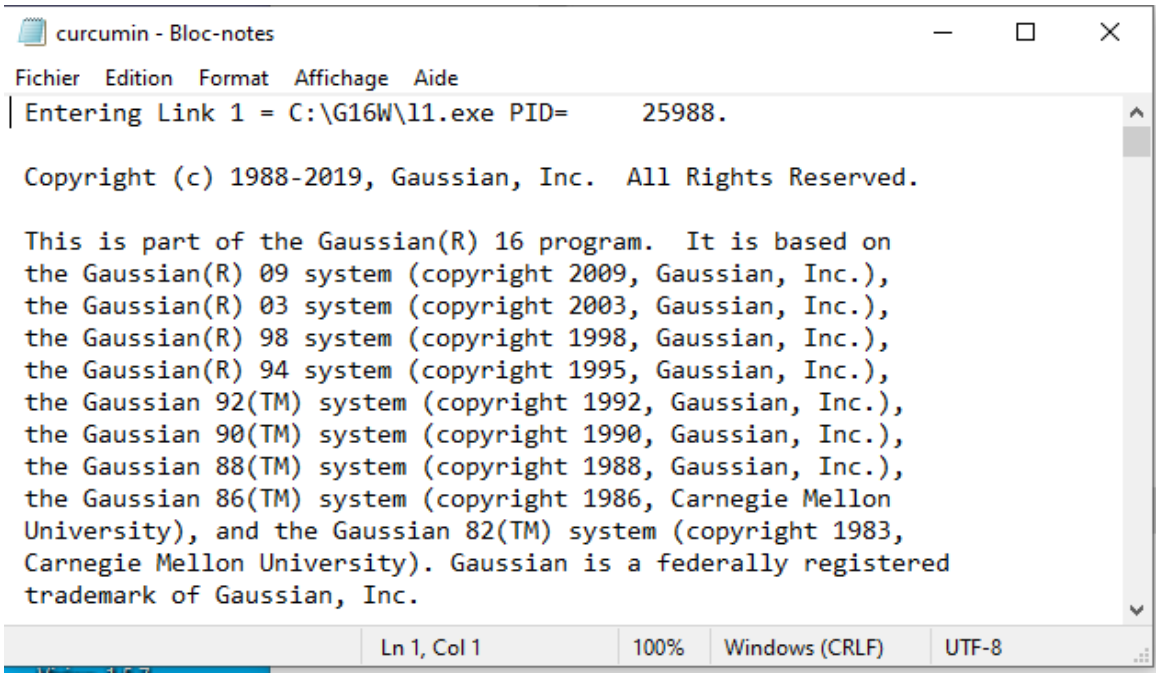
Annex 02: HOMO and LUMO and calculation of energy of GAP



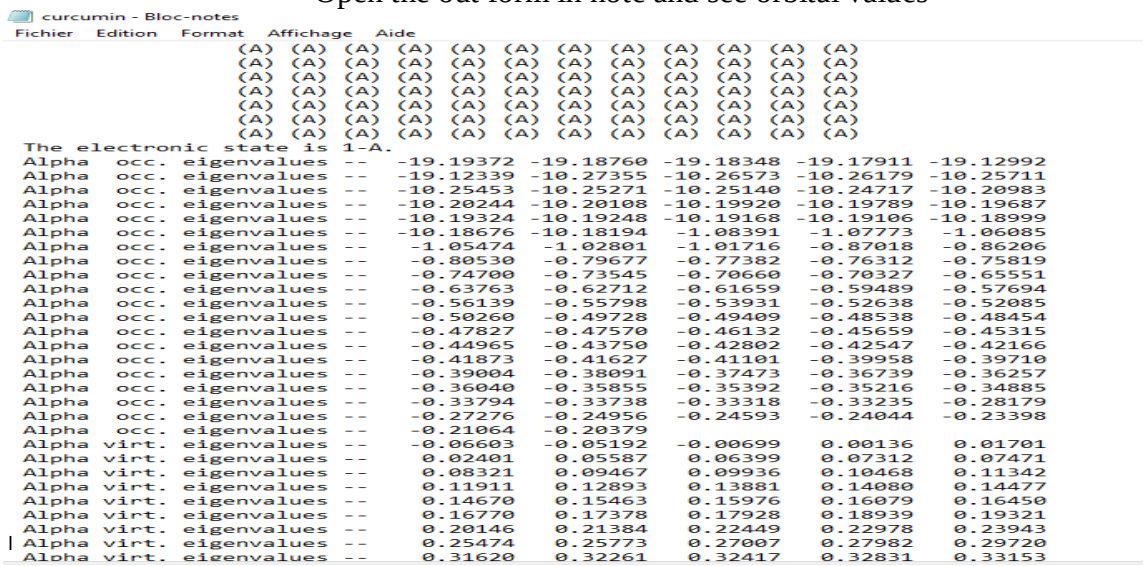
- Open molecule in GAUSSIAN app



- Choose energy and see ortibal sites



- Open the out form in note and see orbital values



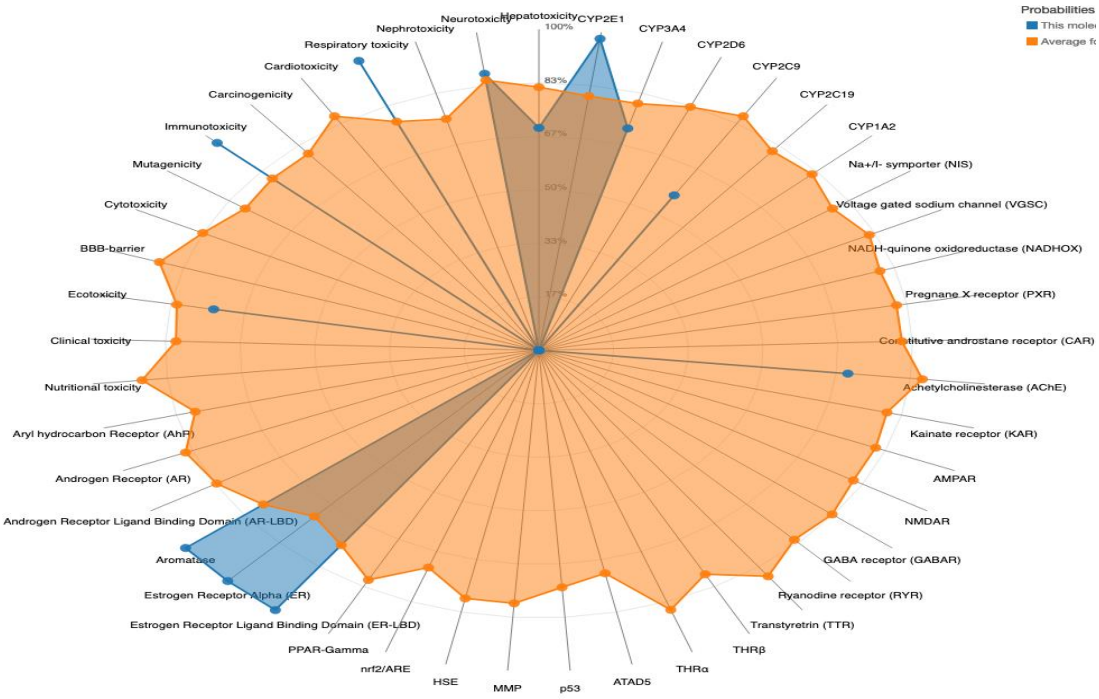
- Choose the orbital HOMO and LUMO
  - And calculate energy of GAP

E GP=LUMO-HOMO

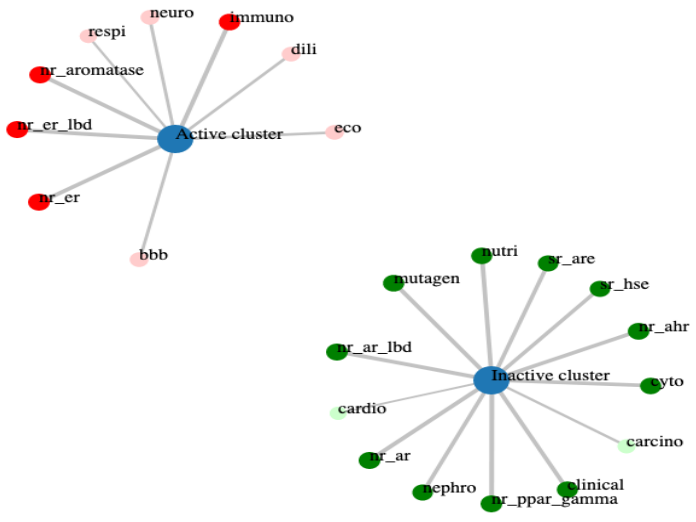
ANNEX 03 .

Rutin

The toxicity radar chart is intended to quickly illustrate the confidence of positive toxicity results compared to the average of its class

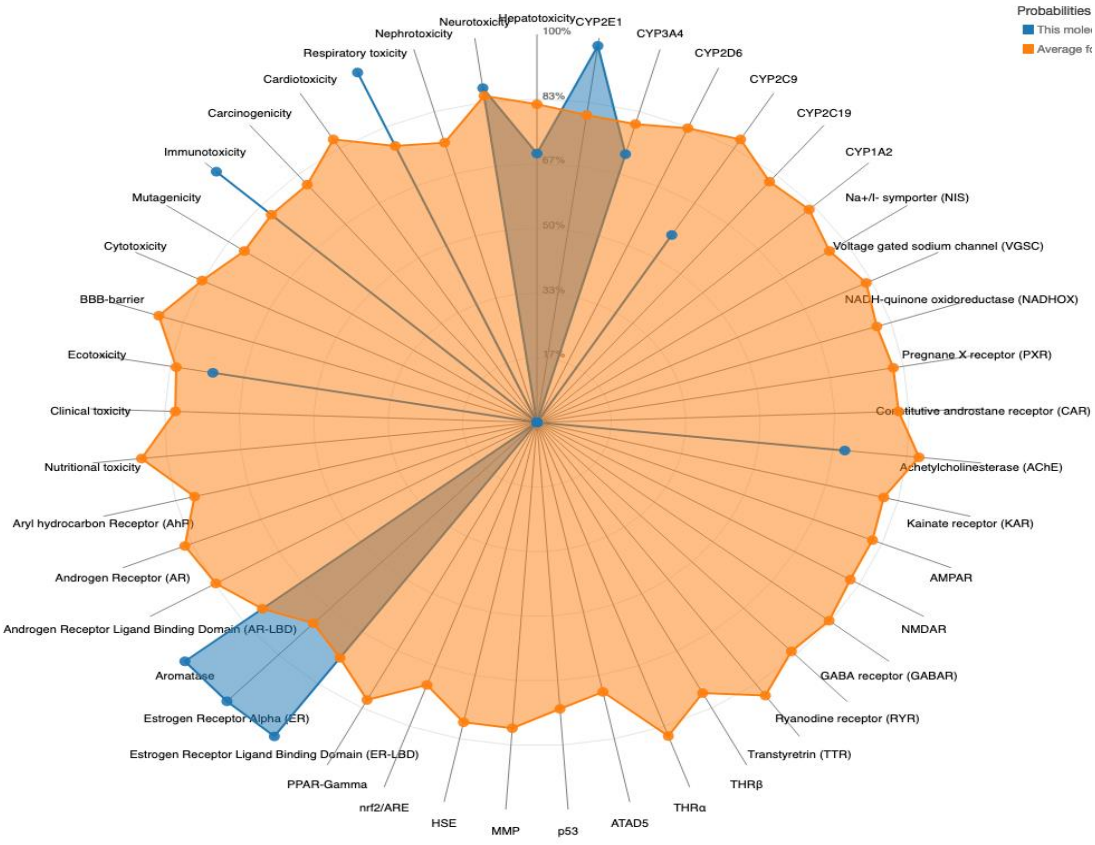


The network chart is intended to quickly illustrate the connection between the selected compound and predicted activities



Hesperidin

The toxicity radar chart



The network chart