République Algérienne Démocratique et Populaire
وزارة التعليم العالي والبحث العلمي
Ministère de l'Enseignement Supérieur et de la Recherche Scientifique
جامعة الجيلالي بونعامة خميس مليانة
Université Djilali Bounaâma de Khemis Miliana
Faculté des Sciences et de la Technologie
Département des Sciences de la Matière

Mémoire de fin d'étude

En vue de l'obtention d'un diplôme de ***Master*** *en Chimie*

Spécialité *: Chimie Pharmaceutique*

Thème

In silico and in vitro approach of anti-diabetic effect of cinnamon extracts

Devant le jury composé de :

– **M^eme M. Fizir**	**Présidente**
– **M^er M.Hammoudi**	**Encadreur**
– **M^er R.Moumen**	**Co-Encadreur**
– **M^eme A. Itatahine**	**Examinatrice**

Présenté par :

– M^elle SLIMI Khaoula

Année universitaire : 2021/ 2022

DEDICATION

I dedicate my dissertation work to you. A special feeling of gratitude to you, « Mom ». I don't want anything from you. I just want to hug you for a moment and tell you how much I miss you and how the days are without you. I would like to thank you for everything you had done for me over the past years, especially when I got diabetes, and how difficult it was for you to visit me in the hospital despite your severe illness. All your sacrifices will remain engraved in my whole memory until my death. Rabi yrhmak my dear sweetheart. I love you.

ACKNOWLEDGEMENT

First and foremost, I would like to thank God Almighty who has granted countless blessing, knowledge, and opportunity to me, so that I have been finally able to accomplish the thesis. Then I would like to thank my parents and family especially my aunt Fatiha for their tireless efforts and support in every path I take to achieve my dreams and goals.

Apart from the efforts of me, the success of this thesis depends largely on the encouragement and guidelines of many others. I take this opportunity to express my gratitude to the people who have been instrumental in the successful completion of this thesis.

I would like to give special thanks to Universitas Djilali bouneama (UDBKM) for giving me the opportunity to complete my studies successfully and I give many thanks to the Faculty of sciences and technology, Department of Material Sciences, administration staff and lecturers for their kindness and cooperation with me during my studies at the university.

Special thanks are extended to my supervisors M^{er} M. Hammoudi and M^{er} R. Moumen for the continuous support of my research, for their patience, motivation, enthusiasm, and immense knowledge. their guidance helped me in all the time of research and writing of this thesis.

I would also like to express my deepest appreciation to professor M^{eme} M. Fizir and professor M^{eme} A. Itatahine for serving as my committee members. I also want to thank you for your brilliant comments and suggestions, thanks to you.

Finally, I thank all my friends who have encouraged me and anyone who has contributed to the development of this work with a bit of advice or even a smile..

ملخص

الهدف من عملنا هو دراسة مساهمة القرفة في الأنشطة المضادة للبكتيريا، المضادة للأكسدة والمضادة للسكري (في دراسة السليكو). تم إستخلاص مركبات النبات بطريقتي التقطير المائي والنقع .أسفرت الدراسة عن استخلاص زيت أساسي بعائد 1.214٪، وقد أظهر تحليل الزيت الأساسي أن خواصه الفيزيائية والكيميائية تتوافق مع المعايير وتشير إلى عدة مكونات مسؤولة عن أنشطة بيولوجية معينة. أظهر نشاط مضادات الأكسدة بفحص DPPH قيم IC_{50} تبلغ 12.89 ميكروغرام /مل و41.68 ميكروغرام /مل للزيت الأساسي ومستخلص القرفة على التوالي، مقارنة بمضاد الأكسدة القياسي، حمض الأسكوربيك IC50 12.77 ميكروغرام/ مل. كشفت هذه الدراسة عن قدرة تخفيض مهمة للزيت الأساسي. أظهر النشاط المضاد للميكروبات أن السلالتين (الإشريكية القولونية والزائفة الزنجارية) سالبتا الغرام والبكتيريا العنقودية الذهبية موجبة الغرام التي تمت دراستهم كانو أكثر تثبيطًا بواسطة الزيت الأساسي ومستخلص الإيثانول بينما كانت بقية المستخلصات غير مثبطة. أظهرت الدراسة في المختبر لمكافحة مرض السكري أن زيت القرفة الأساسي له تأثيرات مثبطة بنسبة 83.7٪ ضد نشاط α-amylase مع قيمة IC_{50} 22.8 ميكروغرام / مل بينما لوحظ في دراسة السيليكو أنه من بين 27 مركبًا مختلفًا تم تحديدها، كانت سبعة مركبات نشطة بيولوجيًا وتفاعلت بشكل كبير مع PPARα/γ في عملية محاكاة الالتحام الجزيئي.

الكلمات المفتاحية :القرفة، زيت أساسي، مضاد البكتيريا، مضاد الأكسدة، مضاد السكري، DPPH، في السيليكو، PPARα/γ.

Résumé

L'objectif de notre travail est d'étudier la contribution de la cannelle dans les activités antibactérienne, antioxydant et antidiabétique (Étude in silico). Les composés de la plante ont été extraits par la méthode d'hydrodistillation et la macération. L'étude a abouti à l'extraction d'une huile essentielle (HE) avec un rendement de 1,214 %, l'analyse de l'HE a montré que ses propriétés physico-chimiques sont conformes aux normes et indiquent plusieurs composants responsables de certaines activités biologiques. L'activité antioxydant par dosage DPPH a montré des valeurs IC_{50} de 12,89 µg/ml et 41,68 µg/ml pour l'HE et l'extrait de cannelle respectivement, par rapport à l'antioxydant standard (IC_{50} ~ 12,77 µg/ml). Cette étude a révélé une capacité réductrice très importante pour l'HE. L'activité antimicrobienne a montré que les deux souches (E. coli et Pseudo.aeruginosa) Gram-négatif et la bactérie S. aureus gram-positive étudiées étaient plus inhibées par l'HE et l'extrait de l'éthanol alors que le reste des extraits n'était pas inhibé. Une étude antidiabétique in vitro montre que l'HE de cannelle a 83.7% d'effets inhibiteurs contre l'activité α-amylase avec une valeur IC_{50} de 22,8 µg/ml, Alors qu'il a été noté dans l'étude in silico que parmi 27 composés différents identifiés, sept composés étaient biologiquement actifs et ont fortement interagi avec PPARα/γ dans les simulations d'amarrage moléculaire.

Mots clés : huile essentielle, antibactérienne, antioxydant, DPPH, antidiabétique, in silico, PPARα/γ.

Abstract

The objective of our work is to study the contribution of cinnamon in the antibacterial, antioxidant and antidiabetic activities (in siluco study). The study resulted in the extraction of an essential oil (EO) with a yield of 1.214 %. The analysis of the EO has shown that its physico-chemical properties comply with the standards and indicate several components responsible of certain biological activities. The antioxidant activity by DPPH assay showed IC_{50} values of 12.89 µg/ml and 41.68 µg/ml for EO and extract of cinnamon respectively, compared with standard anti-oxdiant (IC_{50}~ 12.77 µg/ml). This study revealed a very important reducing capacity for the cinnamon essential oil (CEO). Antimicrobial activity showed that the two strains (E. coli and Pseudo.aeruginosa) Gram-negative and the gram-positive S. aureus bacteria studied were more inhibited by CEO and ethanol extract while the rest of the extracts were uninhibited. In vitro anti-diabetic study shows that the CEO has 83.7 % inhibitory effects against α-amylase activity with an IC_{50} value 22.8 µg/ml while it was noted in the in silico study that out of 27 different compounds identified, seven compounds were biologically active and have highly interacted with PPARα/γ in molecular docking simulations.

Key words : essential oil, antibacterial, antioxidant, DPPH, antidiabetic, in silico, PPARα/γ

General Introduction

General introduction

At present, The development of new therapeutics is an expensive and time consuming process. Normally it will take years to get the newly developed drug for the treatment. Drug repurposing is an efficient strategy in medicinal chemistry to bring faster and effective solutions to the unmet medical needs **[1].** Given the rapid development of diabetes throughout the world, repurposing pharmaceuticals originating from natural sources is seen as a critical therapeutic method for its treatment.

Diabetes is a chronic metabolic disorder characterized by elevated blood glucose level resulting from a defect in insulin secretion, insulin action, or both. Long-term complications of diabetes mellitus (DM) include retinopathy, nephropathy, neuropathy, periodontal disease, sexual dysfunction, and increased risk of cardiovascular diseases. Over 90% of all DM cases are type 2 DM (T2DM).

Management of T2DM involves the use of modern antidiabetic drugs. However, the anti-diabetic drugs are expensive, have significant adverse effects and fail to effectively control the glycemia. Herbal medicines become the alternative way for DM management as they have few adverse effects and readily accessible to the majority of the populations **[2].**

In vivo and in vitro studies constitute the experiments for simulating them in the wet lab whereas in silico (computer aided) methods do not need animal models or enzymatic methods **[3].** In silico computational models are one of the fastest and newest approaches that are involved in the process of drug discovery and development. Due to the accelerated development in the field of pharmaceutics, an increasing demand has arisen regarding the development of more reliable techniques for predicting the pharmacokinetic properties of the new drug candidates as a way to reduce costs and the time that are usually associated with the development of new drugs **[4].**

In our study we have chosen Cinnamon, which is among the many herbal medicines used for the treatment of DM where clinical trials evaluated the efficacy of cinnamon in glycemic control among people with T2DM **[2].**

In this context we aim to answer the following questions :

- has cinnamon the ability to lower blood sugar and helping manage common diabetes complications ?
- What does cinnamon contain that is responsible for its antidiabetic properties ?

- Is it possible to assess the pharmacological qualities of our plant through molecular docking simulations (in silico approach), and what are the possible mechanisms of glucose metabolism modulation by cinnamon essential oil ?

What reinforces our thesis, is the Studies before on cinnamon essential oil which demonstrate improvement of fasting blood glucose, fasting insulin, and improvement in both anatomy and function of kidney and liver cells. Improvement in enzyme function, both enzymes involved directly in glucose metabolism and enzymes involved in excretion, was also consistently noted. Studies including cinnamaldehyde also showed improvement of fasting blood glucose, increased insulin sensitivity, decreased appetite, and both up- and down-regulation of myriad proteins associated with glucose metabolism **[5].**

The following aspects are covered in our thesis :

- First part deals with the literature review which includes 3 chapters :
  1. General information about cinnamon ;
  2. diabetes mellitus ;
  3. silico approach ;
- Second part defines the experimental study which includes 3 parts :
  1. Extraction methods and physico-chemical characterizations of cinnamon extract ;
  2. Study of the antibacterial and antioxidant activity of Cinnamon extracts ;
  3. In vitro and in silico study of cinnamon extract on antidiabetic activity.
- The last part contains the general conclusion

Chapter 1

Cinnamon

CHAPTER 1 :Cinnamon

1.1. Introduction

Cinnamon is a spice mainly obtained from cinnamon bark and leaves. It is an evergreen tree belonging to the Lauraceae family **[6].** The bark of various cinnamon species is one of the most important and popular spices used worldwide not only for cooking but also in traditional and modern medicines. Overall, approximately 250 species have been identified among the cinnamon genus, with trees being scattered all over the world **[7, 8].** There are two types of cinnamon in the world market. The first is the inner bark of C. zeylanicum blume which is called Ceylon cinnamon, Sri Lankan cinnamon or true cinnamon (fig 1.1) and is grown only in Sri Lanka, Madagascar and the Seychelles. The other type is generally known as cassia or cassia cinnamon, and is derived mainly from two other species of the genus Cinnamomum: C. cassia (L) from China and Vietnam (fig 1.2) **[9].** Cinnamon is mainly used in the aroma and essence industries due to its fragrance, which can be incorporated into different varieties of foodstuffs, perfumes, and medicinal products **[10].**

Figure 1.1. Cinnamomum verum, from Koehler's Medicinal-Plants (1887) and Close-up view of raw cinnamon bark

Figure 1.2. Cinnamomum cassia from Koehler's Medicinal-Plants (1887) Dried cassia bark

1.2. Etymology

There are several possible derivations for the name cinnamon. One school of thought is that it comes from Phoenician through the Greek word kinnámōmon, meaning sweet wood **[11].**

Table 1.1 shows some of the appellation of cinnamon according to several languages of the world.

Table 1.1 : Different appellations of cinnamon in several languages **[11].**

Language	Local name
Arabic	Qurfar
English	Cinnamon
French	Cannelle
Hindi	Elavagnum, vayana, karu va, karuwa, twak
Indonesian	Kayu manis
Malay	kayu manis
Spanish	Kanelero, canela legítima,canela
Luganda	Budalasini
Sri Lankan	Kurundu

1.3. History

Historically, the presence of cinnamon has attracted foreign invaders to Sri Lanka (previously known as Ceylon). Arabs were involved in the trading of cinnamon across the globe until the 10th– 15th century and were careful to keep the origin of the product a closely guarded secret **[12]** Gaining access to cinnamon was a prime motivation for the Portuguese to invade Sri Lanka in the early 16th century **[13, 14].** The Dutch commenced systematic cultivation of cinnamon in plantations after they captured the island in the mid 17th century and this was prompted when the Sri Lankan king obstructed the collection of cinnamon from the forests **[13].**

After the British captured Sri Lanka in 1796, cinnamon exports to Europe continued, with the British East India Company being the main exporter **[15].** Because of high export duties imposed by the Dutch, there was a significant reduction in the export of Ceylon cinnamon, which was replaced by the Dutch, there was a significant reduction in the export of Ceylon cinnamon, which was replaced by cheaper Cassia cinnamon. Sri Lanka still continues to be an important supplier of Ceylon cinnamon to customers throughout the world cheaper Cassia

cinnamon. Sri Lanka still continues to be an important supplier of Ceylon cinnamon to customers throughout the world.

1.4. Botanical Description

Although the *Cinnamomum* genus consists of many species of trees and shrubs distributed in the world, it belongs to one family (Laureaceae family). Regarding the cinnamon trees system :

1.4.1. Taxonomic Hierarchy

- **Kingdom :** *Plantae*
- **Subkingdom :** *Viridiplantae*
- **Infrakingdom :** *Streptophyta*
- **Super division :** *Embryophyta*
- **Division :** *Tracheophyta*
- **Sub division :** *Spermatophytina*
- **Class :** *Magnoliopsida*
- **Super order :** *Magnolianae*
- **Order :** *Laurales*
- **Family :** *Lauraceae*
- **Genus :** *Cinnamomum*

1.4.2. Description of cinnamon

Cinamomum tree is evergreen, grows to around 10 m (30 ft). Its branches are strong and bark is smooth and yellowish in colour. It has leathery leaves, 11 to 16 cm (4.5 to 6.25 in) Cinnamon 169 long, with pointed tips. The leaves are dark green on top and light green at the bottom. The inconspicuous yellow flowers with a disagreeable odour, which are tubular with 6 lobes, grow in panicles (clusters) that are as long as the leaves. The fruit is a small, fleshy berry, 1 to 1.5 cm (0.25 to 0.5 in) long, that ripens to black, partly surrounded by a cup like perianth (developed from the outer parts of the flower) **[16].**

1.5. Cultivation and Consumption

Since both cinnamon and cassia have similar requirements of soil, agro climate, and, agricultural practices, the cultural practices described below are applicable to both the crops **[17].**

- ✓ Soil and Climate: Both cinnamon and cassia fourish in humid tropical regions receiving an annual rainfall of 150–250 cm with an average ambient temperature of 27 degree centigrade ;
- ✓ They can come up in places from almost sea level to an elevation of 1000 m above msl **[17] ;**
- ✓ Soil conservation, weed control, manuring, pest and disease management, and training of the plant are important aftercare operations **[18] ;**
- ✓ The stems are ready for the first harvest in about 2.5–3 years, when the bark turns a brown color ;
- ✓ The subsequent shoots are ready to be harvested in about 1.5 years. Traditionally, harvesting takes place when the new leaves turn a light green color because, at this stage, the bark is easier to peel ;
- ✓ Peeling is difficult at times when plants bear red-colored immature leaves, flowers or fruits, and during dry periods **[19] ;**
- ✓ Stems are cut during rains to facilitate peeling. The best time for peeling is when new fushes and leaves are hardened after rains.

The following table represents the different world cinnamon production areas :

Table 1.2 : Top 5 cinnamon producing countries **[20]**

Producing country	Production in tons	Percentage of world production
Chine	47000	44.00%
Indonesia	39000	37.00%
Sri Lanka	12200	11.00%
Vietnam	6000	6.00%
Madagascar	1500	1.00%

1.6. Parts Used

Bark (quills), bark powder, bark essential oil, leaf essential oil, and oleoresin. The part of the plant which is most commonly used for the production of cinnamon includes the thin inner bark of the evergreen tree. Cinnamon oil and powder are commonly acquired from the dried bark of the plant **[21, 22].**

Figure 1.3. Part of cinnamon bark tree

1.7. Active Constituents

Cinnamon bark contains essential oil (up to 2%), with cinnamaldehyde (60–80%) as the major constituent. Other minor constituents are trans-cinnamic acid, o - methoxycinnamaldehyde, eugenol, and monoterpenoids. The bark also contains procyanidins, diterpenes, phenylpropanoids, mucilage, and polysaccharides. The leaf oil has eugenol (70–90%) as the major constituent. The methanol extract had tannins, fl avonoids, glycosides, terpenoids, coumarins, and anthraquinones **[23].**

1.8. Cinnamon Oil Extraction Methods

The essential oils of cinnamon can be extracted using different extraction techniques used to isolate volatile constituents. The methods given below can be employed to extract the essential oils from cinnamon in small scale. Cinnamon stem bark, leaf, root, and fruits are the common parts used for extraction of essential oils, and chemical constituents in essential oils of the four parts are different from each other **[24].**

Among the different extraction processes (Fig1.5), we'll mainly quote the most used of them ;

1.8.1. Hydro-distillation :

Hydro-distillation is relatively cheap, simple, ecofriendly, and produce great oil quality **[25].** The drawback of using hydro-distillation is the slow extraction process. Furthermore, it usually requires four hours to extract the essential oil as it took three hours to obtain the peak yield percentage (fig 1.4) **[26, 27, 28].**

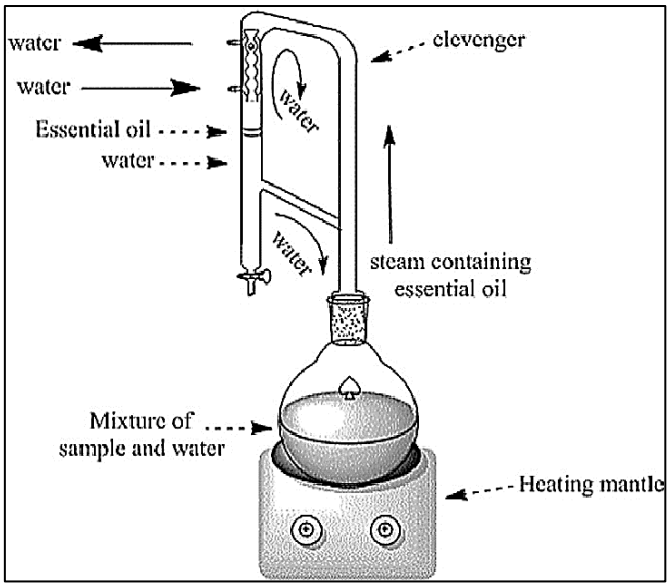


Figure 1.4. Essential oil extraction by hydrodistillation

1.8.2. Solvent extraction :

Common solvents used for bioactive compounds extraction are ether, methanol and hexane. It is often used for heat sensitive and fragile material such as flowers which would not be able to handle the heat of high temperature extraction such as steam distillation. It is one of the simplest methods for isolating volatile compounds, but it uses a large amount of solvent **[29].**

Figure 1. 5. Essential oil extraction methods

1.9. Basic pharmacological properties of cinnamon

Cinnamon is a medicinal plant and has been used as a traditional remedy around the world. The potential pharmacological effects of cinnamon in modern medicine such as antidiabetic, anti-inflammatory, antioxydant and antimicrobial activities are summarized As following :

1.9.1. Pharmacological properties :

Cinnamon is well known in Western natural medicine as a natural species with healing properties. Nowadays, it is also reported as a plant with special attributes used to treat many disorders (Fig1.6).

It is a traditional remedy for dyspeptic conditions like flatulence, gastrointestinal spasms, loss of appetite, and diarrhea. It is also used to improve the flavor of other nonmedicinal products. In folk medicine it is used to treat colds, nausea, inflammation, rheumatism, vomiting, and menstrual disorders. it has carminative and astringent properties.

The available in vitro and animal in vivo evidence suggests that cinnamon has anti-inflammatory **[30],** antimicrobial **[31, 32],** antibacterial **[33],** antioxidant, antitumor, cardiovascular, cholesterol-lowering, and immunomodulatory effects **[34, 35, 36, 37].**

Cinnamaldehyde from cinnamon displayed significant antiproliferative effects on human colon cancer cells in concentration and kinetic-dependent manners [38].

The essential oil of cinnamon showed promising larvicidal and repellant agent against C. tritaeniorhynchus and A. subpictus **[39].**

Cinnamon oil had a regulative role in blood glucose level and lipids, and improved the function of pancreatic islets and thus may be useful in the treatment of type 2 diabetes mellitus **[40].**

Cinnamon bark is effective in the alleviation of diabetes because of its antioxidant and insulin-potentiating activities, and other activities, and this is attributed to the water-soluble polyphenolic oligomers **[41, 42].**

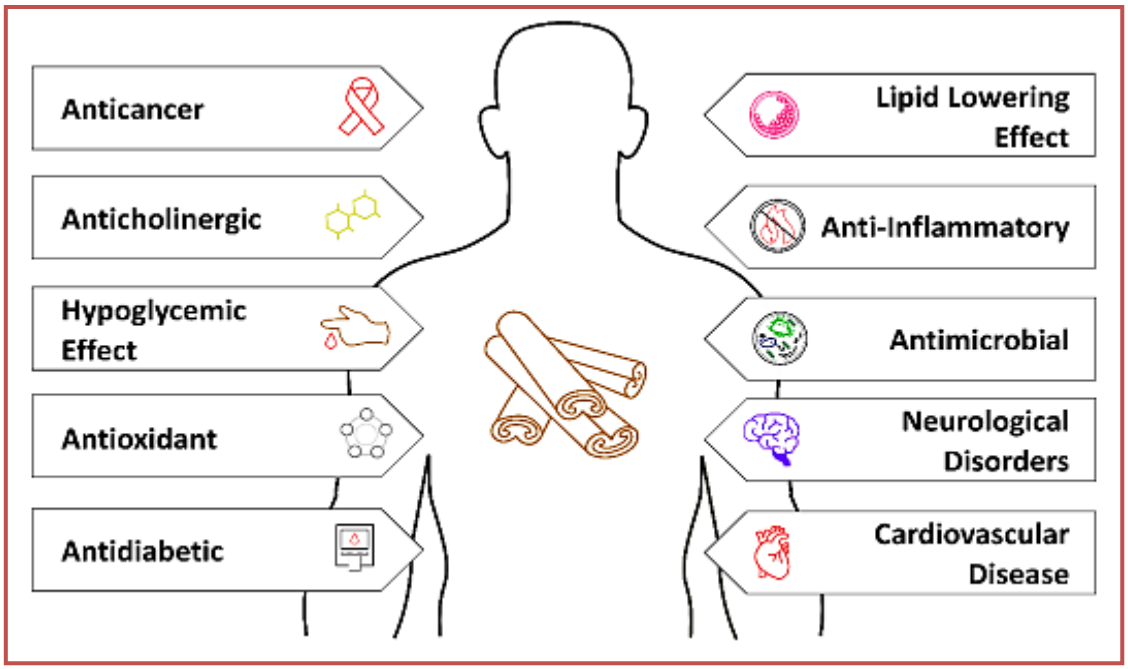


Figure 1.6. Cinnamon in the treatment of diseases and disorder

1.9.2. Biological activities

1.9.2.1. Antioxidant Activity

Cinnamon spice is a source of natural antioxidants that play a key role in the process of aging and diseases. Cinnamomum zeylanicum and Cinnamomum cassia (L.) J. Presl have the highest potential. Extract from the bark of this kind contains biologically active compounds such as eugenol, trans-cinnamaldehyde, and linalool. Phytonutrients have been investigated in the stabilization of palm oil. They prevent the generation of alcohols, ketone, aldehyde, acids, and hydrocarbons. The addition of antioxidants inhibits episodes of the oxidation process of oil, reducing the production of dangerous oxidative derivates and free fatty acids **[43].**

1.9.2.2. Antidiabetic Activities

In the water extract of Cinnamomum zeylanicum prepared using high pressure and the decoction method, benzoic acid, (E)-cinnamaldehyde, trans-cinnamic acid, eugenol, and o-methoxy-cinnamaldehyde were found. These bioactive compounds extracted by this method have huge potential to inhibit α-glucosidase and control hyperglycemia **[44].**

1.9.2.3. Anti-infammatory Activities

Several studies on medicinal plants and their components have indicated the anti-inflammatory activities of cinnamon **[45, 46].** Various studies reported the anti-inflammatory activity of cinnamon and its essential oils **[47, 48].** To date, there are several flavonoid compounds (e.g., gossypin, gnaphalin, hesperidin, hibifolin, hypolaetin, oroxindin, and quercetin) that have been isolated and have anti-inflammatory activities **[49, 50].**

1.9.2.4. Antimicrobial Activity

To date, several antimicrobial activities of cinnamon and its ils have been reported in various studies **[51, 52, 53].** For example, Matan et al. reported the effects of cinnamon oils on different bacterial (Pediococcus halophilus and Staphylococcus aureus), fungal (Aspergillus flavus, Mucor plumbeus, Penicillium roqueforti, and Eurotium sp.), and yeast species (Candida lipolytica, Pichia membranaefaciens, Debaryomyces hansenii, and Zygosaccharomyces rouxii) **[54],** indicating that cinnamon is a natural antimicrobial agent.

1.9.3. Mechanism of Action

The anti-diabetic activity of cinnamon has been established using various in vitro and in vivo models. Bark is the most investigated part for antidiabetic activity. The in vivo anti-diabetic activity of bark has been shown to be mediated through various mechanisms **[55,56].**

These mechanisms included reduction of postprandial intestinal glucose absorption inhibiting the activity of pancreatic α-amylase and α-glucosidase enzymes, stimulation of glucose metabolism, glycogen synthesis, insulin release and cellular glucose uptake (membrane translocation of GLUT-4), inhibition of gluconeogenesis (affects key regulatory enzymes) and potentiating of insulin receptor activity.

Further, the in vivo studies confrmed that the anti-diabetic activity observed works through multiple mechanisms. Such mechanisms include reduction in fasting blood glucose and HbA1c, increase in circulating insulin levels, attenuation of weight loss associated with diabetes and reduction of LDL cholesterol and increase of HDL cholesterol **[55].**

In addition, bark of Cinnamon also demonstrated benefcial effects against diabetic neuropathy and nephropathy **[57].**

1.10. Toxicity

Evidences exist, though very few, on safety concerns and toxicity of cinnamon consumption. ***Dugoua et al., (2007)*** reported that cinnamon is safe to be used in medicinal amounts but may be of concern when it is used in excessive amounts or in long term **[58],** while ***Anderson et al., (1996)*** specified that the amount of cinnamaldehyde consumption should not exceed 700µg/kg **[59, 60 ,61].** Research conducted by ***Shah et all., (1998)*** stated that there was no significant acute and chronic mortality during their toxicity study on the ethanolic extract of Cinnamomumzeynalicum bark **[62].** According to the United States Food and Drug Administration (USFDA), Cinnamomum spp., including common and cassia cinnamon are generally recognized as safe when used in amounts commonly found in food. ***Cao et al., (2008)*** also reported that there have been no adverse events accounted in all human studies involving cinnamon, or aqueous extracts of cinnamon **[63, 64].**

Finally, after taking this part, we will try to answer the following questions empirically in order to better understand the benefits of cinnamon :

1. Does cinnamon have therapeutic effects on human health ?
2. Does cinnamon extract really reduce blood sugar ?
3. What are the compounds responsible for determining the properties of cinnamon ?

Chapter 2

Diabeties Mellitus

Chapter 2 : Diabetes Mellitus

2.1. Definition

Diabetes mellitus is a combination of heterogeneous disorders commonly presenting with episodes of hyperglycaemia and glucose intolerance, as a result of lack of insulin , defective insulin action, or both **[65].** Such complications arise due to derangements in the regulatory systems for storage and mobilization of metabolic fuels, including the catabolism and anabolism of carbohydrates, lipids and proteins emanating from defective insulin secretion, insulin action, or both **[66].**

In addition, a key factor in the development of diabete complications is glycemic level, both at diagnosis and an "upward drift" (Table 2.1) in glycemic level over time. People with higher initial Hb A1c levels had higher cumulative costs than people with lower levels **[67].**

Table 2. 1 : Blood sugar and Hemoglobin A1c optimal levels **[68].**

Hypoglycemia		Excellent		Hyperglycemia			Diabetes			
HbA1C	**0.04**	**0.05**	**0.06**	**0.07**	**0.08**	**0.09**	**.10**	**.11**	**.12**	**.13**	**.14**
Mean Blood mg/dl	**50**	**80**	**115**	**150**	**180**	**215**	**250**	**280**	**315**	**350**	**380**
Glucose mmol/l	**2.6**	**4.7**	**6.3**	**8.2**	**10.2**	**11.9**	**13.7**	**15.6**	**17.4**	**19.3**	**21.1**

2.2. History

Diabetes mellitus has been known since antiquity, its treatments were known since the Middle Ages, and the elucidation of its pathogenesis occurred mainly in the 20th century **[69].**

The discovery of the role of the pancreas in diabetes was made by Joseph Von Mering and Oskar Minkowski in 1889. They found that upon complete removal of the pancreas from dogs, the dogs exhibited all the signs and symptoms of diabetes and died shortly afterwards. In 1910, Sir Edward Albert Sharpey-Schafer (Fig 2.1) of Edinburgh in Scotland suggested that diabetics lacked

a single chemical which was normally produced by the pancreas. Name of this chemical was later proposed to be insulin **[70].**

Following these discoveries, other landmark discoveries followed viz ; identification of sulfonylureas in 1942, the radioimmunoassay for insulin, as discovered by Rosalyn Yallow and Solomon Berson, Reaven's introduction of the metabolic syndrome in 1988, and identification of thiazolidinediones as effective antidiabetics in the 1990s **[69].**

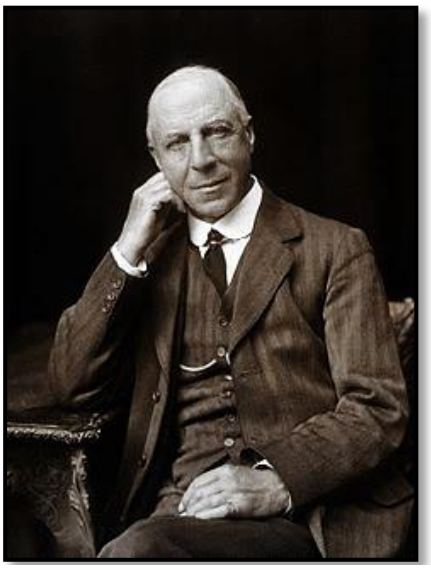


Figure 2.1. Sir Edward Albert Sharpey-Schafer
(2 June 1850 – 29 March 1935).

2.3. Classification of Diabetes Mellitus

There are two main kinds of diabetes, type 1 and 2. Type 1 diabetes (T1DM) or Insulin-dependent Diabetes Mellituss (IDDM) is due primarily to autoimmune-mediated destruction of pancreatic-cell islets, resulting in absolute insulin deficiency.

People with this kind of diabetes must take exogenous insulin for survival to prevent the development of ketoacidosis. The onset of T1DM is rapid, with classical symptoms, as described below **[70].**

The onset of type 2 diabetes (T2D) or non Insulin-dependent Diabetes Mellitus (NIDDM) is usually slow, with an asymptomatic stage that may last for several years. As glucose levels gradually increase, classic symptoms appear, such as increased thirst (polydipsia), an increased need to urinate (polyuria), increased appetite (polyphagia), weight loss and, to some extent certain infections **[71].** T2DM usually develops in adulthood and is primarily related to obesity, lack of physical activity, and unhealthy diet **[70].**

2.3.1. Type 1 Diabetes Mellitus (T1DM)

Type 1 Diabetes Mellitus (T1DM) is one of the most common chronic childhood conditions, with a prevalence of 3 per 1000 in Europe and rising at approximately 2-5% per year **[72].** which results from autoimmune beta-cell destruction in the pancreas and is characterized by a complete lack of insulin production **[73].**

T1DM is characterised by a nonexistent supply of insulin, such that the body cannot control blood glucose levels. Individuals are advised to regulate their own blood glucose levels through daily monitoring and injections of insulin, and matching insulin to diet and exercise **[74].**

2.3.2. Type 2 Diabetes Mellitus (T2DM)

T2DM accounts for between 90% and 95% of diabetes, with highest proportions in low- and middleincome countries. It is a common and serious global health problem that has evolved in association with rapid cultural, economic and social changes, ageing populations, increasing and unplanned urbanization, dietary changes such as increased consumption of highly processed foods and sugarsweetened beverages, obesity, reduced physical activity, unhealthy lifestyle and behavioural patterns, fetal malnutrition, and increasing fetal exposure to hyperglycaemia during pregnancy. T2DM is most common in adults, but an increasing number of children and adolescents are also affected **[75].**

β-cell dysfunction is required to develop T2DM. Many with T2DM have relative insulin deficiency and early in the disease absolute insulin levels increase with resistance to the action of insulin **[76].**

For most people with T2DM, insulin treatment is not required for survival but may be required to lower blood glucose to avert chronic complications. T2DM often remains undiagnosed for many years because the hyperglycaemia is not severe enough to provoke noticeable symptoms of diabetes **[77].**

2.4. Diabetes Symptoms

Diabetes may present with characteristic symptoms such as thirst, polyuria, blurring of vision, and weight loss. Genital yeast infections frequently occur. The most severe clinical manifestations are ketoacidosis or a non-ketotic hyperosmolar state that may lead to dehydration, coma and, in the absence of effective treatment, death.

However, in T2DM symptoms are often not severe, or may be absent, owing to the slow pace at which the hyperglycaemia is worsening.

As a result, in the absence of biochemical testing, hyperglycaemia sufficient to cause pathological and functional changes may be present for a long time before a diagnosis is made, resulting in the presence of complications at diagnosis **[78].**

2.5. Diabetes complications

The most common complication of diabetes is vascular disease, which is also the main cause of morbidity and mortality in diabetic patients **[79].** Vascular complications are mainly divided into macrovascular complications including cerebrovascular, cardiovascular and peripheral vascular disease, and microvascular complications including retinopathy, nephropathy, and diabetic foot (Fig 2.2) **[80].**

2.5.1. Macrovascular complications

Despite the well-known increased risk of CVD among individuals with diabetes, the pathophysiology linking the two conditions is poorly understood. Depending on the cardiovascular event or disease (for example, coronary heart disease (CHD), myocardial infarction, heart failure or stroke) and diabetes subtype, individuals with diabetes have anywhere from a twofold to tenfold increased risk of a cardiovascular event compared with individuals without diabetes **[81- 84].**

2.5.2. Microvascular complications

Although hyperglycaemia-induced damage of the macrovascular system, including the coronary and cerebrovascular arteries, is the leading cause of death in individuals with diabetes **[85],** hyperglycaemia-induced damage to the microvascular network in the kidney, eyes and nerves is by far more common **[86]** and also has a substantial effect on mortality **[85].**

2.5.2.1. Diabetic nephropathy

Diabetic nephropathy (DN) is the most common and frequent microvascular complication in the later stage of diabetes, and it is also a common cause of end-stage renal disease **[87].**

2.5.2.2. Diabetic retinopathy

Diabetic retinopathy (DR) is a common microvascular complication of diabetes, and it is also the main cause of blindness in the vision of 20e 75-year-olds worldwide **[88].**

2.5.2.3. Diabetic foot

Diabetic foot (DF) is one of the serious complications of diabetes. The main reason is that the sustained hyperglycemia in the patient will cause damage to the endothelial cell membrane, and a large number of lipid deposits in the intima, increasing blood coagulation and hardening the arterial wall. DF microvascular and neurogenic lesions are vulnerable to infection and invasion, resulting in the diabetic foot **[89].**

2.5.2.4. Diabetic neuropathy

Diabetic neuropathy is one of the chronic complications of diabetes. This condition is relatively hidden and can involve any part of the whole nervous system, including a variety of neurological syndromes such as diabetic peripheral neuropathy (DPN), autonomic neuropathy, small fiber neuropathy, and multiple radiculopathies. Among them, DPN is one of the most common types **[90].**

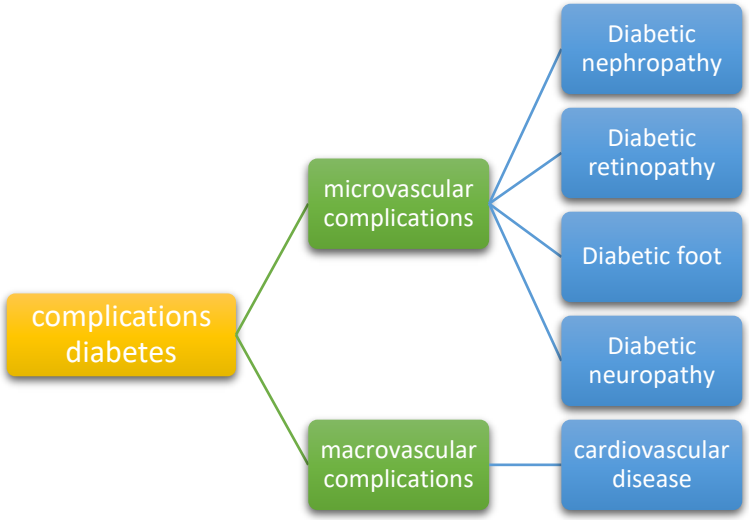


Figure 2.2. Vascular complication of diabetes

2.6. Management of Diabetes Mellitus

Life style management is apparently the cornerstone of management of diabetes mellitus. It is recognized as being an essential part of diabetes and cardiovascular disease prevention. Life style modification programs have demonstrated encouraging improvement in risk factors for diabetes; however, the effect on diabetes incidence has not been reported **[91].** The dietary management of diabetes mellitus is a complement of lifestyle management. In type 2 diabetes, the dietary objective is for improved glycemic and lipid levels and weight loss as appropriate **[92].**

In spite of the underscored importance of lifestyle measures in diabetes therapy, most diabetics cannot escape the value of pharmacotherapy to achieve target glucose concentrations. Different oral hypoglycemics have been in use to aid in maintenance of blood glucose level at the requisite threshhold in diabetics through distinct mechanisms **[93] :**

- ✓ Sulfonylureas and the nonsulfonylurea secretagogues establish normoglycemia by upregulating endogenous insulin secretion ;
- ✓ Alpha-glucosidase inhibitors work by delaying intestinal carbohydrate absorption ;
- ✓ Thiazolidinediones (TZDs) maintain normoglycemia by enhancing insulin sensitivity primarily by increasing peripheral glucose disposal, and suppressing hepatic glucose production ;
- ✓ Metformin works by decreasing hepatic gluconeogenesis while at times also increasing peripheral glucose mobilization and disposal **[94] ;**
- ✓ Synthetic insulin injections are also a therapy against type I diabetes mellitus ;

[94] reports that despite many effective oral hypoglycemic agents available to manage type 2 diabetes, 5% to 10% of the population with diabetes experience secondary failure. This bottleneck can be arrested if clinicians understand the limitations of some therapies currently in use. Secondary failure arises as a result of deteriorating beta cell function, poor compliance to treatment, weight gain, reduced exercise, dietary changes, or illness.

2.7. Medicinal Plants For Treatment Of Diabetes Mellitus

A major drawback associated with hypoglycemic agents is that they are expensive and harbor adverse effects on patients Plant derived medications have also found immense use in the management of diabetes mellitus.

Njagi et all., (2012) notes that there is a new trend in the world to turn to phytodrugs to avoid the adverse effects associated with conventional hypoglycemic agents. Many plant species have been used to treat lifethreatening diseases including diabetes mellitus. A World Health Organization (WHO) study shows that 80% of the world population solely relies on medicinal plants for their primary health care needs **[95].**

To date, the catalogue of antidiabetic medicinal plants is growing at a pleasantly high rate particularly in the African continent. Perhaps this is advised by the economic situation in African, which has driven African diabetics to seek cheaper treatment and management options. This overreliance on antidiabetic medicinal plants has probably invoked scientists to bioassay

these plants in an effort to elucidate more hypoglycemic medicinal plants. The antidiabetic potential of some medicinal plants extracts has been demonstrated in human and animal models of type II diabetes. However, more detailed research on the antidiabetic plants is inevitable to ameliorate the concerns of in vivo safety and efficacy **[95].**

Lasty, after having touched on diabetes in general in this chapter and especially the last part of it, we will try to answer the following questions empirically as usual in order to understand more about the mechanism of diabetes treatment by medicinal plants :

1. Are medicinal plants considered a real alternative to treatment for diabetic ?
2. Which type of diabetes is most affected by this type of treatment ?
3. Being a popular plant in the popular market for diabetics, is cinnamon a natural alternative to lowering blood sugar ?

Chapter 3

In Silico Approach

Chapter 3 : In Silico Approach

3.1. Introduction

Classically, scientific research has been divided into two types – in vivo (‘within the living’) and in vitro (‘within glass’). The development of in vitro techniques was a response to several considerations, including the ethics of experimenting on live subjects, the cost (time and resources) associated with experimenting on live subjects and the inability to control conditions properly.

The invention of the microprocessor and subsequent development of the personal computer has led to numerous scientific advances. One of the most exciting, and least developed, is in the field of in silico (Fig 3.1) (‘in silicon’) research **[96].**

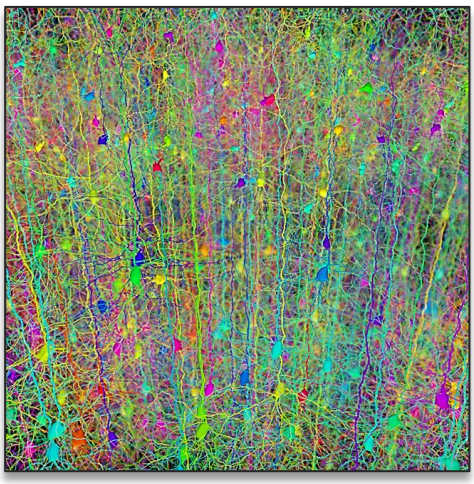


Figure 3.1 : A forest of synthetic pyramidal dendrites generated in silico using Cajal's laws of neuronal branching

3.2. Definition

Imitating the common biological terms in vivo and in vitro, the term in silico refers to performing experiments using computers. Although the historical origin of this term is not clear, it is safe to assume that silico is a reference to the chemical element Silicon (Si), a key component of computer chips. The majority of the in silico methods are primarily used in parallel with the generation of in vivo and in vitro data for accurate modeling and validation of a wide range of applications from the ligand design and optimization to the characterization of fundamental pharmacological properties of molecules such as absorption, distribution, metabolism, excretion and toxicity **[97].**

3.3. Origin of the term

Origin of the 'in silico' term is poorly defined, with several researchers claiming their role in its origination. However, some of the earliest published examples of the word include the use by **[98, 99].** In a more recent book, ***A. Danchin, (2002)*** provides a quotation that offers a concise and cogent depiction of the potential of computational tools in chemistry, biology and pharmacology :

''[…] Informatics is a real aid to discovery when analyzing biological functions […]. […] I was convinced of the potential of the computational approach, which I called in silico, to underline its importance as a complement to in vivo and in vitro experimentation'. **[100].**

3.4. In Silico modelling properties

- ⇨ In silico modelling, in which computer models are developed to model a pharmacologic or physiologic process, is a logical extension of controlled in vitro experimentation. It is the natural result of the explosive increase in computing power available to the research scientist at continually decreasing cost ;
- ⇨ In silico modelling combines the advantages of both in vivo and in vitro experimentation, without subjecting itself to the ethical considerations and lack of control associated with in vivo experiments. Unlike in vitro experiments, which exist in isolation, in silico models allow the researcher to include a virtually unlimited array of parameters, which render the results more applicable to the organism as a whole ;
- ⇨ In silico modelling is best known for its extensive use in pharmacokinetic experimentation, the best-known example of which is the development of the three-compartment model. In addition, complex in silico models have been applied to pathophysiological problems to provide information which cannot be obtained practically or ethically by traditional clinical research methods **[101].**

3.5. Comparing In silico with the different modelings In vitro and In vivo

In silico modeling seems to be the next natural development of in vivo and in vitro modeling (fig 3.2). Where in vivo represents the ultimate and most comprehensive model environment to examine a complex biological phenomenon, in vitro tries to convert the complex in vivo models to smaller, simpler, and well-defined in vitro models that can represent an in vivo phenomenon **[102].** In-silico promises to overcome problems involved with in vivo and in vitro modeling. In silico experiments allow precise observation and control of experimental conditions while modeling the ''process'' of interest in the full biological contex **[103].**

Thus in theory, once an investigator fully understands the behavior of a biological process, by precise manipulation and experimentation in vitro, an ‘in silico model’’ of that process can be created. If an investigator can abstract models for all of the components involved in a complex biological system, it will be possible to synthesize a model of the whole complex by linking together the various models (or levels of complexities in a hierarchically organized system) - outputs of one to inputs of other- and iterating them forward in each other’s presence in a computer. Therefore, an in silico model will not have the drawbacks of in vitro models of being merely abstracted from complex in vivo systems **[102].** However, examples of this modeling approach in different modeling contexts are rare. To date, the most emergent advances of in silico modeling are reported by pharmaceutical approaches to predict toxic and ADME (absorption, distribution, metabolism, and elimination) values of different chemical compounds **[104].**

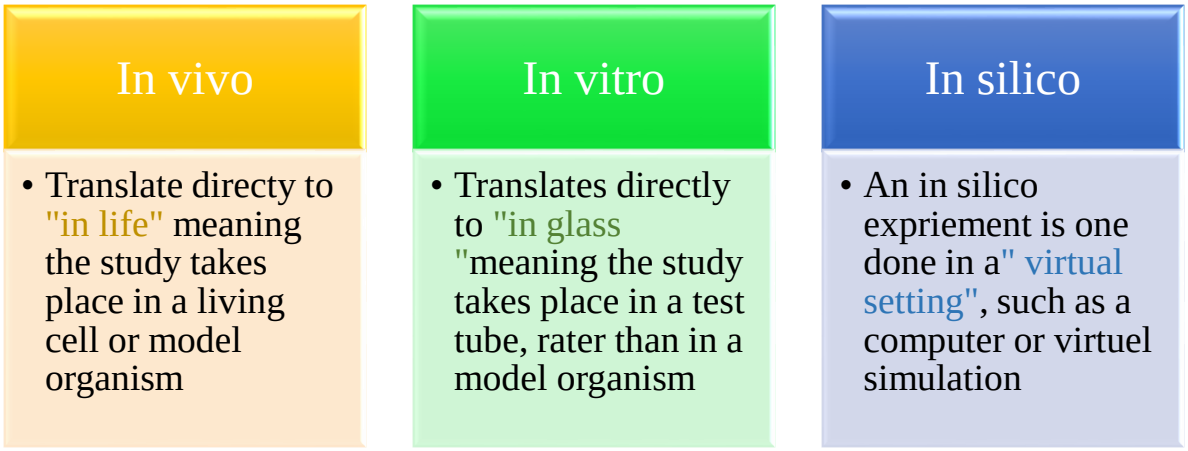


Figure 3.2. In vivo, in vitro, in silico and outs of research

3.6. Application of In Silico approach

As the number of in silico tools has expanded, so too has their application across different industrial and regulatory sectors. This is advantageous, as new information on the development or application of tools in one sector can be leveraged by another. This cross disciplinary sharing of ideas and practice enables more rapid advancement, acceptance and uptake of new in silico methods **[105].** Historically, in silico models have been widely used for predicting the toxicity of chemicals to environmental species, particularly fish, aquatic invertebrates, algae and more recently bees **[106].** However, there are multiple examples of their application within medicinal chemistry, in the design of bioactive chemicals, predictive toxicology and safety assessment **[107].** In the pharmaceutical industry, models have been used extensively to maximise the efficiency of the drug discovery process industrial and herbal **[105].**

3.6.1. In silico methods and tools for drug discovery

In silico pharmacology (also known as computational therapeutics, computational pharmacology) is a rapidly growing area that globally covers the development of techniques for using software to capture, analyse and integrate biological and medical data from many diverse sources. More specifically, it defines the use of this information in the creation of computational models or simulations that can be used to make predictions, suggest hypotheses, and ultimately provide discoveries or advances in medicine and therapeutics **[97].**

Basically, there are two outcomes when bioactive compounds and biological systems interact (Fig 3.3) **[108].** Note that 'biological system' is defined here very broadly and includes functional proteins (for example, receptors), monocellular organisms and cells isolated from multicellular organisms, isolated tissues and organs, multicellular organisms and even populations of individuals, be they uni- or multicellular. As for the interactions between a drug (or any xenobiotic) and a biological system, they may be simplified to 'what the compound does to the biosystem' and 'what the biosystem does to the compound.' A drug that acts on a biological system can elicit a pharmacological and/or toxic response, in other words a pharmacodynamic (PD) event **[97].**

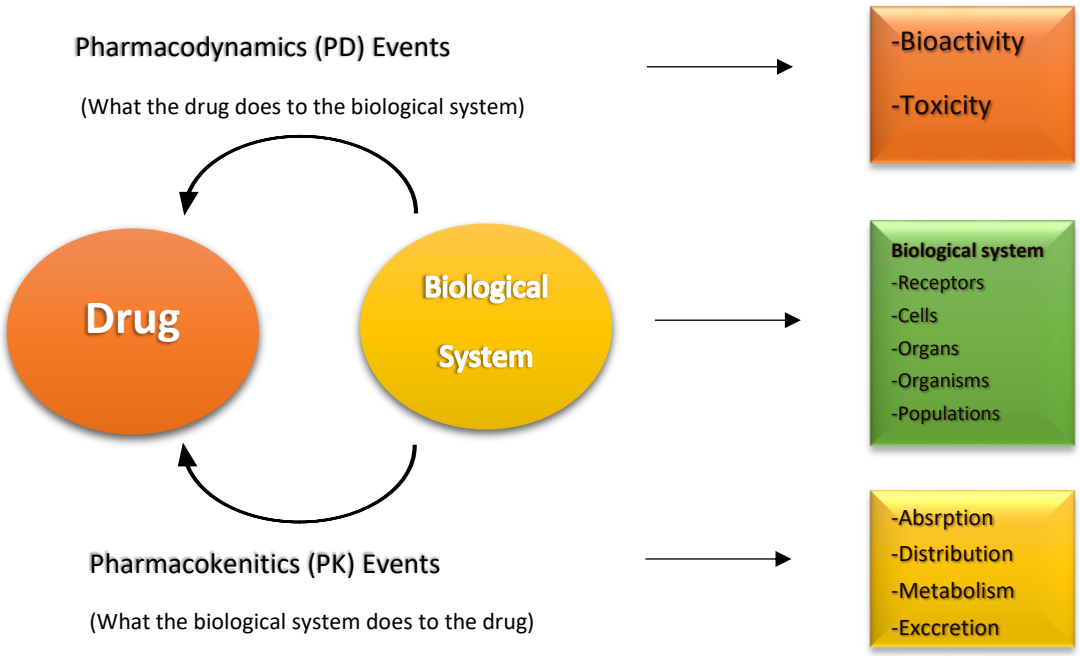


Figure 3.3. Bioactive compounds and biological systems interact **[108].**

3.6.1.1. History and evolution of in silico approaches

Drug design and related disciplines in drug discovery did not wait for the advent of informatics to be born and to grow as sciences **[97].** As masterfully summarised by Albert (1971, 1985) **[109, 110],** he earliest intuitions and insights in structure–activity relations can be traced to the nineteenth century. A relation between activity and a physicochemical property was firmly established by ***Meyer., (1899***) and ***Overton., (1901)*** **[111,112],** who proposed a 'Lipoid theory of cellular depression' such that the higher the partition coefficient between a lipid solvent and water, the greater the depressant action. Such papers paved the way for the recognition of lipophilicity and electronic properties as major determinants of PD and PK responses, as best illustrated by the epoch-making and still ongoing work of Corwin Hansch **[113, 114],** a founding father of drug design. In parallel with our growing understanding of the concept of molecular structure, a few visionary investigators in the late nineteenth and early twentieth centuries (for example, John Langley, Paul Ehrlich and Alfred Clark; reviewed by (Arı¨ens, 1979; Parascandola, 1980) **[115, 116]** developed the concept of receptors, namely the targets of drug action. The analogies between receptors and enzymes were outlined by ***Albert (1971)*** **[109].**

Such was the birth of quantitative structure–activity relationships (QSARs), followed in the 1980s and 1990s by computer graphics and molecular modelling. However, computer sciences rapidly ceased to be a simple tool in drug discovery and pharmacology and became a major contributor to progress. The chemistry– biology–informatics triad has now evolvedin to a life of its own and is bringing pharmacology to new heights **[97].**

3.6.1.2. Increase in biological data on chemical molecules for drug discovery

Over the past few decades, large-scale data has been generated on hundreds of thousands of small molecules through biological screening, and this data is compiled in online repositories that are available for research **[117].** For example, due to advancements in HTS techniques, largescale experiments of >1 million chemicals have been generated **[118].** In addition, this biological assay data has been compiled in chemical library databases, and the amount of data is increasing rapidly due to advancements in chemical synthesis and HTS techniques. This accumulating data and its public availability have enabled the development of machine learning models and facilitated modern in silico drug discovery **[117].**

Traditional prediction methods, such as quantitative structure activity relationship (QSAR) models, can be used in the early stages of drug discovery to prioritize drug candidates by their pharmacological properties and potential adverse effects **[119].** Recently, due to increasing public resources, many machine learning-based prediction methods have been developed to predict drug-target interactions **[120],** the blood-brain-barrier permeability of compounds **[121],** and ADMET-Tox properties of drug candidates **[122, 123].**

3.6.1.3. Target identification

A drug target is defined as a biological entity, usually a protein, that can modulate disease phenotypes **[124].** Thus, the identification of prime drug targets is the first and most important step in drug discovery. Conventional drug target identification strategies are performed experimentally, such as identifying differentially expressed genes between normal and diseased cells or tissues and proteins that are highly interconnected with disease-related proteins **[117].**

a. Experimental approaches : Conventional experimental approaches for target identification require molecular and biochemical studies of disease pathophysiology. Although such studies expand our knowledge of diseases, they can be time-intensive methods for finding promising drug targets **[117].**
b. Computational target identification : Experimental approaches are expensive and are generally conducted at low-throughput scale because of their complexity. To overcome these hurdles, in silico methods have been developed to identify potential drug targets performed on computer or via computer simulation **[125].**
c. Target validation : Once a target is identified, the next step is to confirm whether the modulation of the biological function of the target affects the disease phenotype **[126].** In such cases, animal models in which the target gene is deleted or mutated can be more informative for target confirmation **[117].**

3.6.1.4. *Successful applications of in silico drug design*

The development of new therapeutic drugs is an expensive and time consuming process. In silico technology has become essential in the contemporary pharmaceutical industry because it can reduce the time and resources required for drug discovery. Due to advancements in computational algorithms and accumulated knowledge databases, computational prediction tools have now been integrated into every stage of the drug discovery process **[117].** Computational drug discovery methods have been successfully used in the design and identification of drug compounds to treat various diseases, including cancer **[127, 128],** diabetes **[129, 130],** and viral **[131]** and bacterial infections **[132, 133].**

3.6.2. In silico methods and tools for Phytochemical Research

Phytochemicals (Greek, phyton means plants) are chemical compounds procured from plants. They are essential for the overall metabolic functioning of the plants, and defend them against microbial infection, infestation by pests, herbivores as well as changes in environmental conditions, thus they have disease preventive and healing properties.

Apart from affecting the physiology of plants, phytochemicals show different types of effects on other organisms exposed to these chemicals. They can be beneficial or deleterious to other organisms including human beings **[134].** Although they are not essential nutrients, many plants are exploited for preparation of traditional herbal medicines in less developed countries **[135]** since ancient times for treating numerous chronic diseases like diabetes, blood pressure anomalies, central nervous system disorders, cancer etc.

The study of phytochemicals includes their separation, extraction, purification, identification, structural as well as functional analyses. The frequently used techniques used for phytochemical research involve different chromatographic techniques along with mass spectrometry as well as nuclear magnetic resonance **[134].**

Most of the phytochemicals are unexplored because their biological effects are not known. In-silico methods could be exploited for discovering the actions of un-investigated phytochemicals by identification of their molecular targets using an amalgamation of chemical informatics and bioinformatics along with systems biology approaches (Fig.3.4), hence advantageous for drug discovery **[136].**

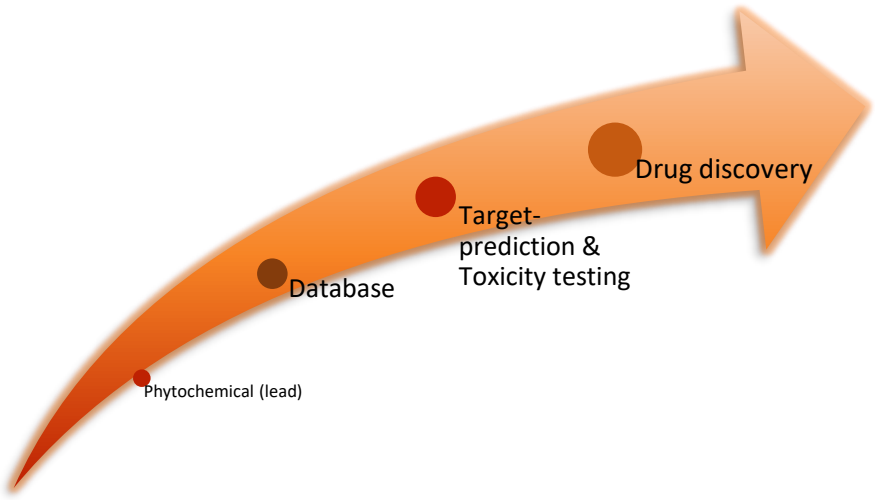


Figure 3.4. Flow-chart representing the use of phytochemical in drug discovery using chemo- and bioinformatics approach

3.6.2.1. Medicinal plant databases

The databases of medicinal plants are collections of particular information about plants used in folk medicine. Dozens databases and Internet sources partially containing such information became available during the last decade. Most of the databases contain information like:

a) Availability (freely accessible or commercial).
b) Plant name.
c) Traditional uses.
d) Plant parts which are used for treatment.
e) Phytoconstituents.
f) Phytoconstituents with their 2D/3D structures.
g) Pharmacological and toxic activities of the phytoconstituents.
h) Possibility of download of phytoconstituent structures and properties **[137].**

Recent initiatives requiring greater use of in silico technologies have called for transparency and development of strong database information that is available to the public at no cost. Electronic information on chemical structure, pharmacological activity, specificity against known molecular targets and traditional uses of the herbs are always helpful and the first learning step to establish a phytochemicals as a drug. This information can serve a wide variety of purposes in the field of virtual screenin **[138].**

3.6.2.2. Quantitative structure activity relationship

In the modern drug design and drug discovery era, application of QSAR is extended to molecular design, prediction of different biological activities, lead compound optimization and virtual screening, classification, diagnosis and elucidation of mechanisms of drug action, toxicity prediction of environmental toxicants and prediction of drug-induced toxicity **[139].**

The methods that correlate the molecular structure to a specific activity or property derived either in vitro or in vivo are known as QSAR, that is, mathematical relationships linking chemical structure and pharmacological activity in a quantitative manner for a series of compounds **[140].** A simple mathematical relationship as noted below is established in any kind of QSAR relations :

Biological activity = f (chemical structure or property)

QSAR analysis statistically connects one or multiple molecular descriptors with the molecular activity. The statistical models hence created are exploited for prediction of the biological activity of newly discovered chemical compounds yet to be tested experimentally. It is also very important to the study of ADME/T (absorption, distribution, metabolism, excretion, and toxicity) properties of phytochemicals used for drug development. There are many software packages available with appropriate QSAR models for the evaluation of ADME/T properties for chemicals including phytochemicals based on their structures **[134].**

The step-wise processes involved in the development of a QSAR model are :

a. Selection of the database of compounds with known functions ;
b. Calculation of molecular descriptors ;
c. Statistical model designing for relating the activity with calculated descriptors ;
d. The assessment of the generated model with a mock set **[141].**

Finaly, after we have done a general study for In silico approach and explain some of its applications, We will try as usual pose the following points or questions to answer later :

1. Is it really possible for The Approach of in silico to be a reliable alternative in scientific and pharmaceutical studies in particular ?
2. Phytochemicals are of great importance as a reservoir of chemical diversity aimed at new drug discovery, does this approach facilitate the detection of that reservoir and determine the pharmacological properties of any plant ?

3. Does in silico approach have a special effect on chronic diseases such as diabetes ?

Chapter 4

Materials and Methods

Chapter 4 : Methods and Materials

4.1. Introduction

Our study project was carried out at the process engineering laboratory, analysis laboratory and research laboratory of khemis miliana, at the level of the Bio-Ressources Naturelles Locales BORN at Chlef University and also at the level of the analysis laboratory of Dr. Zibouche's medical.

The aim of our study is to identify the physico-chemical properties of cinnamon essential oils and its antibacterial and antioxidant activities and finally the Evaluation of hypoglycaemic effect by carrying out two different studies aimed at verifying the antidiabetic activity of our plant substrate represented in cinnamon

Our study is based on the use of essential oil of Vietnamese cinnamon (*Cinnamomum loureiroi*). The part used of cinnamon in this study is the barks

Cinnamon is known for these medicinal characters, their great use by the population and their interesting therapeutic virtues.

4.2. Extraction methods

This part is devoted to the extraction of the essential oil of cinnamon plant by two extraction processes : water distillation or hydro-distillation and Solvent Extraction Method (Maceration).

Bark of *Cinnamomum loureiroi* was obtained from shops. Unfortunately, this species is not planted in Algeria. However, the material is easy to get since cinnamon is commonly used as spice in Algeria's cooking.

The extraction methods used are summarized as shown in the following flowchart :

Figure 4.7. Flowchart representing the two extraction methods using

4.2.1. Steam distillation : (Hydrodistillation)

Steam distillation is the simplest method to extract the essential oil from cinnamon. Steam distillation is mostly used to extract various types of essential oils. The process is cheaper than other extraction methods. It does not require any solvent and is safer than other methods **[142].**

- ***Apparatus :***
  - Flask (1000 ml) ;
  - Graham condenser ;
  - Beaker ;
  - Heating mantle (1000 ml) ;
  - Separatory funnel (250 ml) ;
- ***Reagents :***
  - Distilled water ;
  - Cinnamon bark (we used Vietnamese cinnamon) ;
  - Hydro-distillation is carried out using « Clevenger apparatus » (fig 4.2) ;
- ***Procedure :***

The volatiles organic compounds of cinnamon bark were obtained using clevenger apparatus as shown in following figure :

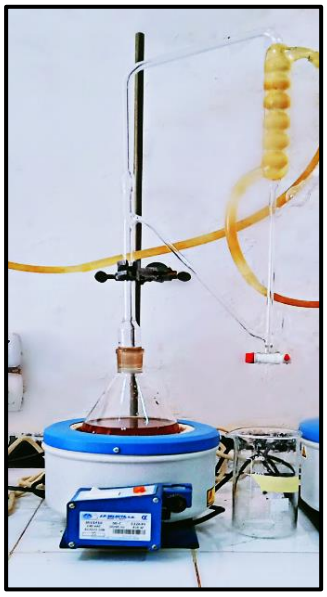


Figure 4.2. shematic of steam distillation extraction process

The Bark of Cinnamon used in this study was purchased from a local supermarket. The fresh sample was washed with the flowing deionized water for 5 min and then dried naturally at an ambient temperature of 25-30 °C for 24 hr. After that, the samples were crushed and ground physically. Cinnamon bark powders were dried until the weight did not change (fig 4.3). A finely-weighted quantity of 150 gm of cinnamon bark powder was put into a 1L distillation flask, and 500 ml of distilled water was poured into the flask, which was connected to the steam generator via a glass tube and to a condenser to retrieve the oil. The essential oils were

volatilized with boiling water for 2 to 3 hours. The recovered mixture was allowed to settle, and the oil was withdrawn.

Figure 4.3. Weighing of cinnamon bark powders

After the steam distillation process, the product was collected and separated using a separatory funnel. The essential oils settled at the upper layer of the separatory funnel and were separated several times until no oil was left in the separatory funnel (fig 4.4) [143]. Essential oils are recovered and stored in the refrigerator at 4 ^{o}C.

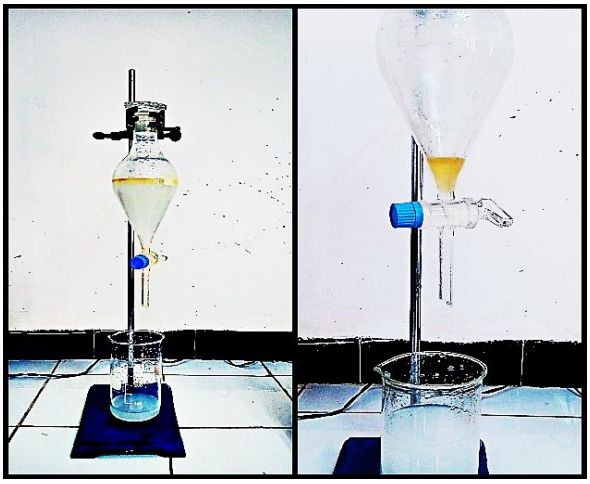


Figure 4.4. Separator the essential oil of cinnamon

The percentage of essential oil yield was defined as the weight of essential oils divided by the weight of bark powder **[144].**

The yield was calculated using the equation bellow :

$$\text{Yield}(\%) = \left(\frac{M_{E.O}}{Bm}\right) \times 100 \qquad (4.1)$$

Where :

- $M_{E.O}$: Mass of the extracted oil (g)
- Bm : Initial plant biomass (g)

4.2.2. Solvent Extraction Method (Maceration) :

Maceration is one of the conventional methods of extraction that is very simple and the cheapest because it only requires a simple container as the place for extraction with the disadvantage of long extraction time **[145].**

- ***Apparatus :***
  - Blender ;
  - Erlenmeyer flask with stopper ;
  - vacuum filtration ;
  - rotary vacuum evaporator ;
- ***Reagents :***
  - Ethanol ;
  - Acetone ;
  - Distilled water ;
- ***Procedure :***

The bark of cinnamon sample was ground to powder by using a blender. Twenty grams of sample plant in powder form was weighed in an Erlenmeyer to which 100 ml of each solvent "ethanol, acetone and distilled water" (fig 4.4). The erlenmeyer flask is placed in dark for three days at room temperature. The mixture was recovered by filtration. This process need the vacuum filtration (fig 4.5) is used to separate the waste and filtrate. The filtrate was separated using a rotary vacuum evaporator RE 200 (fig 4.6), to partially separate the bioactive compounds in the extracts.

Figure 4.5. Solvent Extraction Method (Maceration).

With :

(1) : Distilled water

(2) : Acetone

(3) : Ethanol

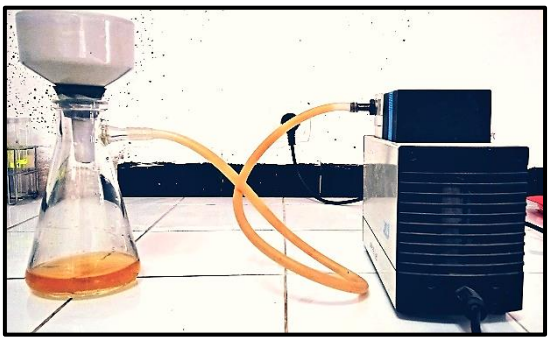


Figure 4.6. Vacuum Filtration System for General Filtration in Lab.

The filtrate was separated using a rotary vacuum evaporator RE 200 (fig 4.7), to partially separate the bioactive compounds in the extracts.

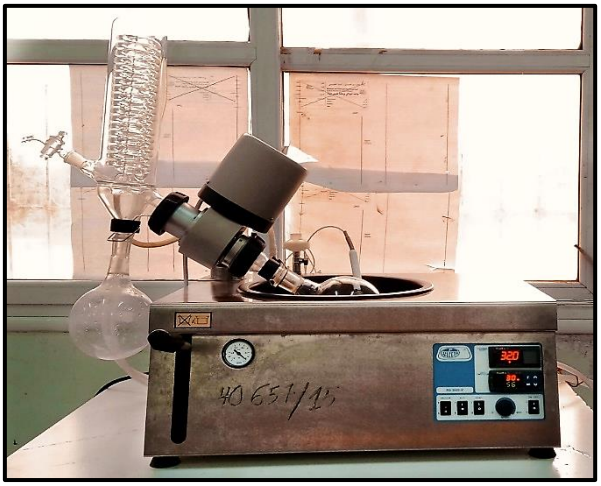


Figure 4.7. Rotary vacuum evaporator process RE 200

The filter residue was dried in the open air for 4 days and then weighed, the yield is calculated as follows **[146] :**

$$\text{Yield}(\%) = \left(\frac{W0-W1}{W0}\right) \times 100 \qquad (4.2)$$

Where :

- W0 : Initial weight
- W1 : Weight after extraction and drying

4.3. Characteristics of cinnamon essential oil

In order to evaluate the quality and composition of the extracted essential oils of cinnamon during this study, analyzes were carried out to determine their organoleptic and physico-chemical properties, these properties constitute a means of checking and controlling the quality of the EO.

4.3.1. Organoleptic properties of cinnamon :

The organoleptic test used the scoring tests on taste, texture, color, and aroma.

4.3.2. physico-chemical properties of cinnamon

Today, the physico-chemical properties (density, refractive value, optical value, acid and ester value) are required for their commercial evaluation.

4.3.2.1. physical properties of cinnamon

This analysis aims to determine the physical properties of EO such as density, relative humidity, relative calcination, refractive index and pH according to international standards.

a. Relative density :

Relative density is the ratio of the mass of a given volume of the oil at 20 °C to the mass of an equal volume of distilled water at 20 °C. This quantity is dimensionless and its symbol is RD [147].

- ***Apparatus** :*
  - Eppendorf Tubes (3 ml) ;
  - Electronic Balance ;
- ***Procedure :***

Using an electrical balance, we consecutively weigh equal amounts of oil and water at a temperature of 20°C.

Relative density is calculated as follows :

$$RD = \frac{(m2 - m0)}{(m1 - m0)} \qquad 4.3$$

With :

- RD : The value of the relative density according to the standards.
- m_o : the Mass in grams of empty eppendorf.

- m_1 : The mass in grams of eppendorf, filled with distilled water.
- m_2 : The mass, in grams of eppendorf, filled with the EO.

b. Refractive index :

The refractive index of an essential oil is a unique number which is the ratio of the sine of the angle of incidence to the sine of the angle of refraction, when a ray of light of defined wavelength passes from air into the essential oil kept at a constant temperature **[147].**

➢ ***Apparatus :***

- Abbe refractometer ;

➢ ***Procedure :***

Refractive indexes of the essential oils were measured by Refractometer (Reichert, AR200) according to the method described by Chophi and Pirbalouti **[148, 149].** The prism of the Digital refractometer was cleaned and the red button was pressed first to ensure that it was cleaned well. The cinnamon EO sample was applied to the prism of a Digital refractometer using a micropipette. Finally, the result of the refractive index was read and recorded.

Refractive index is calculated as follows :

$$n_D^t = n_D^{t'} + 0.004\ (t'-t) \qquad 4.4$$

Where :

- $n^{t'}_D$: The value obtained at the temperature t'= 20°C.
- t': Refraction temperature (20°C).
- t : Exprement temperature (20°C).

Figure 4.8. Abbe refractometer

c. pH value of cinnamon

In chemistry, pH denoting "potential of hydrogen, is a scale used to specify the acidity or basicity of an aqueous solution. Acidic solutions (solutions with higher concentrations of H+ ions) are measured to have lower pH values than basic or alkaline solutions **[150].**

There are two methods for measuring pH: colorimetric methods using papers, and the more accurate electrochemical methods using electrodes and a millivoltmeter (pH meter).

i. colorimetric methods (pH paper) :

➢ ***Apparatus :***

- pH-indicator strips

➢ ***Procedure :***

Firstly, we Remove a pH-indicator strip from its container and then Dip one end of the universal indicator strip into the cinnamon essential oil for which we want to test the pH level (fig 4.9). After that, we Set the universal indicator on a dry surface for 60 seconds to allow the pH reading to take place. When the paper dries, we compare the changed color of the universal indicator paper to that of the pH chart that accompanied the paper's packaging.

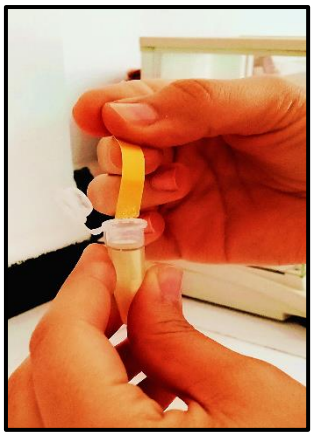


Figure 4.9. pH paper

ii. Electrochemical methods (pH meter) :

➢ ***Apparatus :***

- pH meter.

➢ ***Procedure :***

Before starting we calibrate the pH meter then we take 3 cinnamon bark and put them in a bottle filled with distilled water, after we dropped out it for 15 min, then the pH measurement cell (electrode) is immersed in it to read the pH value at a temperature of 25 C° (fig 4.10) **[151].**

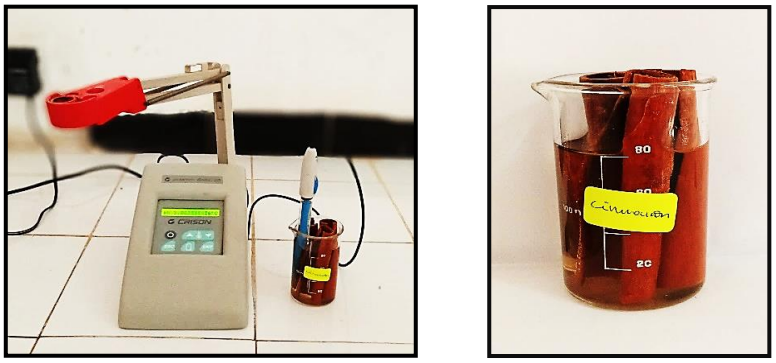


Figure 4.10. pH meter

d. Relative humidity :

- ***Apparatus :***
  - Electronic Balance ;
  - Beaker ;
  - Oven laboraory ;
  - Desiccator ;
- ***Procedure :***

Analysis of water content was conducted using a thermogravimetric method. We weight 5 g of sample in the form of powder in a beaker. Then we dried the refined material in an oven with a temperature of 100-105ºC for 2-3 hours, afterwards we cooled in a desiccator with weighed (fig 4.11). Pre-heat oven for 30 minutes longer cooled and weighed **[152].**

Figure 4.11. Moisture content test

The relative humidity is calculated as follows :

$$RH(\%)=\frac{(m0-m1)}{m}\times 100 \qquad 4.5$$

Where :

- m_0 : beaker +5g of the sample.
- m_0 : beaker after desiccator.
- m : mass of powder.

e. Relative calcination (Ashes) :

➢ ***Apparatus :***

- Platinum crucible ;
- Desiccator ;
- Electronic Balance ;
- Bunsen burner ;

➢ ***Procedure :***

At first, we calcined 1g of cinnamon powder in a Platinum Crucible; then, we passed the hollow over the bunsen burner (fig 4.12) until white ash was obtained. Lastly, we Meter dug it in a desiccator and weighed the residue **[152].**

Figure 4.12. Calcination process

Relative calcination is calculated as follows :

$$\text{Calcination (\%)} = \frac{P1-P2}{m} \times 100 \qquad 4.6$$

Where :

- P1 : Platinum Crucible + ashes
- P2 : Platinum Crucible empty
- m : Mass of powder.

4.3.2.2. Chemical properties of cinnamon :

a. Acid value (neutralization number) :

Acid value (AV) is the milligrams of potassium hydroxide required to neutralize the free faty acide (FFA) in one gram of fat. It is independent of molecular weight and, coincidentally, is almost twice the FFA% when the latter is expressed on an oleic-acid basis **[153].**

- ***Apparatus :***
  - Flask 50 ml ;
  - Burette ;
  - Electronic balance ;
- ***Reagents :***
  - Ethanol 5ml ;
  - Phenolphthalein indicator ;
  - Ethanolic solution of potassium hydroxide (0.1M)
- ***Procedure :***

Firstly we dissolve 100 mg of cinnamon EO in a mixture of 5 ml ethanol (96%) and 3 to 5 drops of Phenolphthalein. The solvent must be neutralized beforehand with a 0.1M ethanolic potassium hydroxide solution. After dissolution, we titrate with Potassium Hydroxide (fig 4.13). The titration is complete when the pink color persists for at least 15s.

Figure 4.13 : Volumetric analysis process

Acide value is calculated as follows :

$$AV = \frac{56.5.M.V}{W} \qquad 4.7$$

Where :

- 56.5 : Molar mass of KOH (g/mol).
- M : Molarity of the base (mol).
- V : Volume of titre for EO sample (ml).
- W : weight of essential oil sample (g).

b. Saponification value :

The saponification value corresponds to the mass in mg of potassium hydroxide (KOH – commonly known as caustic potash or potash lye) needed to neutralize the free fatty acids and saponify the esters contained in a gram of material **[154].**

- ***Apparatus :***
  - Flask 50 ml ;
  - Graham condenser ;
  - Magnetic Hotplate Stirrer ;
  - Laboratory water bath ;
  - Burette ;
- ***Reagents :***
  - Ethanolic solution of potassium hydroxide (0.5N) ;
  - Phenolphthalein indicator ;
  - Hydrochloric acid ;
- ***Procedure :***

The EO sample of 0.5 g was taken into a 250 ml distillation flask and dissolved in 25 ml of ethanolic 0.5 normality of KOH. The same procedure was followed to prepare the blank sample without the fat sample. Then, both the samples were attached to the reflux condenser and heated up to 70oC for about 90 min. After that, the samples were allowed to attain room temperature. Finally, 3 to 5 drops of phenolphthalein indicator were added to samples and titrated against 0.5 normality of hydrochloric acid (fig 4.14).

Saponification value was estimated using the following equation :

$$SV = \frac{56.11.N.(c-d)}{w} \qquad 4.8$$

Where :

- N : the normality of HCI solution.
- c : the 0.5 N HCI solution required for titration of the blank (ml).
- d : the 0.5 N HCI solution required for titration of the sample (ml)
- w : the weight of sample (g).

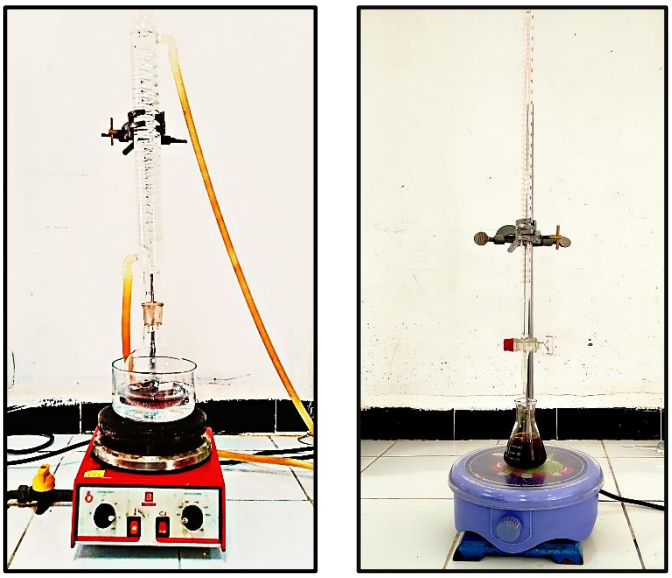


Figure 4.14. Saponification value determination process.

c. Ester Value (EV) :

The ester value of a lipid is the mass of potassium hydroxide, expressed in milligrams, needed to saponify the esterified fatty acids contained in a gram of fatty substance.

The ester value is equal to the saponification value for pure glycerides and is found by calculating the difference between the saponification value and the acid value. Therefore, it is not a property that can be found experimentally **[154].**

Ester value can be obtained by the following formula :

$$EV = SV - AV \qquad 4.9$$

Where :

- EV : Ester value.
- SV : Saponification value
- AV : Acide value.

d. Peroxide Value (POV) :

Peroxide Value (POV) determines all substances that oxidize potassium iodide under the conditions of the test in terms of milliequivalents of peroxide per 1000 g of sample. These substances are generally assumed to be peroxides or other similar products of fat oxidation.

- ***Apparatus :***
  - Electronic balance ;
  - Iodine flask or flask (250 ml) with glass stopper ;
  - Burette ;
  - Magnetic Stirrer ;
- ***Reagents :***
  - Chloroform ;
  - Glacial acetic acid ;
  - Potassium iodide ;
  - Sodium thiosulfate solution (0.01 M) ;
  - Starch solution
- ***Procedure :***

Firstly, approximately 1g of the sample is Transferred accurately into a 250 ml flask closed immediately with a glass stopper; then, we dissolve it in 5 ml of acetic acid chloroform (3:2, v/v) and add 0.5 ml a saturated aqueous potassium iodide solution. The mixture is shaken vigorously for exactly 1 min before distilled (5 ml) water and a magnetic stirrer bar are added. The mixture is titrated with 0.01M sodium thiosulfate solution with constant stirring until the yellow color almost disappears when a starch indicator solution (0.5 ml) is added. The thiosulfate solution is added drop-wise until the blue color just disappears. A sample blank is analyzed similarly.

Peroxide value is obtained from the formula :

$$POV = \frac{(e-f).M.1000}{w} \qquad 4.10$$

Where :

- e : the titration of sample (ml).
- f : the titration of blank (ml).
- M : the molality of thiosulfate solution (mol/l)
- w : the weight of sample (g).

4.3.3. Analysis of the chemical composition of Cinnamon essential oil

With the improvements in instrumental analytical chemistry, the characterization of EO has allowed the scanning of a greater number of molecular constituents of EO. In order to comprehensively analysis of an EO sample, chromatographic procedures which enable component separation and identification should be applied.

Therefore, in this study, we aim to analyze the chemical compounds present in the bark cinnamon EO using Thin-layer chromatography (TLC) and Gas Chromatography-Mass Spectrometer (GC-MS).

4.3.3.1. Thin-layer chromatography analysis (TLC) :

Thin layer chromatography (TLC) is a quick, sensitive, and inexpensive technique used to determine the number of components in a mixture, verify the identity and purity of a compound, monitor the progress of a reaction, determine the solvent composition for preparative separations, and analyze the fractions obtained from column chromatography **[156].**

This part of the study mainly aimed to describe the strategies and principal steps for performing a TLC analysis for cinnamon EO in order to obtain and understand the results accurately.

- ***Procedure :***

a) Sample application (spotting the TLC plate)

Firstly, we obtain two papers of TLC (20 x 9) and (20 x 5) cm square, in this case, a thin layer of powdered silica gel that has been coated onto a plastic sheet. Using a pencil (not ink from a pen), we draw a line ~1 cm from the edge of the bottom side of the paper.

We make seven pencil dots along this line, about 2.5 cm apart. Underneath each dot, then we label the sample we will test on that spot, but because we won't have space to write the full name, we Try for example, "A" for distilled water extract and continue that in the same way with the capital letters. We put 5 µl (100 mg/ml) of each extract by micropipette into the Letter corresponding to each sample. We have to try to keep each dot as small as possible.

b) Development of TLC plates :

In most cases, ascending TLC is applied in a TLC chamber for development once with a single solvent system (single development). In this case, we use a small wide-mouth flatbottom glass jar/bottle with a lid large enough to fit the TLC plate and the mixture of Butanol, acetic acid and distilled water as a mobile phase.

First, we fill the chamber with development solvent to a depth no greater than 0.5 cm, After that, we use tweezers to place the two TLC plates in the prepared development chamber with ts back layer leaning against the chamber's inside wall, and we immediately cover the chamber with the lid fig (4.15).

Development starts once the TLC plate is immersed; when the solvent front has reached an appropriate level (usually within 2 cm of the top of the plate), quickly we remove the lid, and taking out the plate with tweezers, and mark the solvent front with a pencil. At last, we Allow the plates to dry before proceeding to the visualization step.

c) Visualization of colored compounds :

The silica gel on the TLC plate is impregnated with a mixture (50% sulfuric acid and 50% distilled water). When the spots interfere with the mixture and appear as dark spots on a glowing background, we can outline them with a pencil to mark their locations**.**

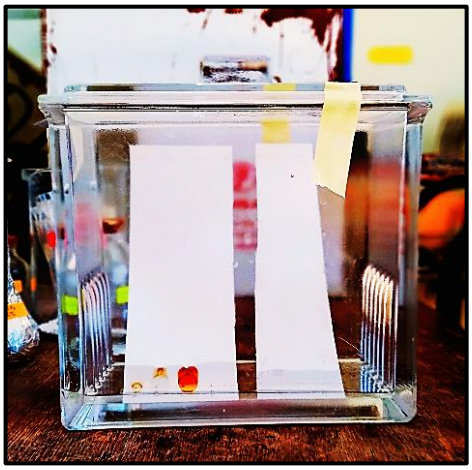


Figure 4.15. Thin-layer chromatography analysis (TLC)

d) Retention factor (R_f) :

The R_f value is used to quantify the movement of the materials along the plate. R_f is equal to the distance traveled by the substance divided by the distance traveled by the solvent. Its value is always between zero and one **[156].**

Retention factor is obtained from the formula :

$$R_f = \frac{di}{D} \qquad 4.11$$

Where :

- R_f : Retention factor.
- di : Distance travelled by component (cm).
- D : Distance travelled by solvent front (cm).

4.3.3.2. Gas Chromatography-Mass Spectrometer analysis (GC-MS) :

Gas chromatography-mass spectroscopy (GC-MS) is one of the analytical techniques which are combined to form a single method of analyzing mixtures of chemicals. Gas chromatography is used to separate the components of a mixture and mass spectroscopy is applied to characterize each of the components individually **[156].**

We used Gas Chromatography (GC) and Mass Spectrometry (MS) in our study because they are excellent tools for the analysis of cinnamon EO and because the semi-volatile and volatile analytes that make up essential oils are readily separated, identified and quantified.

- ***Procedure :***

The essential oil of cinnamon bark was carried out using the Shimadzu GC-MS system (GCMS-TQ8030) (fig 4.16) consisting of a mass selective detector (EIMS, electron energy, 70 eV) and an (Agilent ChemStation) data system. The GC column was an HP-5ms fused silica capillary with a 5% phenyl methylpolysiloxane stationary phase, a film thickness of 0.25 μm, a length of 25 m, and an internal diameter of 0.25 mm. The GC settings were as follows : the initial oven temperature was held at 50 °C for 3 min and then heated at 180 °C at a rate of 2 °C/min, held for 1min, and then heated to 260 °C at 10 °C/min and held for 5 min. The injector temperature was maintained at 250 °C. The sample (1μl, diluted 100: 1 in Hexane) was injected with a split. The carrier gas was helium at a flow rate of 1.0 ml/min. The mass spectral detection was carried out in electronic ionization mode by scanning at 40 to 600 (m / z). Finally, the total time required to analyze a single sample was 35 min.

Most constituents were identified by gas chromatography by comparison of their retention indices with those of the literature or with those of authentic compounds available in our laboratories. Further identification was made by comparison of their mass spectra with those stored in NIST 08 and Wiley 275 libraries or with mass spectra from the literature **[157].**

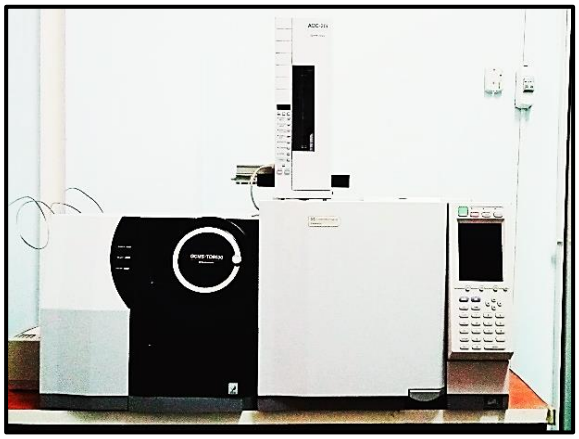


Fig 4.16. Gas Chromatography-Mass Spectrometer analysis (GC-MS)

4.4. Antioxidant Activitie

Essential oils are gaining increasing interest due to their multiple biological activities and great potential for therapeutic use. The antioxidant effect of EO is of special interest in diseases with inflammatory aspects. In our thesis, the antioxidant activities of cinnamon essential oil extract were examined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH).

4.4.1. Free radical scavenging activity : DPPH test :

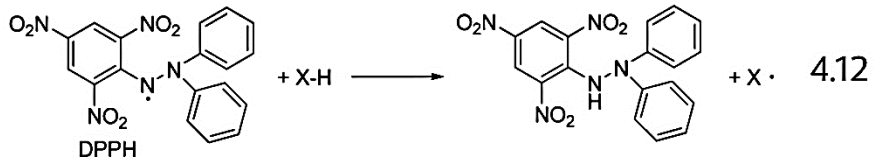
One of the most popular colorimetric assays to estimate the radical scavenging capacity of plants and extracts is the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. This method is accurate, easy to perform, and economical, providing a screening of the general activity of the antioxidants and is based in a stable and synthetic radical, DPPH **[158].**

- ***Mechanism of DPPH test :***

An antioxidant, or any molecule with a weak X−H bond, reacts with the colored and persistent radical DPPH (2,2-diphenyl-1-picrylhydrazyl, λmax between 515 and 517 nm) causing discoloration of the solution from violet to yellow (eq 4.11) **[159, 160].**

Antioxidant activity of standard or essential oils was expressed as IC50, defined as the concentration of the test material required to cause a 50% decrease in initial DPPH concentration **[161].**

Because it depends on the reaction time, taken alone this parameter does not provide meaningful information of the actual reactivity of the antioxidant ; furthermore, data can only be compared when obtained under identical settings **[162].**



4.12

2-diphenyl-1-pycrylhydrazyl (DPPH) 2-diphenyl-1-pycrylhydrazyl (DPPH-H)

➢ ***Apparatus*** :

- Test tubes + holes wooden test tube ;
- Volumetric flasks ;
- Electronic balance ;
- Micropipette ;
- Pipette tips + pro pipette ;
- UV-Visible Spectrophotometer.

➢ ***Reagents :***

- Ethanol ;
- DPPH ;
- EO and extract of Cinnamon ;
- Distilled water.

➢ ***Procedure :***

Free radical-scavenging activity of the EO and extract cinnamon of was evaluated with the modified DPPH (1,1-diphenyl-2-picrylhydrazil radical) assay **[163, 164]** which is based on the measurement of the reducing ability of antioxidants toward the DPPH radical.

Briefly, The DPPH is prepared in 4 mg which is weighed using an electrical balance and then dissolved in 100 ml of methanol. 100 µl of various dilutions of cinnamon essential oil sample diluted in different concentrations (5%,10%,20%,40%,60%,80%100%) were mixed with ml of 0.024 mmol/l methanolic DPPH solution. After an incubation period of 30 min at 25 ◦C, the absorbance at 515 nm was measured. (We prepare the diluted cinnamon extract and return it similarly).

The decrease in absorbance of each essential oil and cinnamon extract was measured at 517 nm using UV/Vis spectrophotomer. Ascorbic acid, a well known antioxidant was used as positive control while DPPH radical solution with 1 ml ethanol was taken as blank **[161].**

The percentage of radical scavenging (%) was calculated by the following formula :

$$\% \text{ Inibition} : ([A_{(blank)}-A_{(sample}]/A_{(blank)}) \times 100 \qquad 4.13$$

Where :

- A(blank) : Absorbance of solution without the test material
- A(sample) : absorbance of sample.

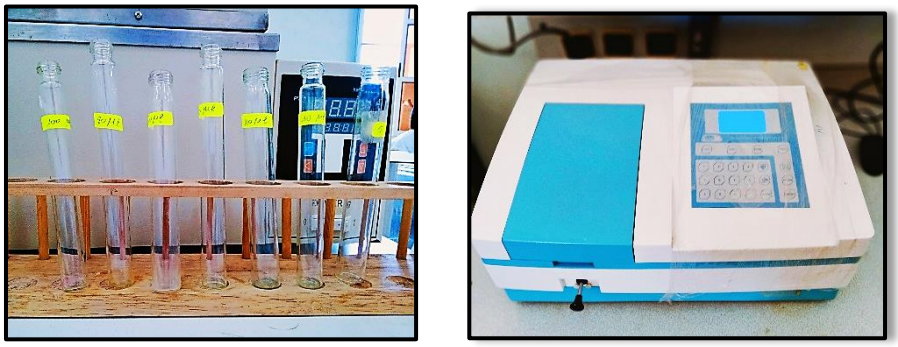


Figure 4.17. DPPH test.

4.5. Antibacterial Activitiy

In the present study, the antibacterial activity of EO and the three extracts of cinnamon were examined against three bacterial strains (fig 4.18) which were obtained from the medicals biologicals analysis laboratory of Dr. A.Zibouche, by two different methods :

- Antibacterial Activity Screening.
- Micro atmosphere assay.

➢ **Bacterial strains :**

- Escherichia coli ATCC 8739 « Gram-negative bacteria ».
- Pseudomonas aeruginosa ATCC 9027 « Gram-negative bacteria ».
- Staphylococcus aureus ATCC 6538 « Gram-positive bacteria ».

These bacteria were selected because they are frequently reported in human infection and are multiresistant to several antibiotics. Strains were maintained in Kligler agar at +4°C.

➢ ***Testing solutions :***

- EO of cinnamon bark extract.
- The different extracts of cinnamon extracted by maceration.

Figure 4.18. Bacterial strains used

4.5.1. Antibacterial Activity Screening

The antibacterial activity of the EO and diffrents extracts of cinnamon was evaluated by the disk diffusion method which is recognized as reliable and reproducible; it is mainly used in a preliminary stage of the in-depth studies because it gives access to qualitative results **[165, 166].** The method was used with slight modifications **[167].**

➢ ***Mechanism of disk diffusion test :***

This method is based on depositing a sterile disk, pre-soaked in EOs, on a bacterial carpet at the beginning of the growth and measuring the zone where the bacteria could not develop: the inhibition zone diameter value, which reflects the antibacterial activity of the EOs.

➢ ***Preparation of bacteria inoculate :***

Bacteria inoculate were prepared by growing cells in Triptic Soy Broth for 24 h at +37°C. The cell suspensions were diluted with peptone water to provide initial cell counts of about 107 to 108 colony forming unit (CFU)/ml.

➢ ***Procedure :***

Firstly we prepared a bacterial suspension in Physiological Sterile Solution [Sodium Chloride 0.9%] and then diluted it to 1/100. 15 ml of Mueller-Hinton agar medium was poured

per Petri dish. 2 ml of the inoculum was deposited on each Petri dish. After 5 minutes of impregnation, the excess inoculum was removed. On the surface of each Petri dish, four sterile filter paper discs of 6 mm (Whatman paper) were impregnated with 15 µl essential oil and The three extracts of cinnamon, then we placed them on the inoculated Petri dishes. After remaining at 4°C for 2 h, the Petri dishes were incubated at 37 °C for 24 h. After incubation, we measured the inhibition zone diameter in millimeters**.**

4.5.2. The micro-atmosphere method [168]

This method evaluates the activity of essential oils vapors on the same strains, technically near the disc diffusion method. It is used to define essential oils' activity, which is to be employed as atmospheric preservatives.

- ***Procedure :***

Solidified medium was inoculated with 100 µl of bacterial inoculums. Then, 10 µl of each testing solutions were added to 6 mm diameter sterile blank filter discs and placed in the center of the cover of the Petri dish in which was previously cast a thin layer of medium to avoid adsorption of testing solutions onto the plastic material of the cover. The dishes were then sealed using sterile laboratory parafilm to avoid eventual evaporation of the essential oils, followed by incubation at 37 °C for 24h.

Finally, the effectiveness of the essential oils was calculated by measuring the diameter (in mm) of the zone of microorganism growth inhibition above the disc.

4.6. Andiabetic Activitie

Diabetes mellitus (DM) is one of the leading metabolic disorder, due to the insufficient or lack of insulin or its action **[169].** The anti-diabetic medications, including sulfonylurea, meglitinide analogue, a-amylase inhibitors, and a-glucosidase inhibitors are generally utilised, which may cause side effects and unfavourable impacts **[170].**

Medicinal plants are used as raw material for the extraction of active secondary metabolites, which is used in the synthesis of the new drugs. Cinnamon is one of the most significant species used by people all over the world. It is extensively utilised in different types of nourishment stuff as well as medicinal products **[171].**

This study gives an overview of in vitro and in silico approaches of various compounds as inhibitors on two proteins related to diabetes mellitus which are α-amylase and Peroxisome proliferators activated receptor gamma (PPARα/γ).

4.6.1. In vitro anti-diabetic study :

This research was designed to evaluate the α-amylase inhibitory activities of CEO on the level of Bio-Ressources Naturelles Locales LBRN at Chlef university.

- ***Determination of alpha-amylase inhibition activity*** :

Alpha amylase inhibitor plays major role in the management of postprandial hyperglycemia **[172].** It inhibits the action of alpha amylase enzyme leading to a reduction in starch hydrolysis to maltose and consequentially lower postprandial hyperglycemia.

The present investigation was undertaken to make a comparative study on the ability of the cinnamon extract to inhibit α-amylase activity.

- ***Apparatus :***
  - Test tubes + holes wooden test tube ;
  - Electronic balance ;
  - Micropipette ;
  - UV-Visible Spectrophotometer.
- ***Reagents :***
  - 1% Starch solution ;
  - α-amylase (2.5 mg/ml) ;
  - potasium phosphate buffer 0.02 M with pH=6.9 ;
  - 50% Citric acid.
- ***Procedure* :**

The assay was carried out following the standard protocol of ***Sindhun et al., 2013*** with slight modifications. The assay mixture containing 0.6 ml of potassium phosphate buffer (0.02 M), 40 µl of α-amylase (2.5 mg/ml) and the essential oils over a various concentrations range (12.5, 25, 50 µg/ml) were incubated for 15 minutes at room temperature, followed by addition of 0.2 ml of starch 1% in all test tubes. The reaction was terminated with the addition of 4 ml Citric acid 50% reagent and placed in a boiling water bath for 5 minutes, cooled and diluted

with 15 ml of distilled water and absorbance was measured at 450 nm using a UV-VIS spectrophotometer. The control samples were prepared without the essential oils.

The inhibition (%) was calculated according to the following formula :

$$\text{Inhibition of α-amylase activity (\%)} = \frac{A(\text{control}) - A(\text{EO})}{A(\text{control})} \times 100 \qquad 4.14$$

Where :

- A (control) : the absorbance of the control at wavelength 450 nm.
- A (EO) : the absorbance of essential oils at wavelength 540 nm.

4.6.2. In Silico anti-diabetic study :

Recently, the strong anti-hyperglycemic effect of cinnamon on mice models has reported but the action mechanism of its bioactive compounds has remained unknown. This study aimed to evaluate molecular interactions existing between various bioactive compounds in cinnamon essential oil and targeted protein related to diabetes mellitus. To do this, we used the following materials :

- ***Micro-computer***

We used a micro-computer with a RAM of 4 GB and all the programs used are installed on Windows 10 operating system.

- ***Programs***

In this study, several programs were used to carry out the practical part (table 4.1) :

Table 4.1. Programs used to carry out in silico study.

Programs	Using	Logo
AutoDockTools V1.5.6	an interactive graphical user interface (GUI) for coordinate preparation, docking and analysis **[173]**	AutoDock 4
Discovery Studio Visualizer V21.1.0	Molecular modeling environment for both small molecule and macromolecule applications, targeted mostly towards the needs in drug discovery and pharmaceutical industry **[174].**	

PyMOL Molecular Graphics System V2.3.2	A cross-platform molecular graphics tool, has been widely used for three-dimensional (3D) visualization of proteins, nucleic acids, small molecules, electron densities, surfaces, and trajectories **[175].**	
AutoDock Vina V1.1.2	a turnkey computational docking program that is based on a simple scoring function and rapid gradientoptimization conformational search **[173].**	/

- ***Preparation of the receptor/target and ligand for the docking***

- ***Receptor preparation :***

Three-dimensional structure of the target protein peroxisome proliferator-activated receptor gamma (PPARα/γ) for the treatment of type II diabetes (PDB ID : 3G9E) were retrieved from Protein Data Bank (www.rcsb.org) (fig 4.19) **[176].**

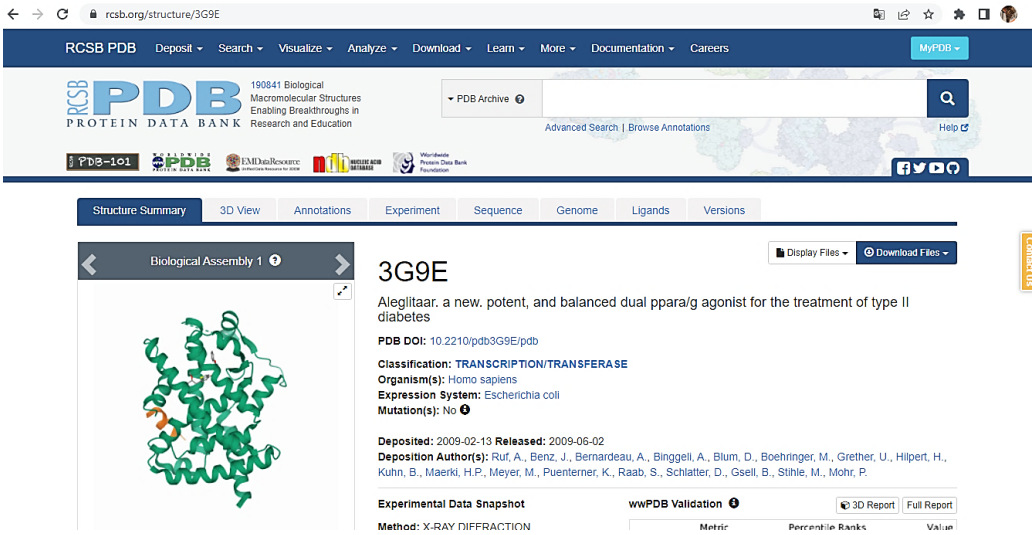


Figure 4.19. Downloading the pdb format file of protein.

The PDB file (3G9E.pdb) contains protein, water, ligands, cofactors, ions, etc., these protein structure inhibitors (water, ligands, cofactors, ions) were separated by releasing atomic coordinates of the PDB file

Firstly, start the ADT program, and use a text editor to remove these Protein structure inhibitors that should not be included in the protein. The file protein.pdb is provided for use as a tutorial for this protocol and it includes protein coordinates taken from PDB entry 3G9E.

Open the file by selecting “Grid->Macromolecule->Open”, use the “Files of type” menu to choose “all files”. Click on coordinate file, in this case, protein, and click “Open”. ADT will read coordinates. Add Kollman charges, merge the polar hydrogens (fig 4.20) because they are required for appropriate treatment of electrostatics during docking, delete water and assign appropriate atom types. Click “OK” to accept the changes. A window will pop up to write the PDBQT file. Click “Save” to register a file protein.pdbqt.

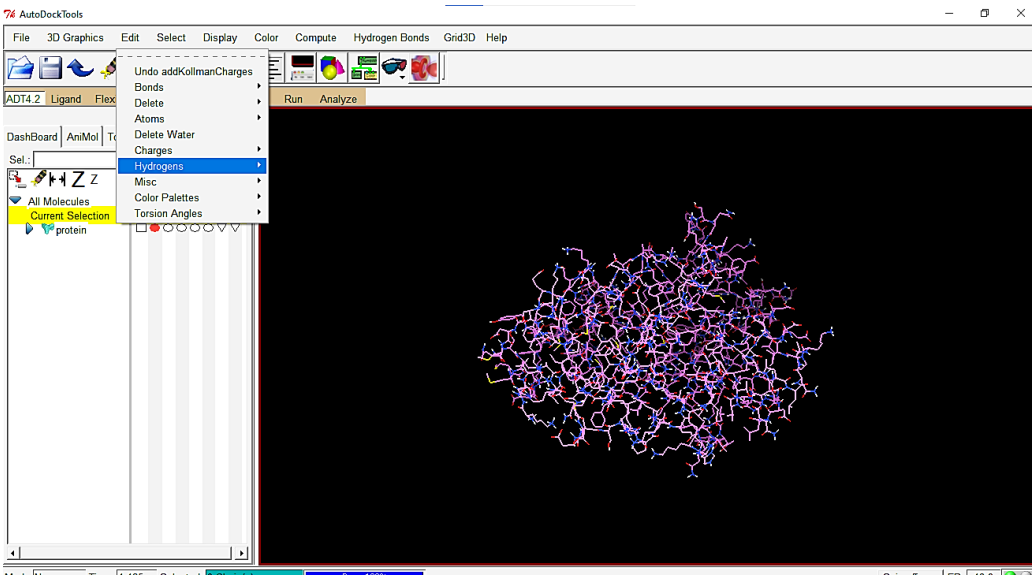


Figure 4.20. Addition of polar hydrogens to protein structure.

- ***Ligand preparation :***

In the drug discovery field, some public repositories (e.g., PubChem) can collect and store chemical information and their biological activities in accessing free of charge to researchers..

The PubChem (https://pubchem.ncbi.nlm.nih.gov/) (fig 4.21) is a free and open resource, which contains information on small molecules and their biological activities **[177].**

The volatile cinnamon compounds (Ligands) are selected according to their therapeutic properties, especially their anti-diabetic activity, Lipinski's Rule of Five are used In order to identify these compounds.

⇨ Lipinski's Rule of Five :

The Lipinski Rule of Five **[178],** was originally conceived to aid the development of orally bioavailable drugs, and was not designed to guide the medicinal chemistry development of all smallmolecule drugs.

The rule states that poor absorption and permeation of a drug is more probable when the chemical structure fulfils two or more of the following criteria :

- Molecular weight (MW) is greater than 500.
- The calculated log P value is above five.
- There are more than five hydrogen bond donors (–NH–, –OH).
- The number of hydrogen bond acceptors (–N ¼, –O–) is greater than ten.

In our work, these parameters were calculated using the SwissADME site (www.swissadme.ch).

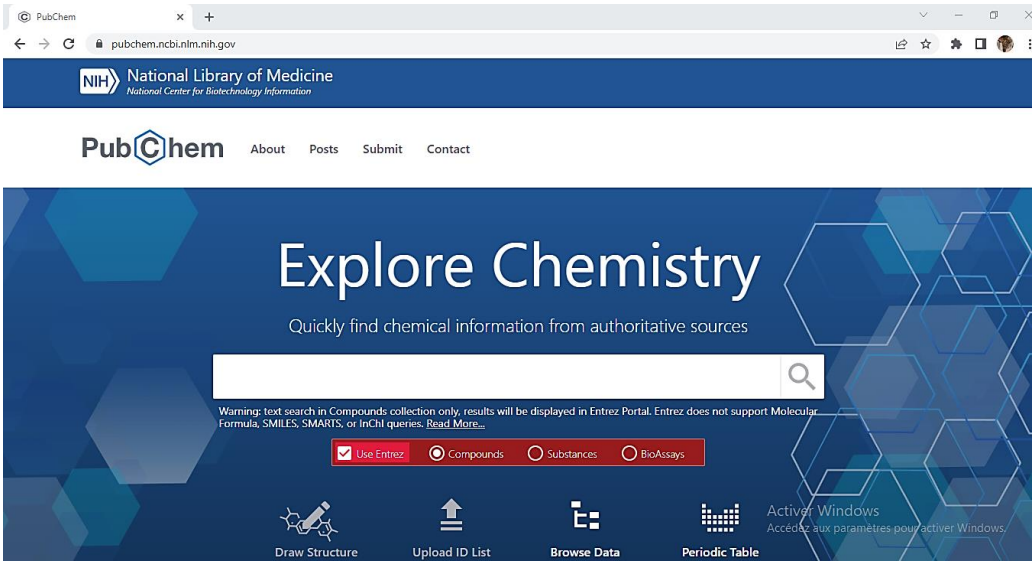


Figure 4.21. Snapshot of the PubChem homepage

After downloading the ligand from PubChemin in sdf format, we change its format to pdb by using a pymol program as follows :

In Pymol open the sdf file then click on **File** > Export Molecule > Save and enter the file name (fig4.22), at last, change the option of SDF and save it as **ligand.pdb** (fig 4.22). The next step is the preparation ligand.pdbqt file.

Note : Be careful with case sensitive of letter and naming of protein, naming as it may affect the follow-up commands execution.

- Close and re-open **AutodockTool** to avoid any confusion.
- Open Ligand and Click on **Input** and then **Open.**
- Change file format from pdbqt to pdb in next pop-up window.

- Select **ligand.pdb** and then click **Open.**
- Again, Open Ligand menu then Click on **Output**, followed by clicking Save as **PDBQT.**
- Save Ligand file as ligand.pdbqt in specified working folder where protein.pdbqt was saved.

After preparation and saving of protein.pdbqt and ligand.pdbqt files, next step is the Preparation of Grid Parameter File.

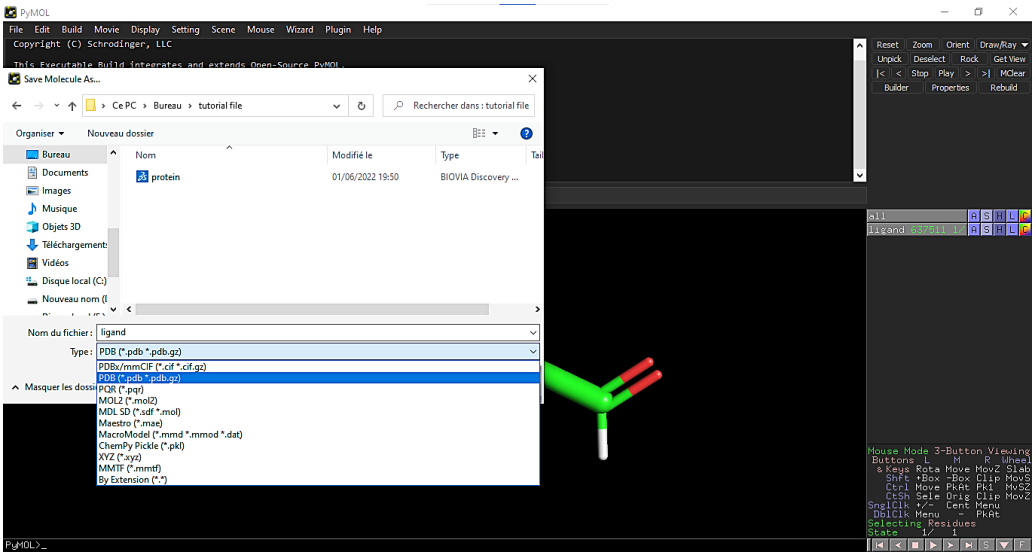


Figure 4.22. convert Sdf format to PDB using Pymol

➢ ***Preparation of Docking Parameter File (conf.txt) :***

Generate a configuration file (Box 1) for AutoDock Vina that specifies the PDBQT files for the ligand and protein, and defines the docking parameters. To do this, restart ADT and set the default working directory.

Select "**Grid->Macromolecule->** Open Protein **PDBQT** file, click "**Yes**" to preserve the existing charges in the file, and "OK" to accept. There may also be a warning window if there are slight irregularities in charges. Click "**OK**" if it appears.

Select "**Grid-> GridBox**", opens a window for defining the center and size of the search space (Fig 4.23). Other options are available, or the values may be changed manually with the thumbwheels.

Important : when finished, choose "**File->output Grid** dimensions file" write the configuration file (**Grid.txt**) then click on "Save".

Make sure to save conf.txt in the same folder or working directory where protein.pdbqt and ligand .pdbqt files were already saved.

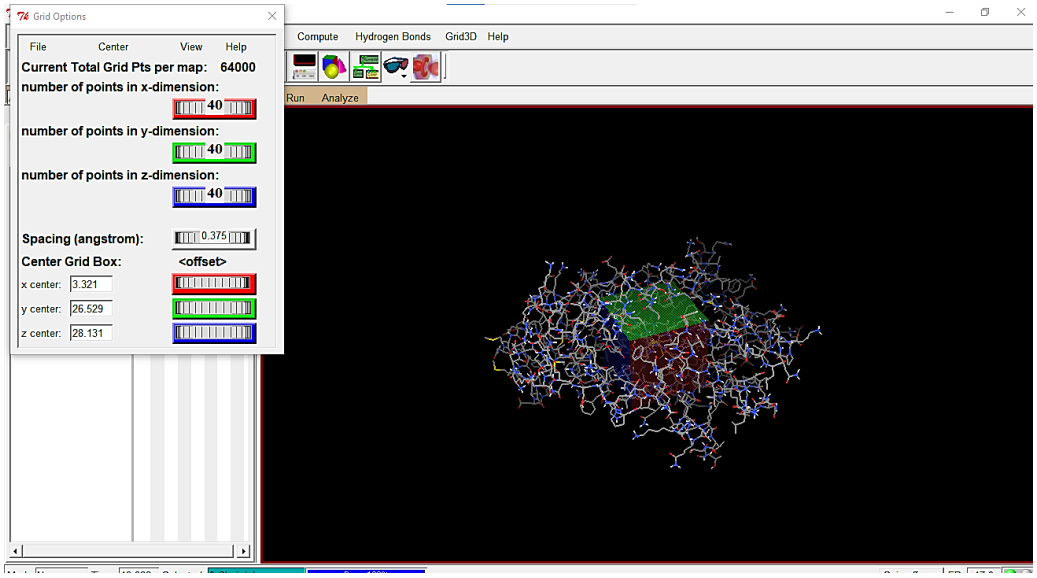


Figure 4.23. The best docking site on our protein using Autodock Tools 1.5.6

Box 1 | Vina configuration file

```
receptor = protein.pdbqt
ligand = ligand.pdbqt
center_x = 3.321
center_y = 26.529
center_z = 28.131
size_x = 40
size_y = 40
size_z = 40
energy range = 4
exhaustiveness = 8
```

Figure 4.24. Docking parameter file (conf.txt).

- ***Running Autodock Vina :***

First, locate the installed vina in our default installation directory. Default installation directory could be “C:\Program Files (x86)\The Scripps Research Institute” Copy that vina.exe,

vina_licence.rtf and vina_split.exe files from the installation folder into the working directory (In current case, it is working directory on Desktop\tutorial file"

Next step is running vina command using Command Prompt (command line in window) for Molecular Docking (fig 4.25).

Open **CMD**, from Window START search for CMD and click on **Command prompt**.

Go to the working directory where required files are present using the command :

« cd C:\Users\HP\Desktop\tutorial file » in this case study, Press enter and then type the following commands « vina.exe - -config conf.txt - -log log.txt » .

It will take quite some time to complete (from seconds to minutes) depending on the performance of system, when processing is finished, the results are saved in the log.txt file and and will go shift back to the working directory location.

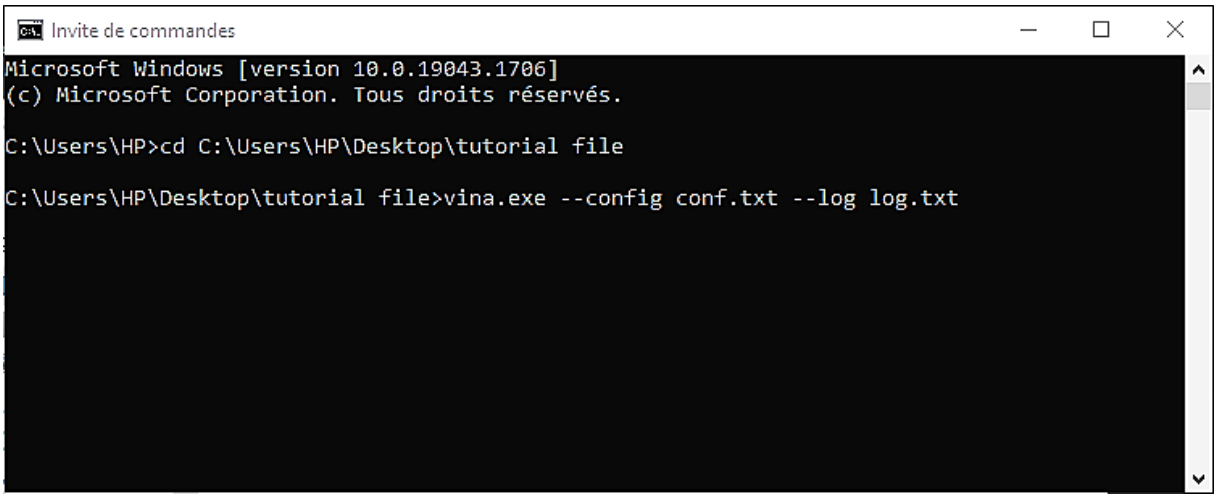


Figure 4.25. Command line window before execution of autodock vina commands.

- ***Evaluation of Docking and Virtual Screening :***

For analyzing the docking results and retrieving the binding interaction of the Ligand-protein complex, Open **protein.pdbqt** in Discovery Studio, then drag and drop ligand.pdbqt from the working directory into the graphical window of discovery studio as shown in Figure 4.26.

Click **Receptor-Ligand Interactions** in DS followed by clicking Ligand Interactions on left hand side to show the 3D interaction.

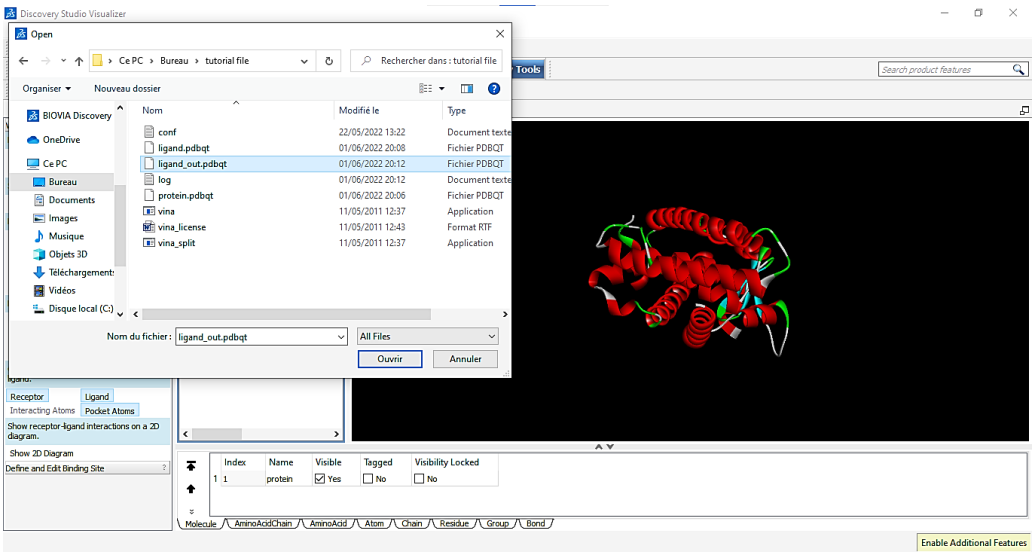


Figure 4.26. Opening and displaying the protein and ligand in DS.

For Saving the interactions diagrams as an Image file, go to File and then Save As, Change the file format from Discovery Studio files to Image Files.

Name the new file as ligand, Click **Save** and then **OK** to Save in the working directory.

To show the 2D interaction, click on Show 2D Diagram on left-hand side in the ligand interaction window ; this will also generate the 2D image of the binding interaction between our ligands and protein.

Chapter 5

Results and Discussion

Chapter 5 : Results and Discussion

5.1. Introduction :

In this work, we extracted essential oils from cinnamon bark and examined their organoleptic, physico-chemical characterizations, biochemical and biological activity, and anti-diabetic effects using two approaches (in vitro and in silico) Then we discussed the results that were obtained.

5.2. Extraction methods

5.2.1. Steam distillation : (Hydrodistillation) :

➢ ***Yield results (%) :***

The first quantification to be made is the yield of essential oil obtained by the steam distillation method. The percentage of cinnamon EO yield is calculated from the weight of the EO compared to the dry weight of the bark powder mass used in the steam distillation; the results are present in the table and the figure below:

Table 5.1. Masses used in steam distillation method

Mass of initial plant	Mass of the extracted oil+ eppendorf tube	Mass of eppendorf empty	Mass of the extracted oil
150 g	2.7403 g	0.92 g	1.8203 g

- Yield value :

$$\text{Yield}(\%) = \frac{1.8203}{150} \times 100 = 1.214\%$$

Table 5.2. Percent yield results in steam distillation method.

Type of Cinnamon	The percent yield	standards
Vietnamese cinnamon	1.214%	0.2-0.4% **[179]**

Figure 5.1. Essential oil obtained of cinnamon by steam distillation.

➢ ***Yield Discussion :***

According to the results and as shown in the table and the figure above, the cinnamon essential oil has yellowish color with a spicy odor, and it has also been extracted with the resulting 1.214% yields.

Compared to other species of the same family (fig 5.2) studied in our laboratory, our yield is considered superior compared to that obtained by ***Taibi and Ben-Hadj-Tahar., (2018)*** which is about 0.8668% for Chinese cinnamon and about 0.8666 % for Moroccan cinnamon. It should be noted that all of these essential oils were obtained by hydrodistillation in a Clevenger for 3 hours.

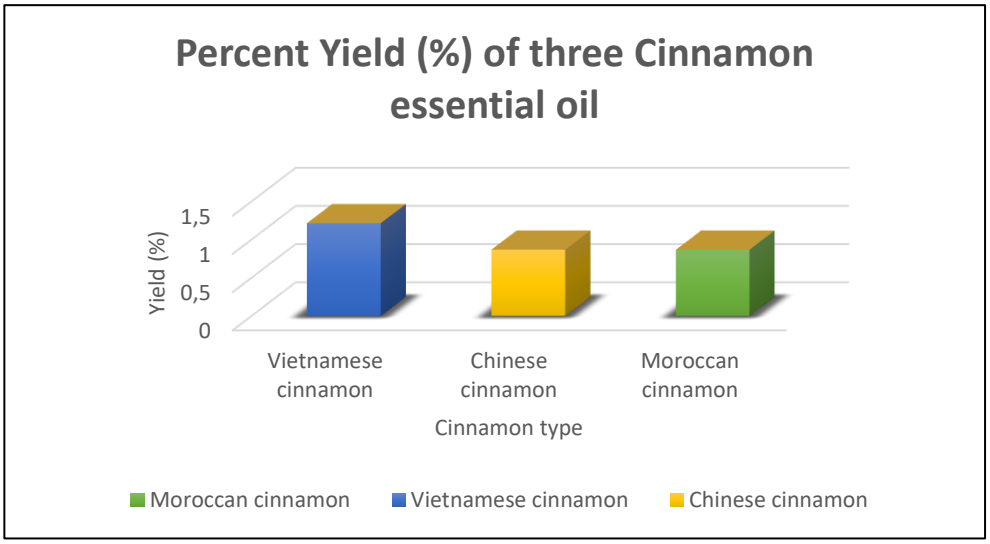


Figure.5.2. Graphic representation of the different yields (%) of the essential oils of the three types of cinnamon.

These differences are due to several factors, ***Seu-Saberno et Blakeway, (1984) ; Svoboda et Hampson, (1999)*** explain that the chemical composition and the yield of essential oils vary according to various conditions, wich is as follow :

- ✓ Climatic environment ;
- ✓ Localization ;
- ✓ Genotype ;
- ✓ Geographical origin ;
- ✓ Harvest period ;
- ✓ Place and duration and temperature of drying ;
- ✓ Parasites ;
- ✓ Viruses and weeds ;

The yield of Vietnamese cinnamon essential oil which recorded in this study was found to be relatively higher. This has revealed that this type of cinnamon spice can be regarded as oil crops.

5.2.2. Solvent extraction method (maceration)

The extraction is carried out with different solvents (at room temperature 25°C) in order to select the best extraction solvent. The solvents that were tested in this part of our research are listed in the following table :

Table 5.2. Some properties of the solvents used.

Solvents used	Chemical formula	Density (20°C)	Boiling point (°C)	Polarity	water solubility
Ethanol	C_2H_5OH	0.81	78.5	highly polar	Miscible
Acetone	CH_3COCH_3	0.79	56.53	Polar	Miscible
water	H_2O	1	100	Polar	Miscible

➢ ***Results :***

We have classified the results in the table and figures below :

Figure 5.3. Extracts obtained by maceration method

- Influence of the nature of the solvent on the yield :

Table 5.3. Results of the extraction of Cinnamon by maceration

Type of sample	Solvent	Initial weight (in g)	Final quantity (in g)	Yield (%)
Powder	Ethanol	20 g	16.95 g	15.25%
Powder	Acetone	20 g	17.02 g	14.9%
Powder	Water	20 g	18.47 g	7.65%

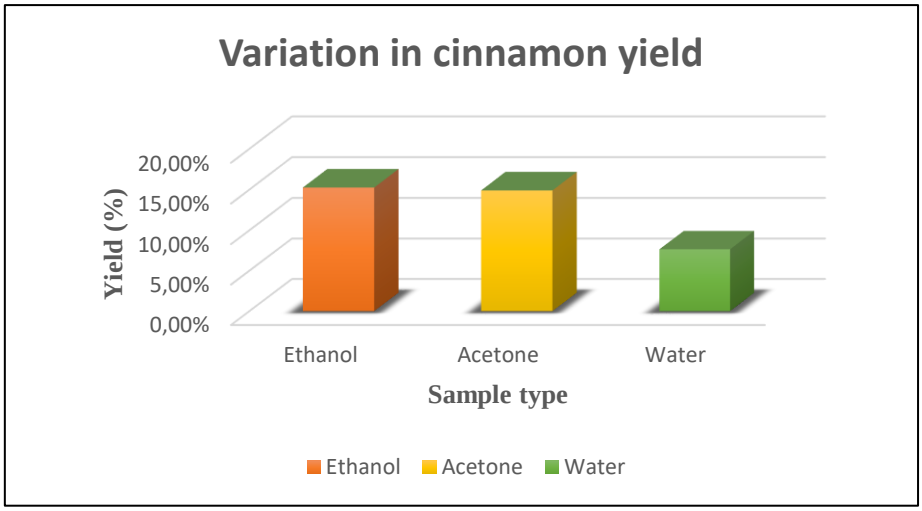


Figure 5.4. Variation in cinnamon yield according to the type of solvent.

➢ ***Discussion*** :

We used in our study three solvents with decreasing polarity: Ethanol 96%, Acetone 96% and distilled water. After studying the table above, it becomes clear to us that the best yields are obtained respectively for the three solvents ; Ethanol 96% (15.25%), Acetone 96% (14.9%) and Water (7.65%).

Reading the extraction yields shows that the highest yields were obtained with polar solvents (96% ethanol and 96% acetone) and the lowest yield is observed for water (7.65%). These results (fig 5.4) can be explained by the differential solubility of different phenolic compounds or polar aromatic plants which are soluble in polar solvents.

In terms of yield, we can say that Ethanol and acetone are the best extraction solvents.

5.3. Characteristics of cinnamon essential oil

5.3.1. Organoleptic properties of cinnamon :

- ✓ ***Taste :*** Spicy and sweet.
- ✓ ***Texture :*** Mobile and mildly viscous liquid.
- ✓ ***Color :*** Golden Yellow.
- ✓ ***Aroma :*** Good smell and woody

5.3.2. Physico-chemical properties of cinnamon :

5.3.2.1. physical properties of cinnamon :

a. Relative density :

The value of cinnamon EO density is calculated from the ratio of the mass of a given volume of the essential oil at 20 °C divided by the mass of an equal volume of distilled water at 20 °C. The results are present in the tables below :

➢ ***Results :***

Table 5.4. Relative density of cinnamon essential oil.

sample type	mass of EO+eppendorf,	mass of water+eppendorf,	Mass of empty eppendorf	Relative density	Standars [180]
CEO	2.6025 g	2.5659 g	0.92 g	1.022	1.000-1.030

➢ ***Discussion :***

At the beginning of the applied study and after we extracted the essential oil of Vietnamese cinnamon, we put the distillate in the separating funnel and we noticed that it was sinking at the bottom of the separatory funnel until we added sodium chloride to make the water denser, This proves that cinnamon essential oil is denser than water and it can be said that the oil conforms to international standards (***According to the European Pharmacopoeia., (2011)***).

Our result are same to those found by ***Poaty et al., 2015*** where they calculated the density of essential oils of plants extracted by steam distillation method and found that two of these oils had a higher density than water which are the essential oil of cloves (1.0561) and Chinese cinnamon (1.0495) and this confirms our results **[181].**

b. Refractive index :

➢ ***Results :***

The refractive index were calculated and brought to 20 °C using a refractometer and are shown in the following table and figure :

Table 5.5. Refractive index for cinnamon essential oil

Sapmle type	$n^{t'}_D$	n^T_D	Standars
Cinnamon EO	1.615	1.6138	1.600-1.615 **[182]**

Figure 5.5. Refractive index as shown on refractometer

➢ ***Discussion :***

Compared to these results, we note that IR of Cinnamon essential oil 1.615 is higher than IR of water 1.333.

This value depends on the chemical composition of the oils and the temperature. It also indicates the ability of essential oils to reflect light (Hellal, 2011). It essentially varies with the content of monoterpenes and oxygenated derivatives (Chouitah, 2012). High content of mono terpenes will give a high index. So we can say that the essential oil of cinnamon is rich in monoterpenes and has the power of refraction to light.

c. pH value :

The pH measurement is done by two methods, the results are shown in the figures below :

Figure 5.6. pH measurement by pH paper and pH meter

The figures show the results obtained with the pH meter is 5.86, while the results of using the pH paper showed a change in the color of the paper between [5-6].

➢ ***Discussion :***

The determination of the pH is carried out using a pH paper for the essential oil and the pH meter for Cinnamon bark, where Cinnamon has a pH of 5.86. Our pH of bark and essential oil is acidic, this conformity is due to the purity of our oil (well separated from water).

d. Relative humidity :

➢ ***Results :***

The moisture content results are classified in the following table :

Table 5.6. Moisture content results are classified in the following table :

Sample type	Beaker +5g of the sample	Beaker after desiccator.	Relative humidity	Standars
Powder of cinnamon bark	45.52 g	44.955 g	11.3%	10% to 12% **[183]**

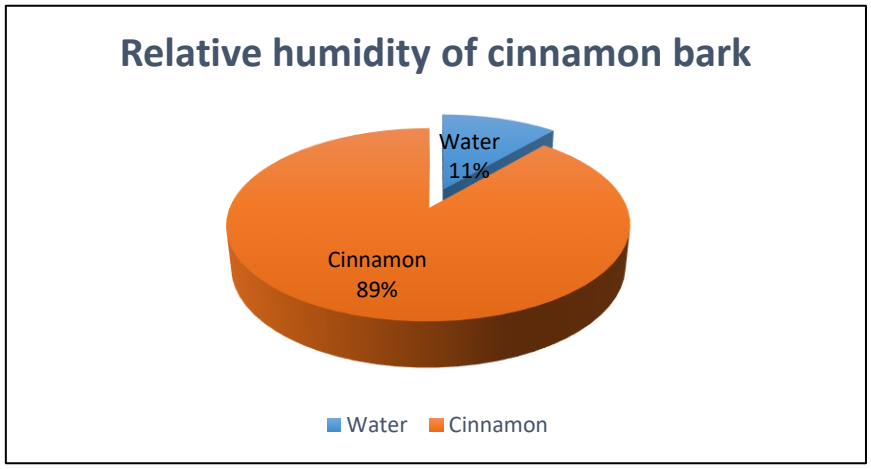


Figure 5.7. Water content in cinnamon.

➢ ***Discussion :***

The analysis results of our sample revealed that the moisture content of cinnamon bark is 11.3%.

According to these results, vietnamese Cinnamon contains few water molecules. What indicates that This cinnamon is of better quality because of several factors such as the method of conservation, the climate, the region or the nature of the soil.

e. Relative calcination (Ashes) :

➢ ***Results :***

The relative calcination results are classified in the following table :

Table 5.7. Represents the calcination for the cinnamon.

Sample type	Platinum crucible + ashes	Platinum Crucible empty	Mass of powder	Relative calcination (%)	Standars
Powder of cinnamon bark	49.3127 g	49.2771 g	1.0002	3.55%	≤ 6 **[183]**

➢ ***Discussion :***

The quantity of ashes present in the cinnamon sample which is 3.55% indicates that this value meets the standards (<6). These results indicate that this type of cinnamon contains more organic matter which also means that it is poor in minerals.

5.3.2.2.Chemical properties of cinnamon :

a. Acid value (neutralization number) :

➢ ***Results :***

Figure 5.8. Acide value results.

The results of acid value are shown in the following table :

Table5.8. Represents the cide value for the cinnamon EO.

Sample type	Volume	AV	Standars
Cinnamon EO	0.3 ml	16.83 mgKOH/g	<20 **[184]**

➢ ***Discussion :***

The acid values for cinnamon was found to be 16.83 mg KOH/g and it complies with the standards **[184].** This acid index indicates the behavior and quantity of free acids present in our oil, which provides informations about the quality susceptibility to alterations of essential oils.

Actually, the higher the acid index, the lower the quality of essential oils because it represents the total concentration of free fatty acids found, this acidity is influenced by the microorganisms and their enzymes and the quantity of water found in the oil. According to our results, the cinnamon essential oil has a low acid value; this shows that our essential oil has an excellent storage quality.

b. Saponification value :

➢ **Results :**

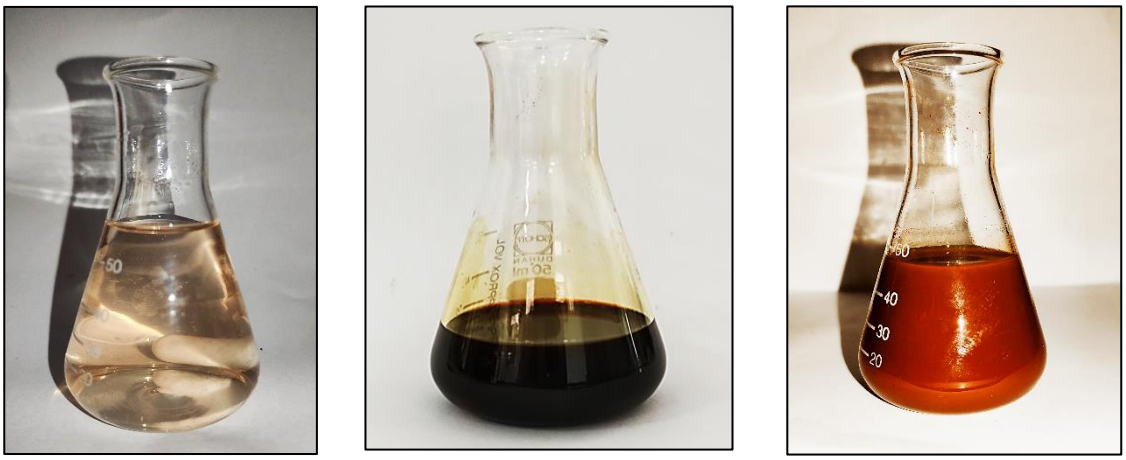


Figure 5.9.Saponification value for titration of the blank and for titration of the essential oil

The results of saponication value are shown in the following table :

Table 5.9. Represents the saponification value for the cinnamon EO.

Sample type	Mass of sample	Volume (c)	Volume (d)	SV	Standars
Cinnamon essential oil	0.5010 g	24.3 ml	23.2 ml	61.71mgKOH/g	33.6 **[180]**

➢ ***Discussion :***

The saponification value of the Cinnamon EO was 61.71 mg KOH/g, which means that it has potential for soap production. This indicates that the EO could be used in soap making since its saponification value is high.

c. Ester Value (EV) :

➢ ***Results :***

The results of the ester index are classified in the following table :

Table 5.10. Result of the ester index for the essential oil of cinnamon

Sample type	Acid value	Saponification value	Ester value	Standars
Cinnamon Essential oil	16.83	61.71	44.88	20.14 **[185]**

➢ ***Discussion :***

The result obtained shows that our ester index which was measured higher than that of the maximum value of the pharmacopeia standard which is 20.14, which indicates that our oil is rich in esters and FFA which combined in the form of triglycerides.

d. Peroxide value (POV) :

➢ ***Results :***

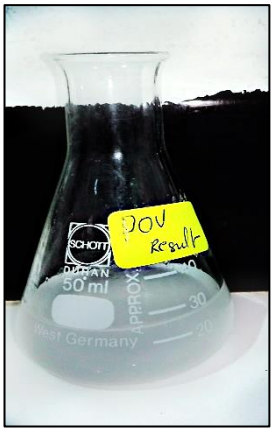


Figure 5.10. Result of POV value

The results of peroxide value are shown in the following table :

Table 5.11. Results of the POV for the essential oil of cinnamon

Saple type	Volume (e)	Volume(f)	POV	Standars
Cinnamon essential oil	20.4 ml	18.1 ml	22.94 meq/kg	<80 **[180]**

➢ ***Discussion :***

Peroxide value measures the deterioration of oil from oxidation. Therefore, the low peroxide value of 22.94 meq/kg obtained from Cinnamon essential oil indicates that the oil can be kept for a long period of time. The peroxidation rate differed from oil to oil according to the different treatments to which the oils were subjected. Experts see that the Oils exposed to both atmospheric oxygen and light showed a much more significant increase in peroxide value during storage.

However, this index is only an indicator of the start of oxidation, which makes it possible to evaluate the quantity of peroxides present in the oil, which provides information on the relative of fatty acids **(Diramanetal., 2010).**

5.3.3. Analysis of the chemical composition of Cinnamon essential oil :

5.3.3.1. Thin-layer chromatography analysis (TLC) :

➢ ***Results :***

The present study was conducted to isolate the main bioactive compounds from the bark of Cinnamon; the results of the characterization of these compounds appear in the figure below:

- ***Mobile phase :***

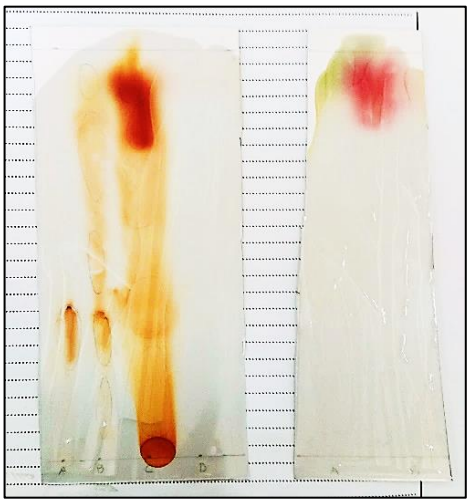


Figure 5.11. Thin layer chromatography chromatogram of cinnamon essential oil after development.

✓ (A) Distilled water extract.
✓ (B) Acetone extract.
✓ (C) Ethanol extract.
✓ (A') Cinnamon EO.

The retention factors (Rf) values for the different extracts are presented in Table 5.12.

Table 5.12. Retention factors (Rf) values for the different extracts.

Sample type	Distilled water extract.	Acetone extract.	Ethanol extract.	Cinnamon EO.
di (cm)	6.7 cm	3.6 cm	4.9 cm	17.8
		6.5 cm	7.9 cm	
		10 cm	11.5 cm	
		15.3 cm	15.3 cm	

		17.4 cm	17.8 cm	
R_f	0.37	0.2	0.27	0.98
		0.36	0.43	
		0.55	0.63	
		0.85	0.85	
		0.96	0.98	

➢ ***Discussion :***

⇨ **Identification by comparison of Rf values of cinnamon compounds**

The R_f values can be affected by a number of different factors such as : layer thickness, moisture on the TLC plate, vessel saturation, temperature, depth of mobile phase, nature of the TLC plate, sample size, and solvent parameters.

From the conclusions based on the R_f-values of cinnamon components compiled from literature, the following compounds (β-caryophyllene; cinnamaldehyde; eugenol; coumarin; linalool; and methyl chavicol) were identified and we matched it with compounds found in our cinnamon extracts.

A summary of comparison of the experimental R_f values of cinnamon powder components to those from literature is tabulated in Table 5.13 below **[186] :**

Table 5.13. Summarized table of visualized cinnamon compounds from literature review

compound	R_f value	Standars
β-caryophyllene	0.9	**[186]**
Cinnamaldehyde	0.5	**[186]**
Eugenol	0.47	**[186]**
Coumarin	0.40	**[186]**
Linalool	0.30	**[186]**
Methyl chavicol	0.20	**[186]**
Carvone	0.50	**[186]**

The elution solvent system used 1% vanillin in ethanol and 5% ethanol solution in sulphuric acid.

We noticed that our three extracts contain several compounds of brown color, represented by a single spot for the extract of distilled water, five spots for the extract of acetone and other five spots for the extract of ethanol.

And for the cinnamon essential oil, we noticed two spots of different colors, one of them is green and is one of the compounds of our oil and the other is pink and it is a reference spot.

After comparing the references in table 5.13, the working conditions and our results we see that our Rf is very close to those of reference,

At first, we can notice just the Linalool spot in water extract and it can be observed in the lower zone (R_f ~0.37) on the standard chromatogram. In the acetone extract, we were able to identify some spots which are as follows: Methyl chavicol (R_f ~0.2), Linalool (Rf ~0.36), Carvone or Cinnamaldehyde with (R_f ~ 0.55) and β-caryophyllene (R_f ~0.96). We noticed in the ethanol extract the presence of several spots, some of which we identified by literateur references, which are : Methyl chavicol (R_f ~0.27), Eugenol or Coumarin (R_f ~0.43), Cinnamaldehyde (R_f ~ 0.63) and β-caryophyllene with (R_f ~0.98). Finaly, β-caryophyllene can be identified in the upper zone as a red light zone (R_f ~0.98) in our oil extract.

As a final result, it can be concluded that the spots of the same extract having a difference of R_f >0.2 can be easily separated by TLC using the appropriate mobile phase (***Still et all., 1978***) **[187].**

5.3.3.2. Gas Chromatography-Mass Spectrometer analysis (GC-MS) :

➢ **Results :**

The volatile components collected from the SD method were separated and identified by GC-MS. Total ionization chromatograms (TIC) of the volatile constituents of CEO extracted are shown in (Fig 5.12), and the identification results of volatile components are listed in (Table 5.14).

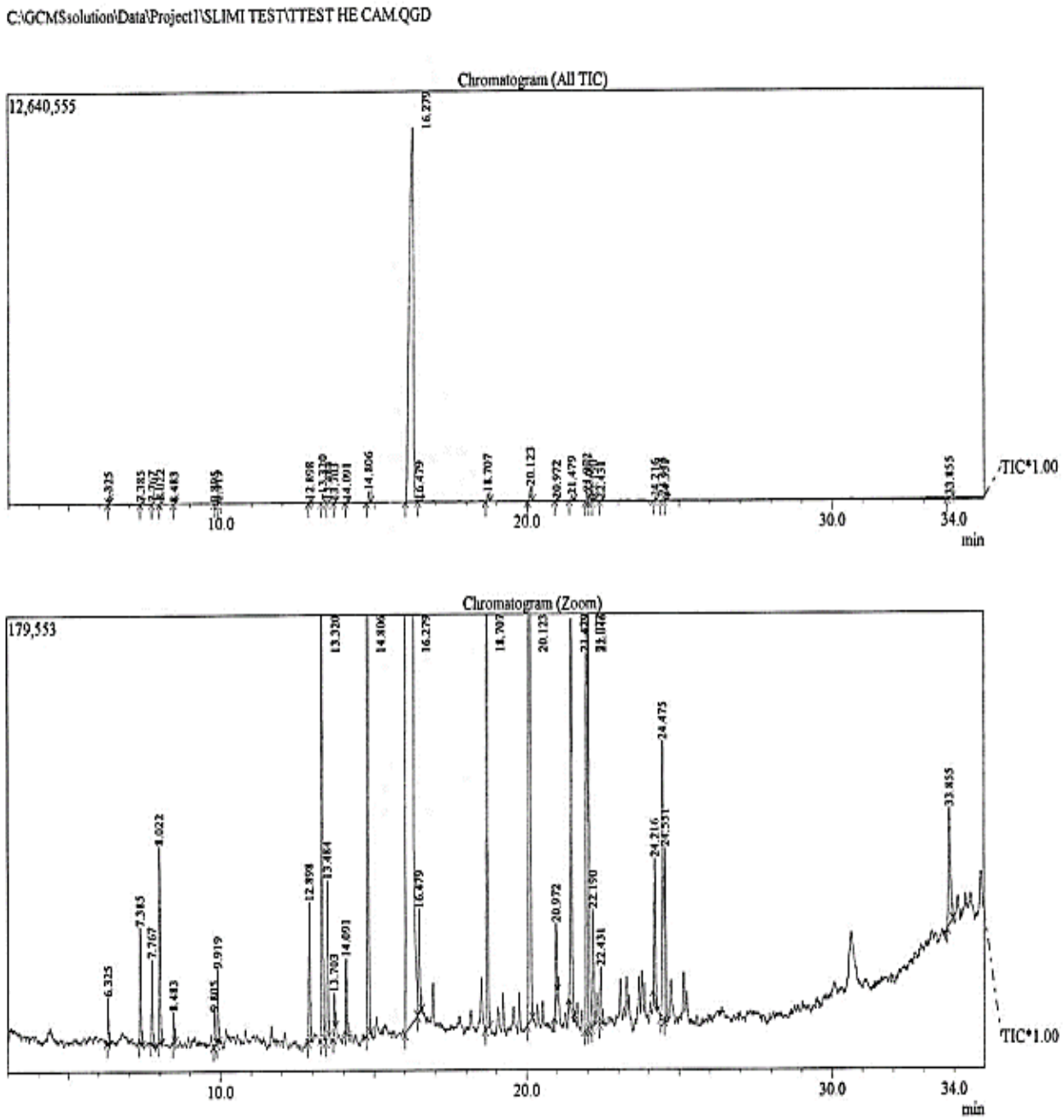


Figure 5.12. Total ions chromatograms (TIC) of cinnamon essential oil extracted by steam distillation.

Table 5.14. Volatile components (%) of cinnamon essential oil extracted

C:\GCMSsolution\Data\Projet1\SLIMI TEST\TTEST HE CAM.QGD

Peaks	Ret.Time (min)	Compound	Synonym	Formula	Erea %
1	6.325	Styrene		C_8H_8	0.03
2	7.385	Bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl-	α-Pinene	$C_{10}H_{16}$	0.09
3	7.767	Camphene		$C_{10}H_{16}$	0.06
4	8.022	Benzaldehyde		C_7H_6O	0.16
5	8.483	β-Pinene		$C_{10}H_{16}$	0.02
6	9.805	Cyclohexane, 1-methyl-4-(1-methylethenyl)-	DL-Limonene	$C_{10}H_{16}$	0.04
7	9.919	1,8-Cineole		$C_{10}H_{18}O$	0.06
8	12.898	2-methylbenzofuran		C_9H_8O	0.13
9	13.320	Benzenepropanal		$C_9H_{10}O$	0.55
10	13.484	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)-	Borneol	$C_{10}H_{18}O$	0.15
11	13.703	2-methylbenzofuran		C_9H_8O	0.03
12	14.091	3-Cyclohexene-1-methanol, α,α4-trimethyl-	α-Terpineol	$C_{10}H_{18}O$	0.07
13	14.806	2-Propenal, 3-phenyl-	cinnamaldehyde	C_9H_8O	0.80
14	16.279	2-Propenal, 3-phenyl-	cinnamaldehyde	C_9H_8O	93.16
15	16.479	Benzene, 1-methoxy, 4-prop-2-enyl	Estragole	$C_{10}H_{12}O$	0.12
16	18.707	Copaene		$C_{15}H_{24}$	0.57
17	20.123	(E)-Cinnamyl acetate		$C_{11}H_{12}O_2$	1.29
18	20.972	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1α,4aβ,8aα)-	γ-Cadinene	$C_{15}H_{24}$	0.06
19	21.479	α-Muurolene		$C_{15}H_{24}$	0.44
20	21.972	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-	δ-cadinène	$C_{15}H_{24}$	0.71
21	22.046	2-Propenal, 3-(4-methoxyphenyl)-	4-Methoxycinnamaldehyde	$C_{10}H_{10}O_2$	0.44
22	22.190	Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-	1,4-Cadinadiene	$C_{15}H_{24}$	0.12
23	22.43	1-Isopropyl-4,7-dimethyl-1,2-dihydronaphthalene	α-Calacorene	$C_{15}H_{20}$	0.06
24	24.216	α-Cubebene		$C_{15}H_{24}$	0.13
25	24.475	tau-Muurolol		$C_{15}H_{26}O$	0.34
26	24.551	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1α,4aβ,8aα)-	γ-Cadinene	$C_{15}H_{24}$	0.17
27	33.855	Tetrapentacontane		$C_{54}H_{110}$	0.20

➢ ***Discussion :***

⇨ ***Identification of volatile compounds of CEO :***

TIC results (Fig 5.12) showed that the retention time of the volatile components ranged between 6 and 35 min, most of which were concentrated between 22 mins to 40 mins.

As outlined in the figure above, 26 peaks were observed in our sample. we can note the two highest peaks than the rest of the compounds which are cinnamaldehyde (16.279 min) then followed by The (E)-Cinnamyl acetate (16.279 min).

Table 5.14 shows that many kinds of compounds were identified, including aldehydes, esters, alcohols, terpenes and aromatics. The major abundant component of CEO was cinnamaldehyde 93.96 %, otherwise, the minor compounds were (E)-Cinnamyl acetate (1.29%), Benzenepropanal (0.55%), Copaene (0.57%), δ-cadinène (0.71%), α-Muurolene and 4-methoxycinnamaldehyde (0.44%), and other compounds (0.03~0.34%).

2-propenal, 3-phenyl or cinnamaldehyde (93.16%) of the essential oil extracted from cinnamon bark is the compound most responsible for the cinnamon's ragrant odor. The quantity of phytochemical present in cinnamon bark that is grown in vitenam is different from other area. This is due to geographical position, cultivation, variety of cinnamon; harvesting time and extraction method affect the actual yield and composition of the essential oil **[188].**

⇨ ***Biological activity of volatile compounds of CEO :***

The cinnamon essential oil are used in the manufacture of perfumes,soaps and also as a flavoring in dentifrices. Besides these, cinnamon has a broad spectrum of medicinal and pharmacological application **[189].**

Table 5.15 is a guide showing some of the biological activities of some of the compounds that we obtained in our CEO :

Table 5.15. Biological activities of cinnamon essential oil compounds

volatile compound	Biological activities	References
α-Pinene	anti-inflammatory, antimicrobial, antioxidant, antidiabetic.	**[190, 191]**
Camphene	Antioxidant, antidiabetic, antifungal	**[192, 193, 194]**

Benzaldehyde	Antioxidant, antimicrobial,	**[195]**
β-Pinene	antimicrobial, antiinflammatory,	**[196]**
DL-Limonene	Antioxidant, antidiabetic, anticancer	**[197]**
1,8-Cineole	anti-inflammatory , antioxidant	**[198]**
2-methylbenzofuran	anti-microbial, anti-diabetic, anti-cancer	**[199]**
Benzenepropanal	Antibacterial, antidiabetic, antitumour	**[200]**
borneol	Antimicrobia, antiinflammatory, antiviral	**[201]**
α-terpineol	Antioxidant, antiulcer, anticonvulsant	**[202]**
Cinnamaldehyde	antioxidant, antibacterial, anti-mutagenic, antidiabetic	**[203]**
Estragole	antioxidant and antimicrobial	**[204]**
Copaene	Antimicrobial, antifungal, antioxidant	**[205]**
(E)-Cinnamyl acetate	Antidiabetic, antioxidant, antimicrobial	**[206]**
γ-Cadinene **α-Muurolene** **δ-cadinene** **1,4-Cadinadiene** **α-Calacorene** **α-Cubebene** **tau-Muurolol**	-Antitumor (anticancer). - Anti-inflammatory - Antibacterial -Antiviral -Antiparasitic -Antiulcer -analgesic	**[207]**
4-Methoxycinnamaldehyde	Antimicrobial, antiviral, anti-inflammatory,	**[208]**

1.1. Antioxidant activity

1.1.1. Free radical scavenging activity (DPPH test) :

➢ **Results :**

The antioxidant activity of our oil and extract has been evaluated by the DPPH test. The evaluation of the antioxidant activity of essential oil and extract was made in comparison with the standard antioxidant ascorbic acid which was prepared in the same conditions as presented in the following figures, tables and curves:

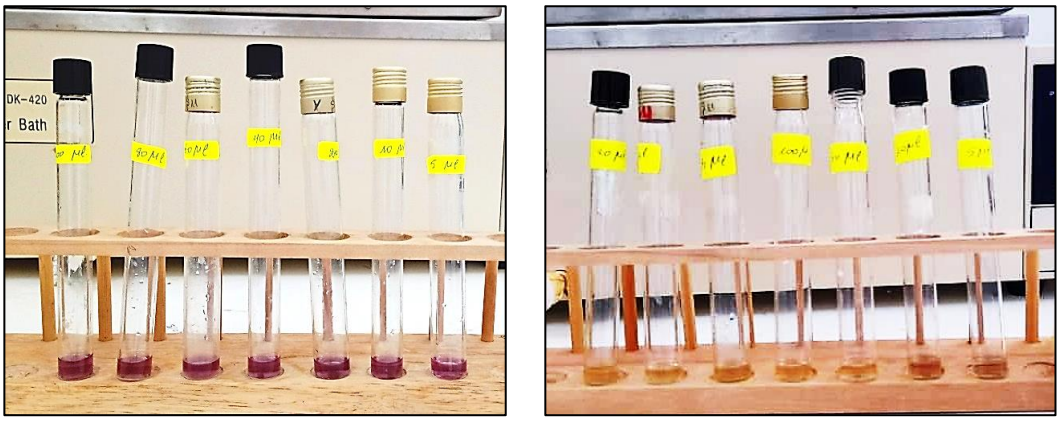


Figure 5.13. DPPH test results for the CEO (purple) and cinnamon extract (yellow)

The Table 5.16 presents the percentages of inhibition of free radicals DPPH at different concentrations in comparison with the synthetic antioxidant ascorbic acid (vitamin C) (Fig 5.14), (fig 5.15), (fig 5.16).

Table 5.16. presents the variation of the absorbance of ascorbic acid according to different concentration.

C (µg/ml)	5	10	20	40	60	80	100
Inhibition% (CEO)	43.44	50.63	54.23	59.09	64.25	67.41	73.90
Inhibition% (CE)	37.88	41.73	43.98	50.56	55.3	59.55	67.73

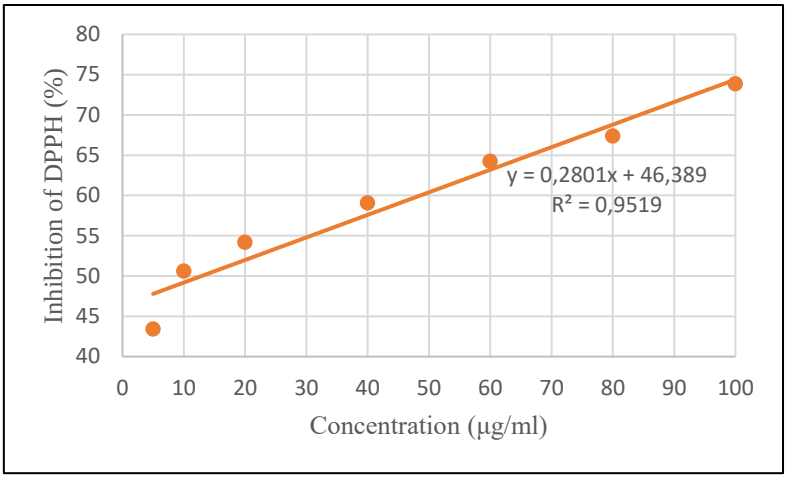


Figure 5.14. Percentage inhibition of cinnamon essential oil by DPPH test.

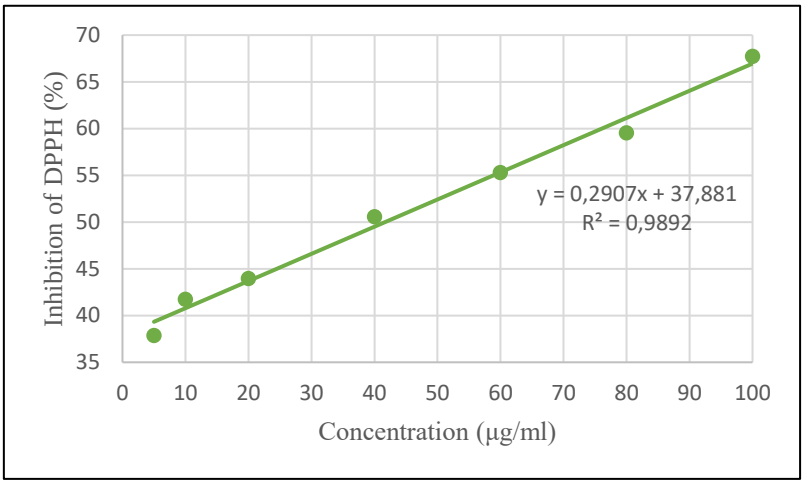


Figure 5.15. Percentage inhibition of cinnamon extract by DPPH test.

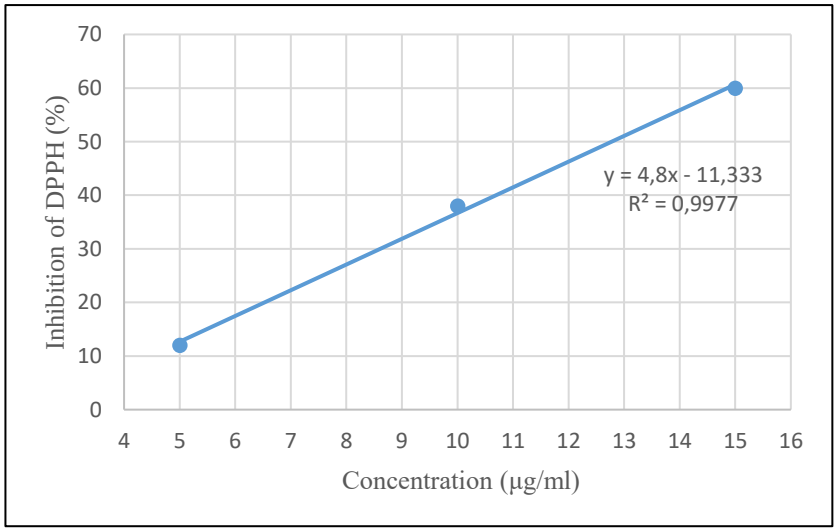


Figure 5.16. Percentage inhibition of ascorbic acid by DPPH test.

- **Determination of IC_{50} :**

From these curves, we can determine the percentages of inhibition obtained according to the concentrations used as well as the value of IC_{50} of each extract (CEO+CE). The IC_{50} value was calculated to determine the concentration of the sample required to inhibit 50% of radical.

The lower the IC_{50} value, the higher the antioxidant activity of samples **[215].**

The values of the effective concentrations of the oil and the extract where ascorbic acid was used as positive control are reported in the table and the following figure :

Table 5.17. represents the IC50 result of the CEO, CE and ascorbic acid

sample type	IC_{50} (µg/ml)
Cinnamon essential oil	12.89
Cinnamon extract	41.68
Ascorbic acid	12.77

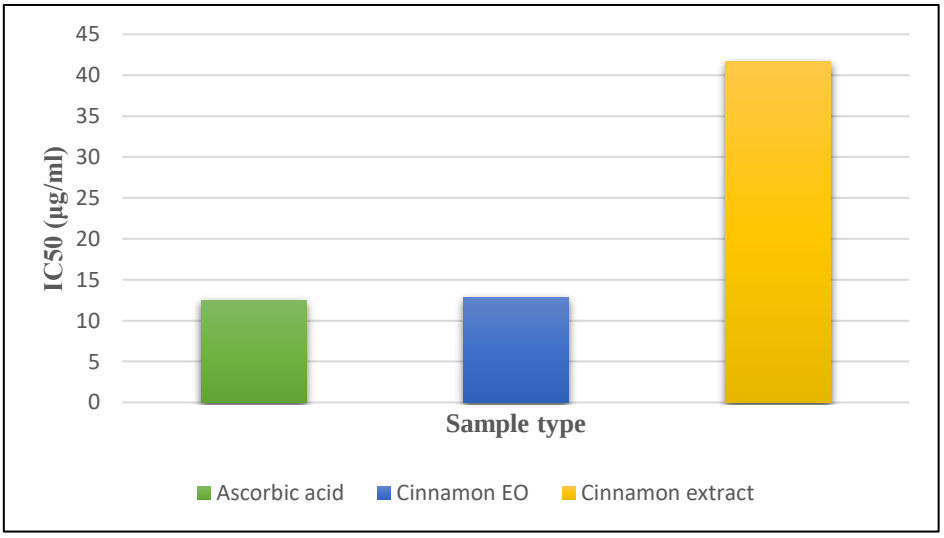


Figure 5.17. IC_{50} values obtained from the different samples.

➢ ***Discussion*** :

Figure 5.17 presents the IC_{50} values of cinnamon studied, although standard ascorbic acid had higher scavenging activity (IC_{50}~ 12.77 µg/ml) than the CEO and the CE, the CEO still showed good free radical scavenging activity (IC_{50}~ 12.89 µg/ml), compared with a cinnamon extract which showed weak inhibition of the extract (IC_{50}~ 41.68 µg/ml).

Cinnamaldehyde which is a major compound of our essential oil with a rate of (93.16%) has a big antioxidant activity **[203].** The influence of the high content of phenolic compounds in essential oil and the low it in the extracts is the reason that explains these results.

A previous study found that the antioxidant potency of a phenolic compounds depends on their chemical structure, mainly due to their redox properties, which can play an important role in free radical adsorption and neutralization **[216].**

1.2. Antibacterial Activitiy

The antibacterial activity of our essential oil extracted by steam distillation and the extracts by maceration was studied on three strains by two methods, both of which showed almost different results, but at the same time, they confirmed that our plant has antibacterial activity.

The three strains included :

- ✓ *Escherichia coli ATCC 8739 ;*
- ✓ *Pseudomonas aeruginosa ATCC 9027 ;*
- ✓ *Staphylococcus aureus ATCC 6538.*

Reading is done by measuring the diameter of the inhibition zone around each disc. The diameter value needs to be checking and comparing to the prescribed values. This conforms whether the bacteria is susceptible or not. The results can be symbolized by signs **[209, 210].**

- ✓ Resistance (-) : D≤ 9 mm.
- ✓ Sensitive (+) : 10 mm≤ D ≤ 15 mm.
- ✓ Intermediate strain (++) : 16 mm ≤ D ≤ 20 mm.
- ✓ Very sensitive (+++) : D > 20 mm.

1.2.1. Antibacterial Activity Screening :

➢ ***Results :***

The measurement of the Zone of Inhibition is carried out by using a physical ruler (fig5.18).

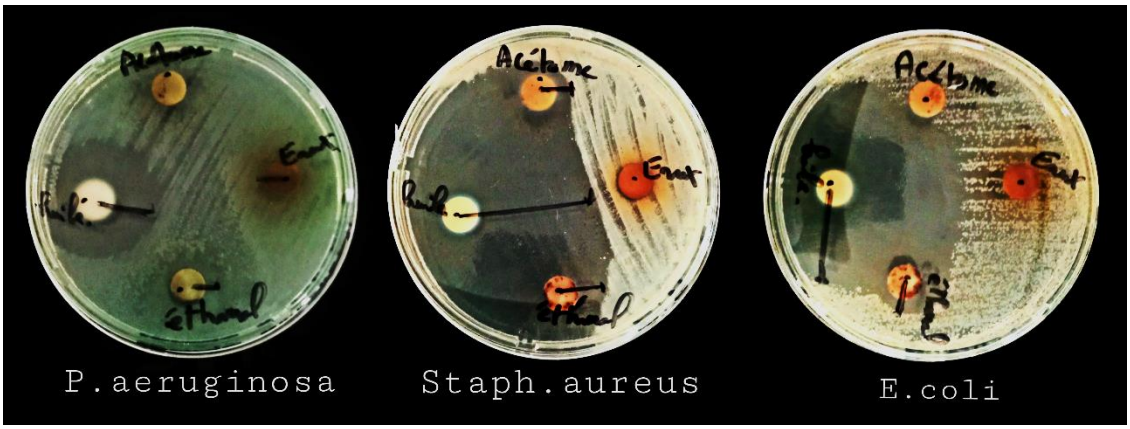


Figure 5.18. Zones of inhibition results of each strain

- The zones of inhibition of the strains are identified in the following table :

Table 5.18. Results of the antibacterial activity of essential oil and different cinnamon extracts

Strains	Time (h)	Zone diameter of inhibition (mm)			
		Essential oil	Water extract	Ethanol extract	Acetone extract
Escherichia coli	24h	50mm	0mm	20mm	0mm
	48h	50mm	0mm	20mm	0mm
Pseudomonas aeruginosa	24h	36mm	14mm	19mm	0mm
	48h	36mm	14mm	19mm	0mm
Staphylococcus aureus	24h	70mm	0mm	30mm	20mm
	48h	70mm	0mm	30mm	20mm

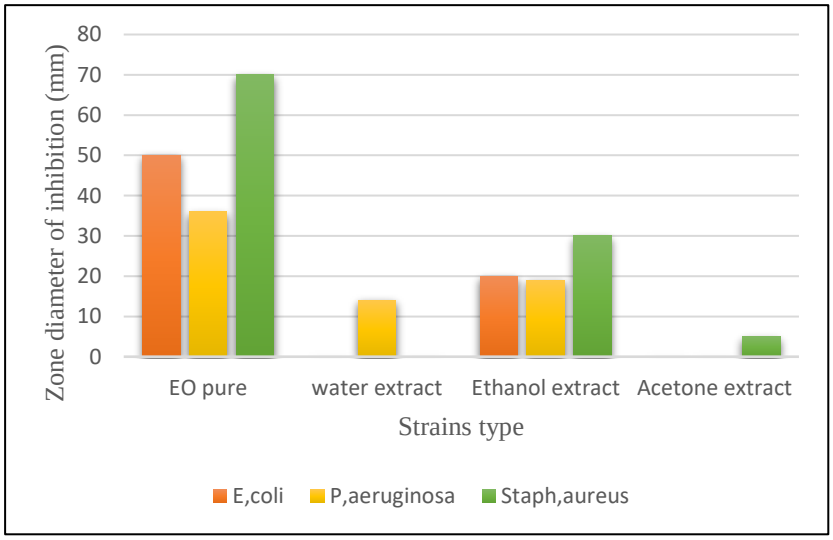


Figure 5.19. Variation in zone diametre of inhibition according to the type of extract.

➢ ***Discussion :***

From previous results, we try to summarize the activity of our EO and various CE on the tested strains in the following table :

Table 5.19. Estimation of the antibacterial activity of our EO and various CE.

Strains	Bacteria sensitivity			
	essential oil	Water extract	Ethanol extract	Acetone extract
E.coli	Very sensitive (+++)	Resistance (-)	Intermediate strain (++)	Resistance (-)
P.aeruginosa	Very sensitive (+++)	Sensitive (+)	Intermediate strain (++)	Resistance (-)
Staph.aureus	Very sensitive (+++)	Resistance (-)	Very sensitive (+++)	Intermediate strain (++)

Depending on the figures above and the results noted in Table 5.19, CEO shows remarkable antibacterial activity where we can see a variation in the zone diameter of inhibition of which CEO prevented the growth all of strains tested. According to the classification made by ***Ponce et al., (2003),*** the three bacterial strains tested on EO are classified as "Extremely sensitive" **[212]** (Table 5.19).

Friedman et al. (2004) shows that there are major factors influencing the results of testing the antibacterial activities of an essential oil and which are :

- The composition and solubility of the essential oil.
- The microorganism and the speed of its growth **[224].**

On another side, we note that the CE has different activities on the tested strains. The water and acetone extracts showed activity on P. aeruginosa and Staph.aureus respectively, but they had no effect on the rest where a zone of inhibition was almost 0 mm , while ethanol extract had an effective role in inhibiting all tested strains especially Staph.aureus strain with a record inhibition diameter of 30mm.

As a simple explanation, the antibacterial activity of CEO oil is mainly due to the majority compound in our EO (cinnamaldehyde component 93.16%), which have hydrophobic properties and can react with bacterial cell membranes, contributing to damage to the

membrane, another action is the ability to inhibit bacterial peptide and protein synthesis, thus having gram-positive and gram-negative bacteria action **[212, 213].**

1.2.2. Micro-atmosphere assay :

➢ **Results :**

Micro-atmosphere results for CEO and CEs are shown in the following table and figure :

Table 5.20. Results of the antibacterial activity of EO and different CEs (micro-atmosphere)

strain	Time (h)	Zone diameter of inhibition (mm)			
		Essential oil	Water extract	Ethanol extract	Acetone extract
Escherichia coli	24h	65mm	0mm	24mm	0mm
	48h	65mm	0mm	24mm	0mm
Pseudomonas aeruginosa	24h	40mm	0mm	0mm	0mm
	48h	40mm	0mm	0mm	0mm
staphylococcus aureus	24h	50mm	0mm	30mm	0mm
	48h	50mm	0mm	30mm	0mm

Figure 5.20. Micro-atmosphere results for CEs

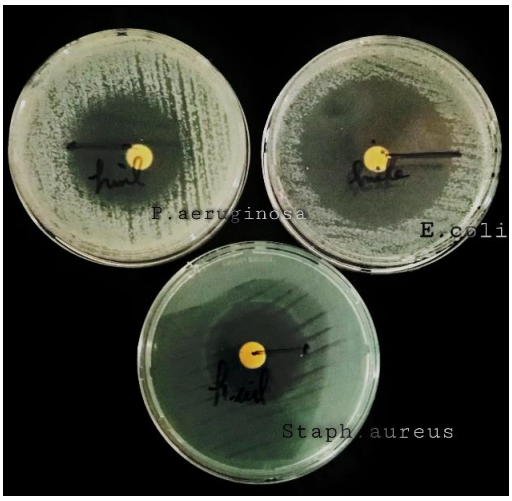


Figure5.21. Micro-atmosphere results for CEO

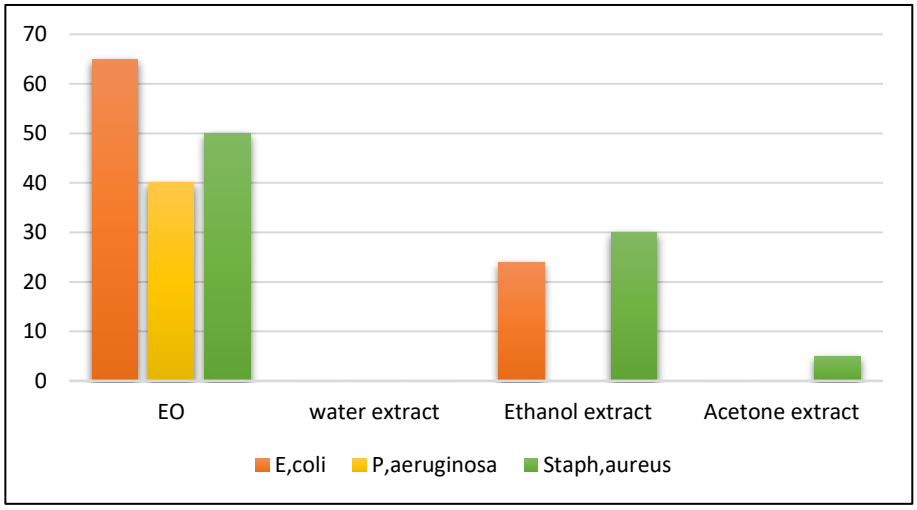


Figure5.22. Variation in zone diametre of inhibition (micro-atmosphere).

➢ ***Discussion :***

After studying the activity of our EO and various CE by micro-atmosphere assay, we try to summarize the results in the following table :

Table 5.21. Estimation of the antibacterial activity of our EO and various CEs (micro-atmosphere).

Strains	Bacteria sensitivity			
	Essential oil	Water extract	Ethanol extract	Acetone extract
E.coli	Very sensitive (+++)	Resistance (-)	Very sensitive (+++)	Resistance (-)

P.aeruginosa	Very sensitive (+++)	Resistance (-)	Resistance (-)	Resistance (-)
S.aureus	Very sensitive (+++)	Resistance (-)	Very sensitive (+++)	Resistance (-)

Depending on table 5.21 and on the results obtained in the table 5.20 and figures 5.21 and 5.22. we can see the effect of the CEO on the growth inhibition of the tested strains. The bacteria were very sensitive to the essential oil where we observed an important activity found for the three strains with a record inhibition diameter ranging from 40mm to 65mm.

On the other side, we noticed a weak effect of the extracts, where we recorded the largest diameter of inhibition was for the ethanol extract against Escherichia coli and staphylococcus aureus with a record inhibition diameter ranging from 24mm to 30mm While we did not record any effect of the rest of the extracts against the tested strains where the inhibition diameter was 0mm. The excessive sensitivity of bacteria may occur due to a high number of monoterpenes in the vapor of EO, where they can attack the bacteria easily. Thus, cinnamon can be used as air decontaminant in fields and it can be a good candidate to be used in agriculture as bio bactericide **[214].**

1.3. Antidiaberic Activity

1.3.1. In vitro anti-diabetic study :

➢ ***Result :***

In the present study, essential oil of cinnamon bark with known antidiabetic activity was investigated for its potential to inhibit α-amylase activity. Three different concentrations viz., 12.5, 25 and 50 mg/ml of essential oil tested for the inhibition of α-amylase activity (Table 5.22).

The results are summarize in the following table and figure :

Table 5.22. Represent the α-Amylase inhibitory activity of CEO

Plant extracts	Concentrations (µg/mL)	Inhibition %	IC_{50} value (µg/mL)
CEO	12.5	26.74	22.8
	25	66.27	
	50	83.7	

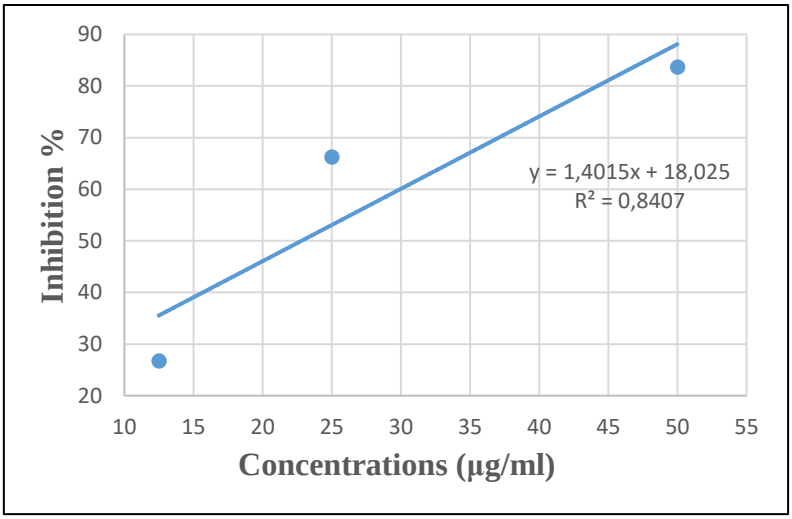


Figure 5.23. α-Amylase inhibitory activity of CEO

Cinnamon EO (at a concentrations 50 µg/mL) showed 83.7% inhibitory effects against α-amylase activity with an IC_{50} value 22.8 µg/mL (table 5.22).

➢ ***Discussion :***

Inhibiting digestion enzyme activity is a favoured therapeutic approach for reducing postprandial glycaemia through retarding starch digestion. Many herbal extracts have been reported to have antidiabetic activities, they have been used directly or indirectly for the preparation of many modern medicines. . Compared to other spices, cinnamon is recorded to have inhibitory effects **[218].**

In this study, in vitro inhibitory effect of cinnamon essential oil on alpha amylase activities was evaluated where we compared IC_{50} values with results obtained in previous studies for the same type of cinnamon estimated at 12.6 µg/ml **[219].**

By comparison, our cinnamon essential oil showed a higher IC50 value (22.8 µg/mL) that was statistically different from Vietnamese cinnamon (previously studied) which showed the lowest IC_{50} at 12.6 µg/ml..

Although both cinnamon types showed appreciable α-amylase inhibitory effects but the Previous studies have observed that the IC50 value is probably due to the correlations between the polyphenol content of natural products and inhibitory enzyme effects.

From the results, it can be concluded that the use of these plant extracts will be greatly beneficial in reducing the rate of digestion and absorption of carbohydrates and thereby contribute to the effective management of diabetes by decreasing postprandial hyperglycemia.

Future studies will provide an insight into the molecular mechanisms by which these plant and their active compounds regulate glucose homeostasis **[220].**

1.3.2. In Silico anti-diabetic study

➢ ***Result :***

⇨ ***Receptor preparation***

In our study, we used the protein peroxisome proliferator-activated receptor gamma (PPARα/γ) in PDB format (ID: 3G9E), it is downloaded from "Protein data bank" and prepared from autodock tools program in PDBQT format (fig 5.1).

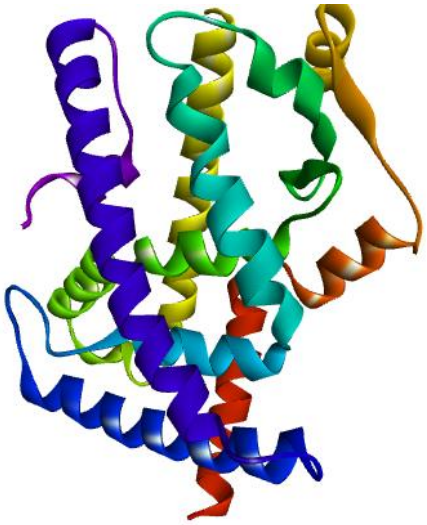


Figure 5.24. PDBQT format of PPARα/γ (ID : 3G9E)

⇨ ***Ligands preparation :***

The 7 cinnamon volatile compounds (CVCs) or (Ligands) having activity against diabetes melitus along were submitted to the SwissADME server to check whether they had potential properties to pass the Lipinski's rule of 5 (Table 5.23).

Table 5.23. Results of Lipinski's rule on 7 compounds

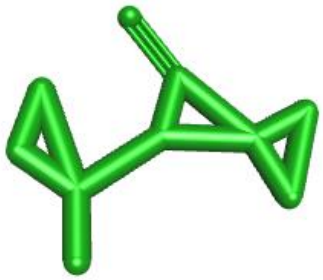
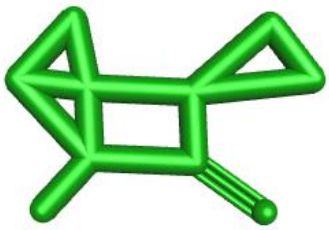
Compounds	PubChem ID	MW (g/mol)	Log $p_{o/w}$	HD	HA
α-Pinene	6654	136.23	3.44	0	0
Camphene	6616	136.23	3.43	0	0
DL-Limonene	22311	136.23	3.37	0	0
2-methylbenzofuran	20263	132.16	2.59	0	1
Benzenepropanal	7707	134.17	1.91	0	1
Cinnamaldehyde	637511	132.16	1.97	0	1

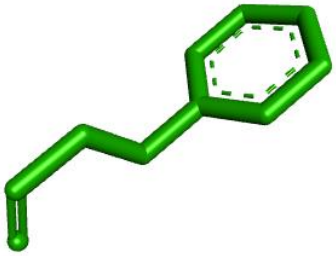
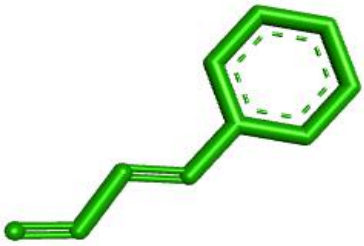
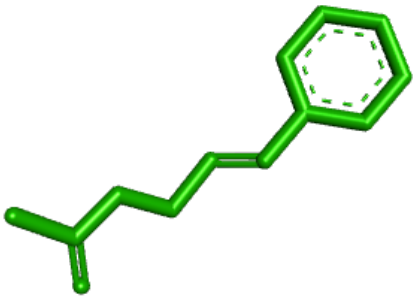
(E)-Cinnamyl acetate	5282110	176.21	2.33	0	2

After submitting 7 ligands, all of them passed the Lipinski's rule of 5 and showed no violations in SwissADME filters and were predicted to be having drug-like properties.

The 7 ligands which in pdb format, they were submitted on the autodock tools program to convert it to pdbqt format and to check after if they had potentially interacted with PPARa/ γ.

Table 5.24. Represents 3D structure of ligands used for docking showing by DS.

Ligands	PDBQT format
α-Pinene (PubChem ID : 6654)	
Camphene (PubChem ID : 6616)	
DL-Limonene (PubChem ID : 22311)	

2-methylbenzofuran (PubChem ID : 20263)	
Benzenepropanal (PubChem ID : 7707)	
Cinnamaldehyde (PubChem ID : 637511)	
(E)-Cinnamyl acetate (PubChem ID : 5282110)	

⇨ **AutoDock Vina results :**

To see the bond strength of the ligands studied against the (PPARα/γ) Protein., their binding affinity has been analyzed using the Autodock Vina program, and the results are shown in the following Table :

Table 5.25. Binding Energy results for Ligands Docked With (PPARα/γ) Protein.

ID	compounds	Binding Energy (kcal/mol)
1	α-Pinene	-6.7
2	Camphene	-6.8
3	DL-Limonene	-7.0
4	2-methylbenzofuran	-5.9
5	Benzenepropanal	-5.2
6	Cinnamaldehyde	-5.9
7	(E)-Cinnamyl acetate	-5.6

The calculated binding free energies for the compounds 1–7 are in the range between -7.0 and -5.2 Kcal/mol. The binding energy strength for the compounds obeys the order of: 3>2>1>4>6>7>5, where the DL-Limonene showed the highest binding affinity (-7.0 kcal/mol) using Autodock Vina than the rest of the compounds.

⇨ **Molecular dockig study :**

The Results of the interaction details of the compounds (ligand) studied with target protein PPARα/γ (PDB=3G9E) are summarized in the following table :

Table 5.26. Results of the interaction details of the target enzyme PPARα/γ

compounds	Ligand type	Receptor pocket	Interactions Category	Distance(Å)
α-Pinene	C = O	ILE249	Hydrophobic	5,25855
	C = O	ILE341	Hydrophobic	5,34314
	C = O	PHE247	Hydrophobic	5,4488
Camphene	C = O	ILE249	Hydrophobic	5,05778
	C = O	ILE341	Hydrophobic	5,24202
	C = O	ILE249	Hydrophobic	5,11084
	C = O	ILE341	Hydrophobic	5,39094
	C = O	PHE247	Hydrophobic	5,23112
DL-Limonene	C = O	ARG288	Hydrophobic	4,62993
	C = O	ALA292	Hydrophobic	3,88971

	C = O	LEU330	Hydrophobic	4,14183
	C = O	ILE326	Hydrophobic	4,35993
	C = O	MET329	Hydrophobic	4,58437
2-methylbenzofuran	CZ - NH1	ARG288	Electrostatic	3,23545
	CZ - NH2	ARG288	Hydrogen Bond	2,55872
	C = O	MET329	Hydrophobic	4,54852
	SD - CE	MET329	Other	5,36621
	C = O	MET329	Hydrophobic	3,50969
	C = O	LEU330	Hydrophobic	4,42734
	CA - C	ALA292	Hydrophobic	3,6933
	C = O	ALA292	Hydrophobic	4,0916
	C = O	LEU333	Hydrophobic	5,28651
	CA - C	ILE296	Hydrophobic	4,93345
	CA - C	ILE326	Hydrophobic	4,82902
	C = O	ILE326	Hydrophobic	4,7108
Benzenepropanal	HG - OG	SER342	Hydrogen Bond	2,16352
	HE21 - O	GLN345	Hydrogen Bond	2,24099
	CD – OE1	GLU259	Electrostatic	3,8908
	CG1 - CD1	ILE341	Hydrophobic	3,91769
Cinnamaldehyde	CB - OG	SER332	Hydrogen Bond	3,55072
	C = O	LEU228	Hydrogen Bond	3,29913
	HE - NE	ARG288	Hydrogen Bond	3,8236
	C = O	MET329	Hydrophobic	4,51275
	C = O	ALA292	Hydrophobic	5,30025
	C = O	ILE326	Hydrophobic	5,48393
	C = O	LEU330	Hydrophobic	4,46984
(E)-Cinnamyl acetate	HG - OG	SER342	Hydrogen Bond	2,5009
	CD - OE1	GLU259	Electrostatic	3,75584

The compounds showed different bonds after docking using Discovery Studios program. The 3D and 2D results are shown in Figures 5.25(A)-5.31(A) and 5.25(B)-5.31(B) respectively, while the interpolated charge surfaces around the ligand are shown in figures 5.32-5.37.

Figure 5.25. Interaction of alpha-pinene with target protein PPARα/γ

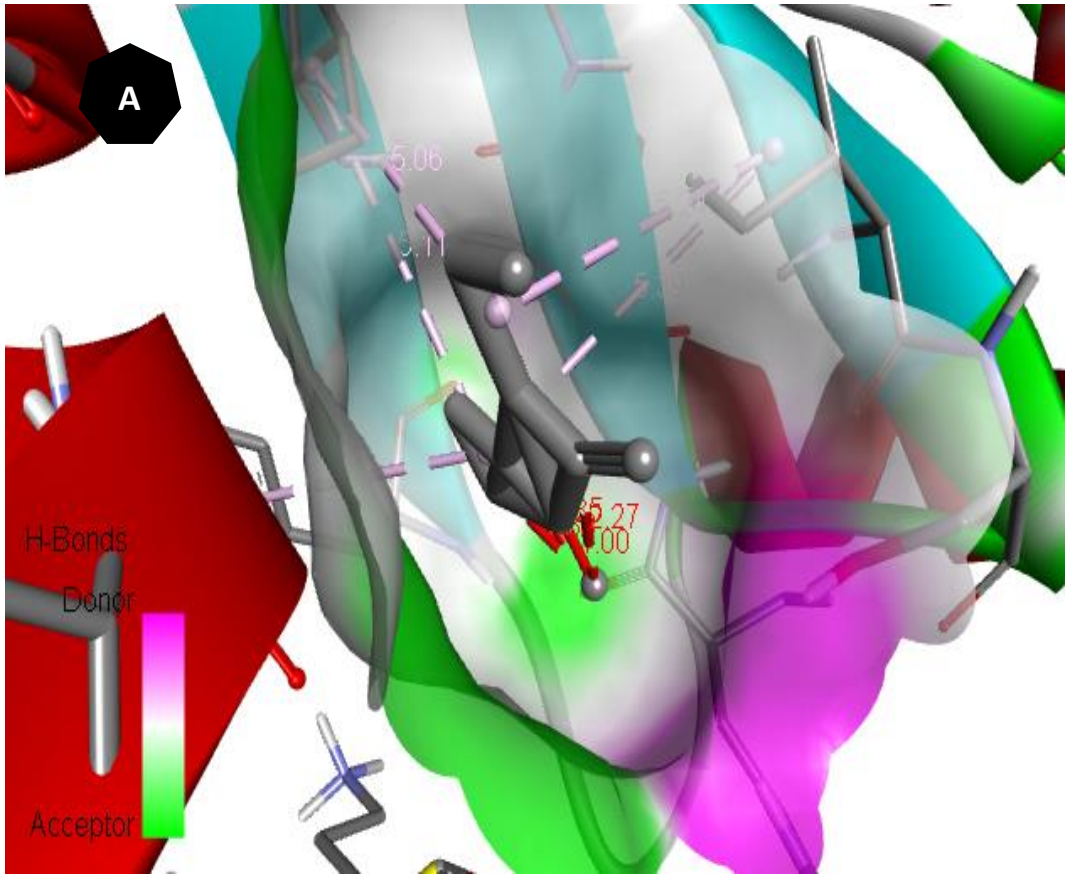


Figure 5.26. Interaction of Camphene with target protein PPARα/γ

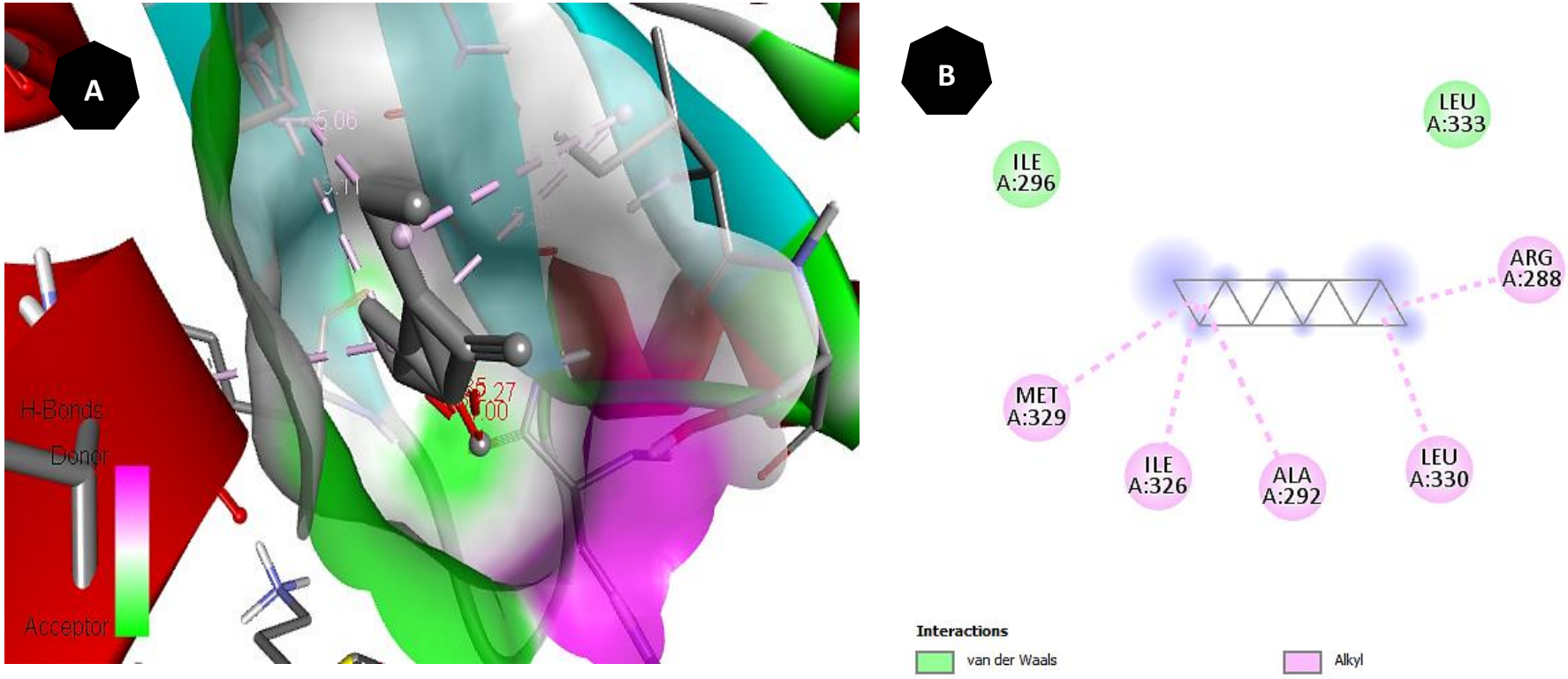


Figure 5.27. Interaction of DL-Limonene with target protein PPARα/γ

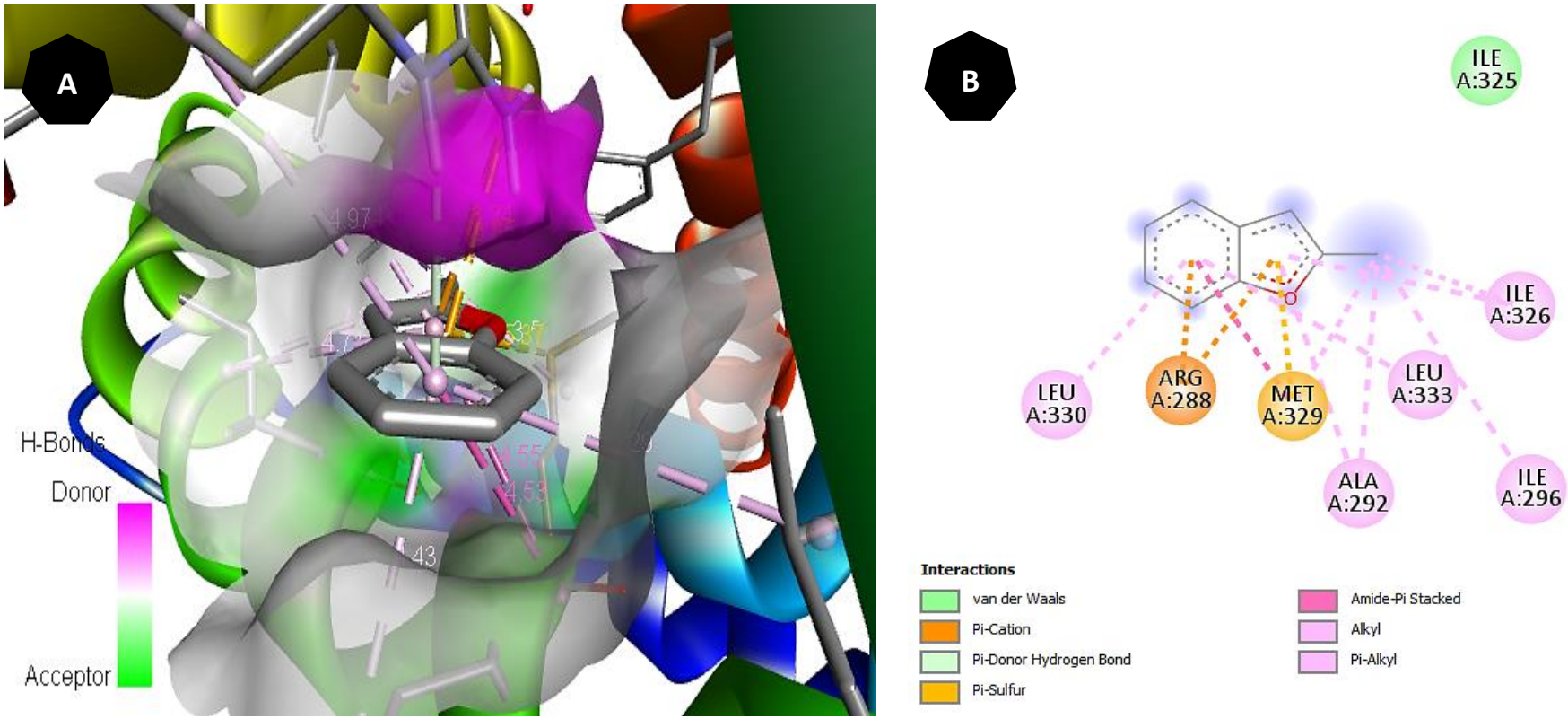


Figure 5.28. Interaction of 2-methylbenzofuran with target protein PPARα/γ

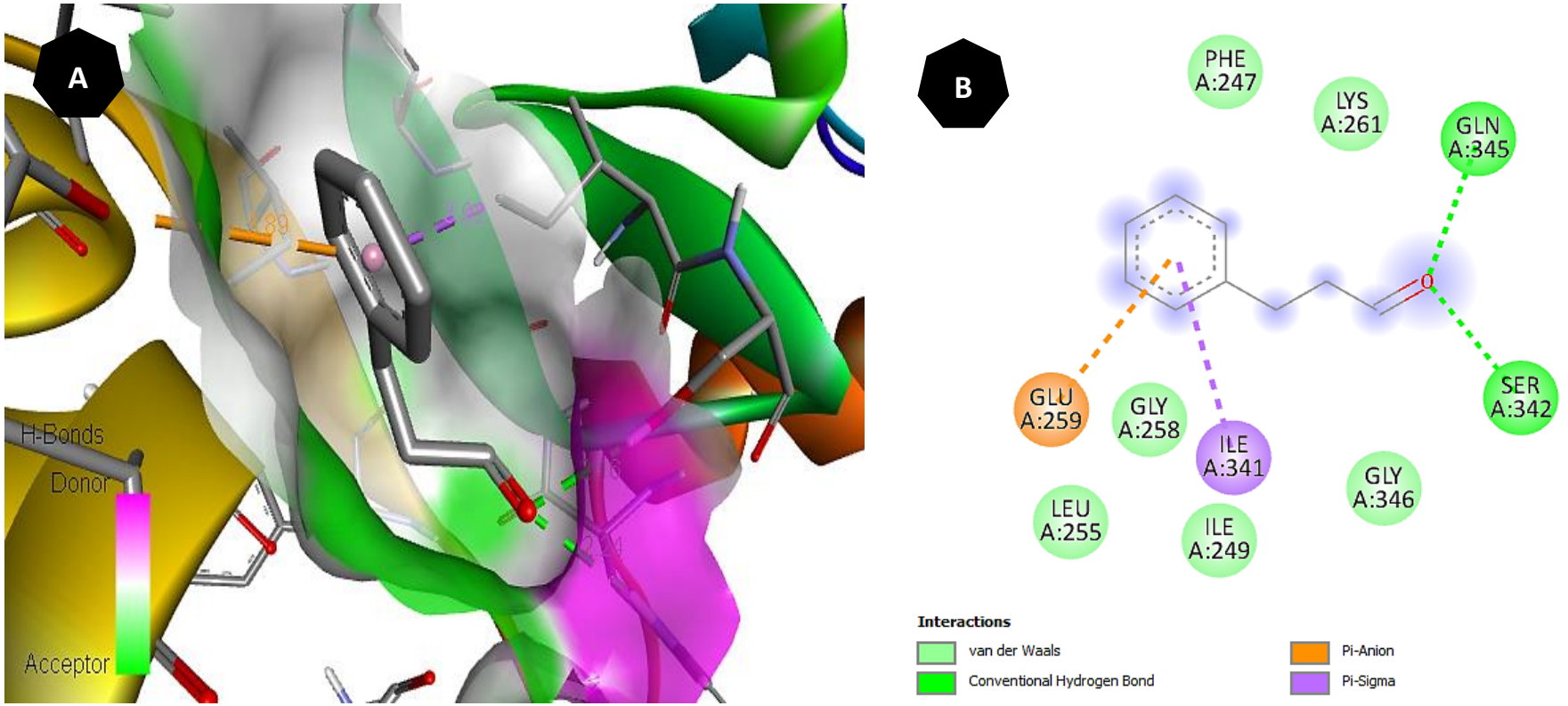


Figure 5.29. Interaction of Benzenepropanal with target protein PPARα/γ

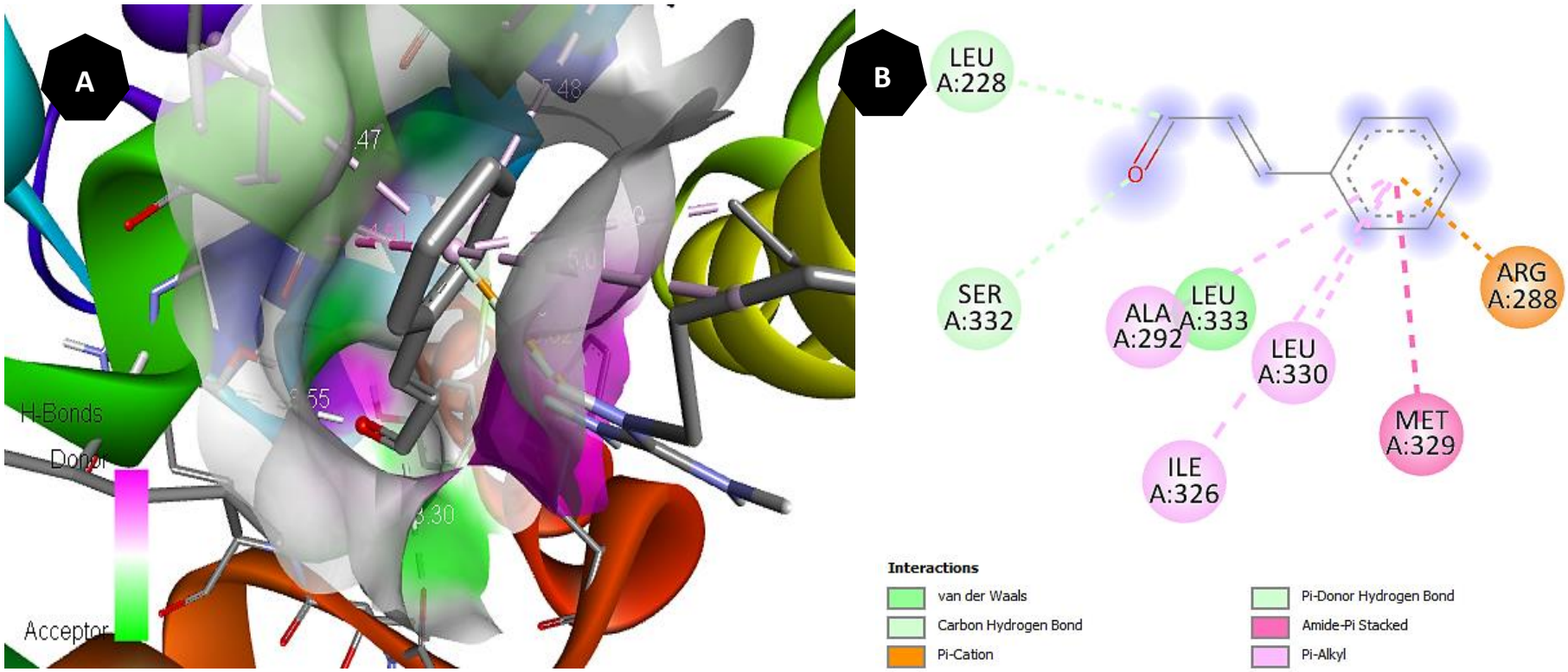


Figure 5.30. Interaction of Cinnamaldehyde with target protein PPARα/γ

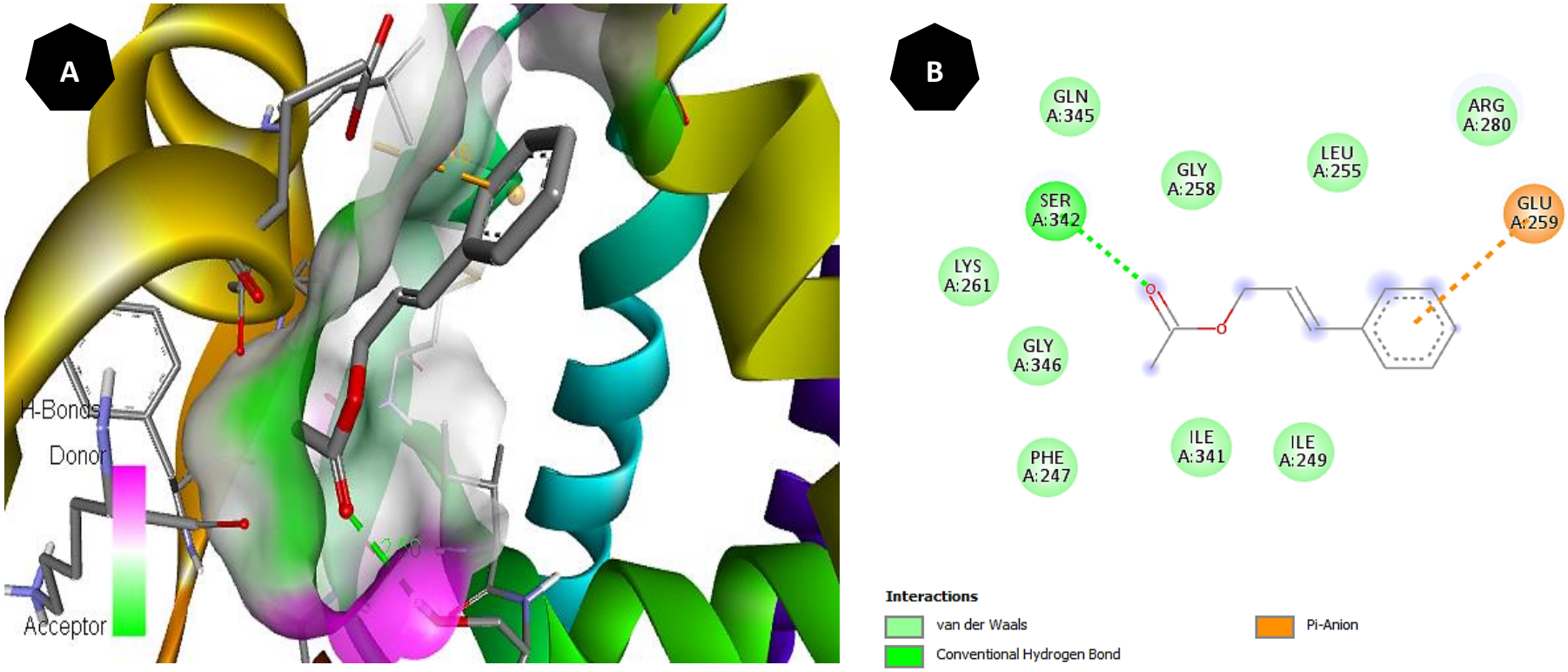


Figure 5.31. Interaction of (E)-Cinnamyl acetate with target protein PPARα/γ

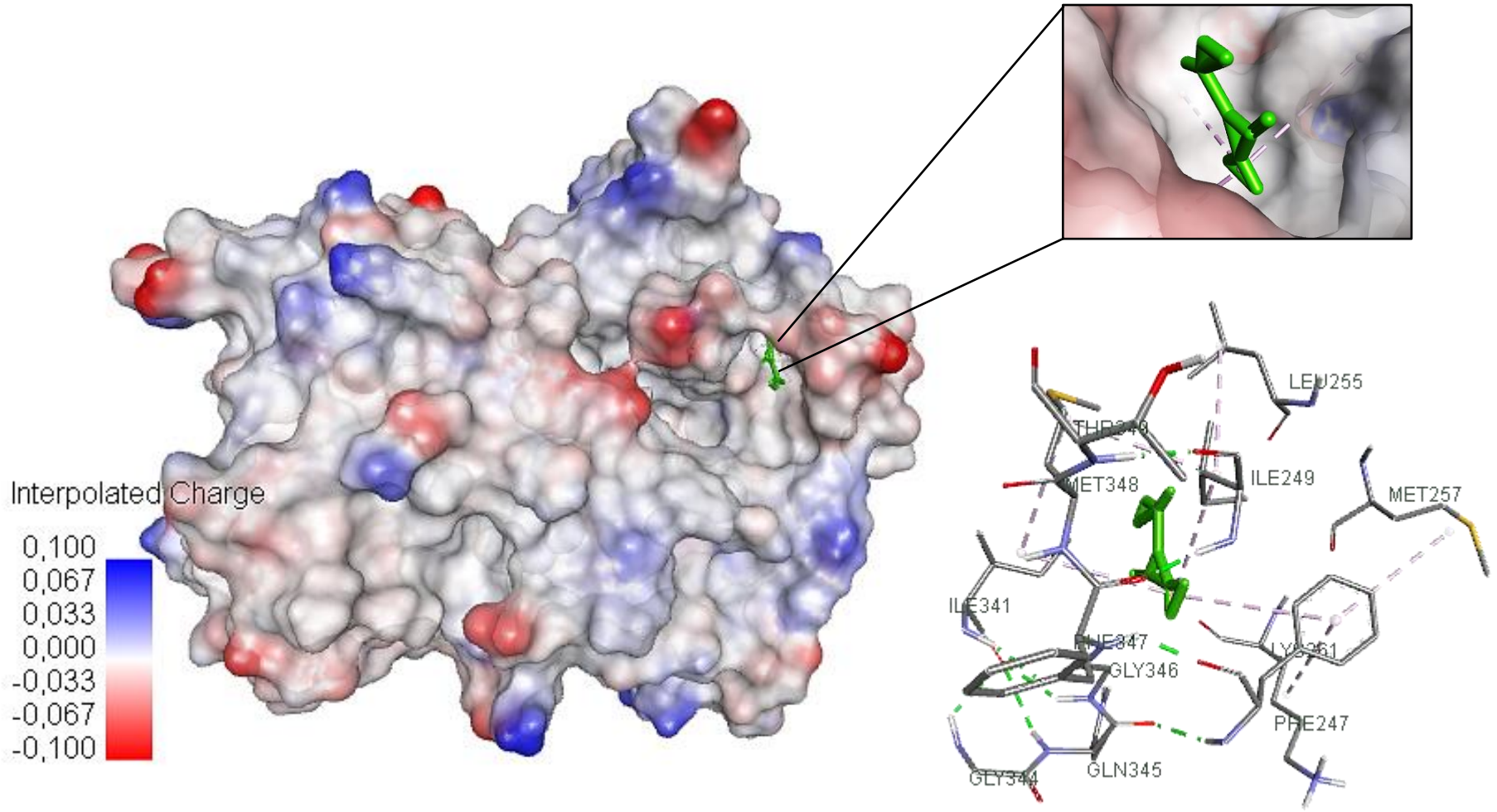


Figure 5.32. Molecular electrostatic potential map between α-Pinene and PPARα/γ

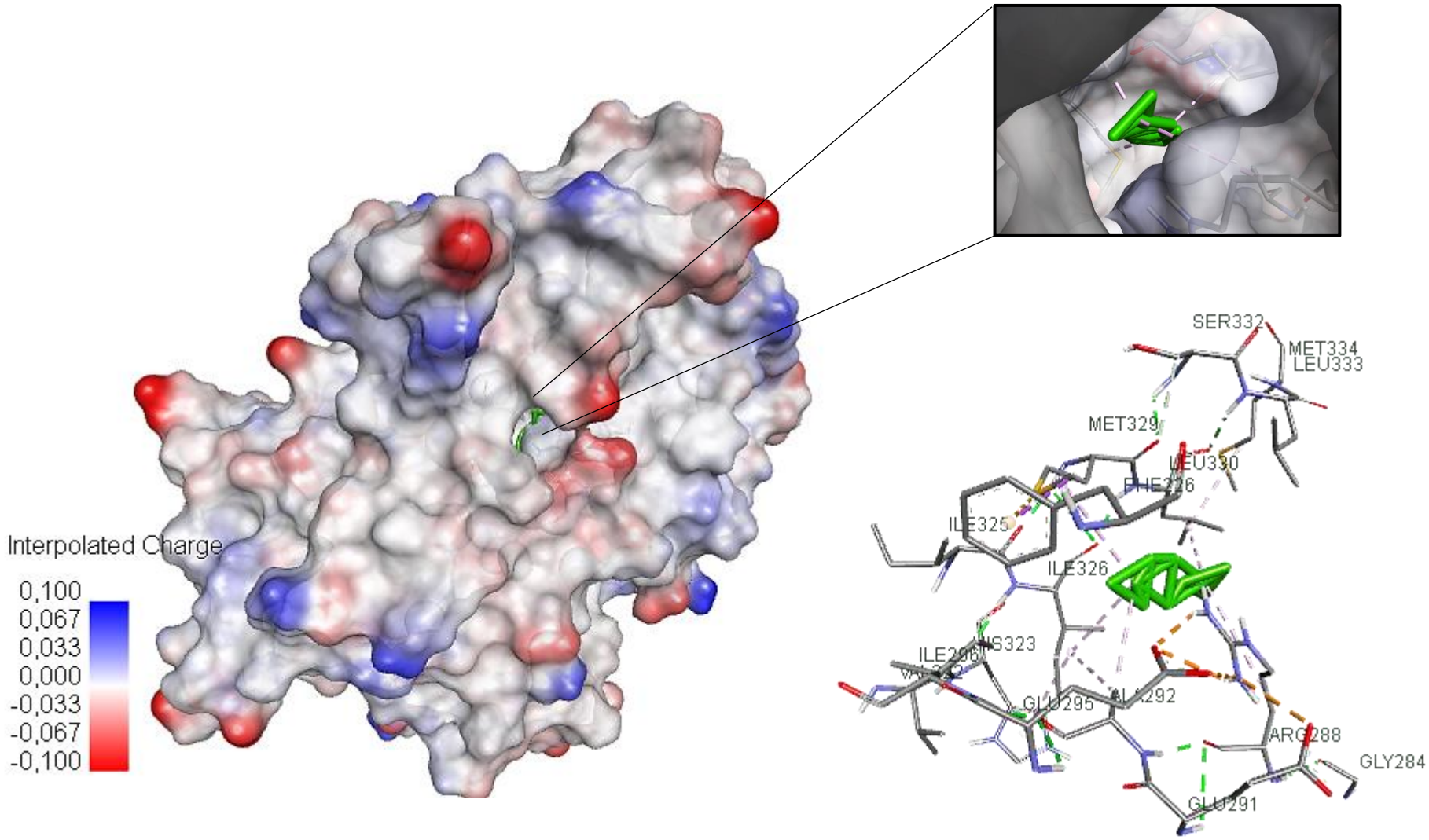


Figure 5.33. Molecular electrostatic potential map between DL-Limonene and PPARα/γ

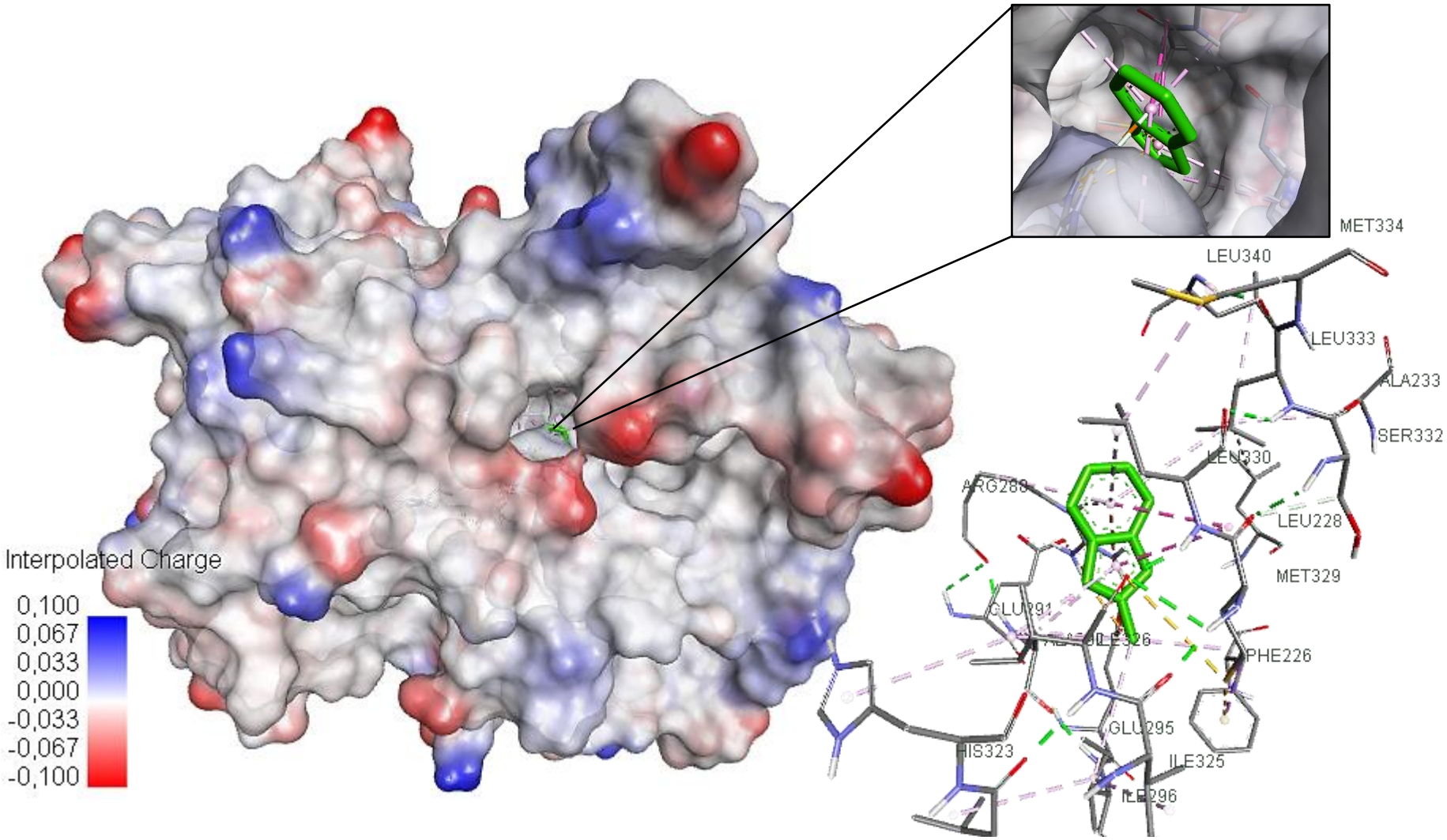


Figure 5.34. Molecular electrostatic potential map between 2-methylbenzofuran and PPARα/γ

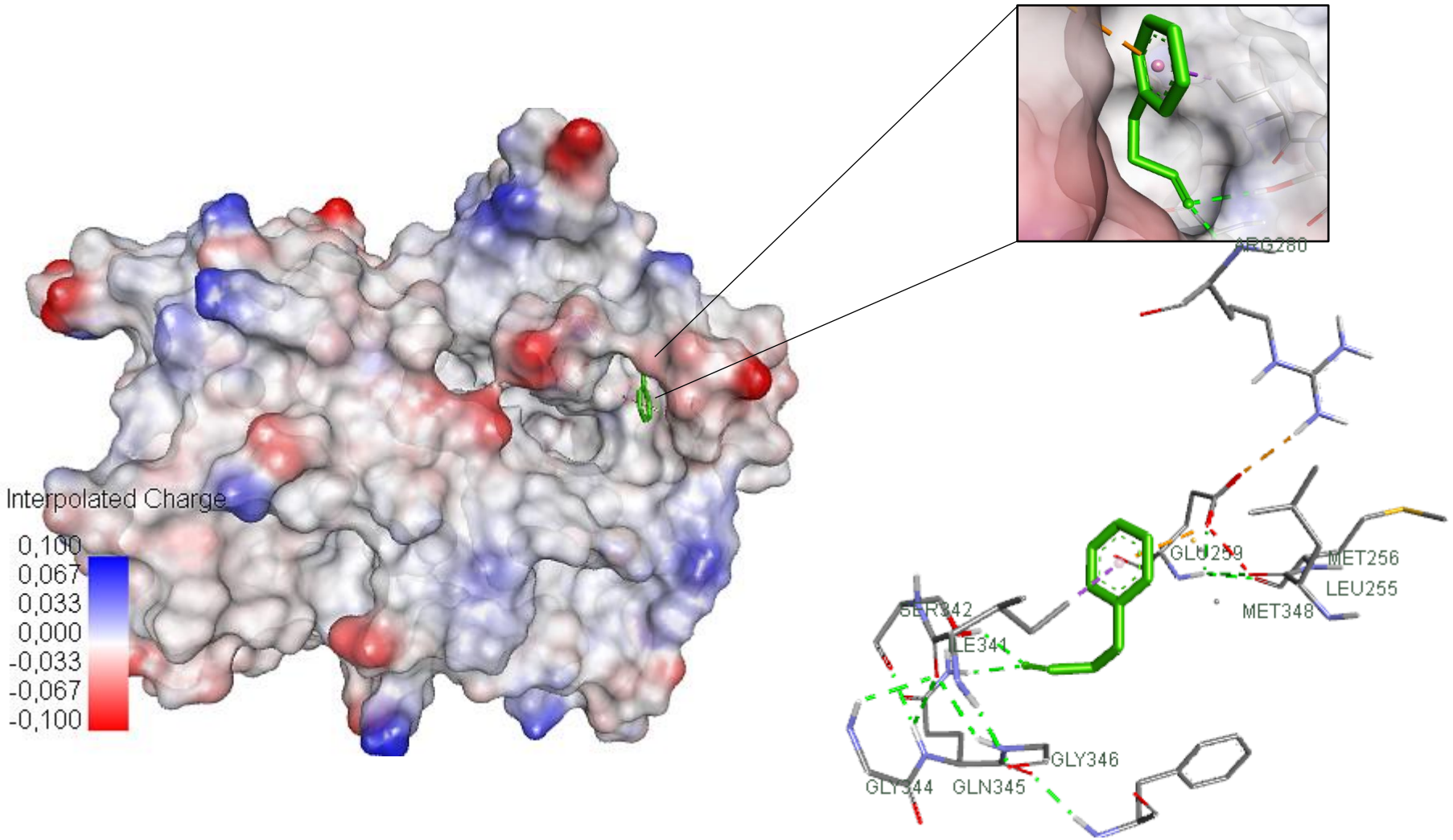


Figure 5.35. Molecular electrostatic potential map between Benzenepropanal and PPARα/γ

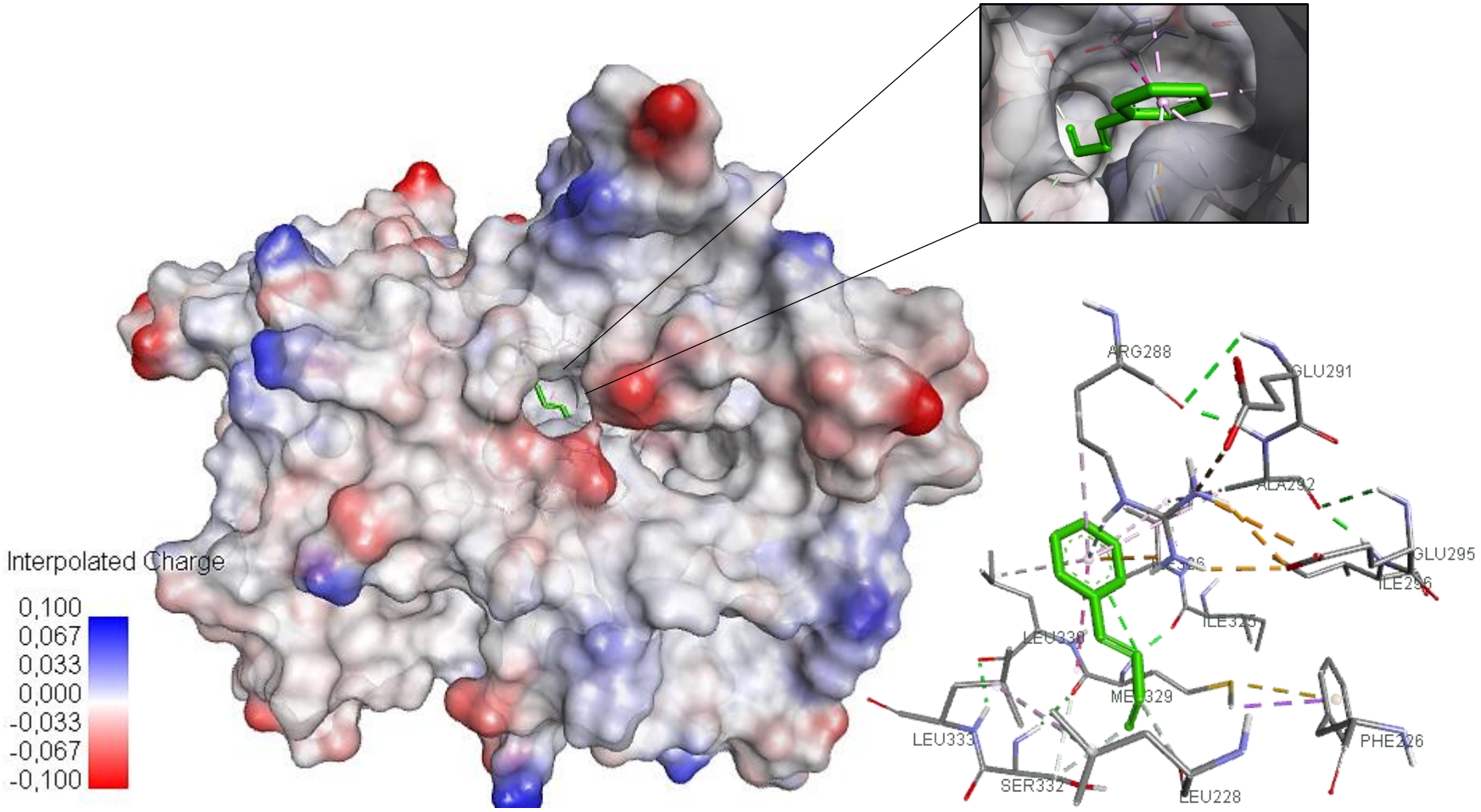


Figure 5.36. Molecular electrostatic potential map between Cinnamaldehyde and PPARα/γ

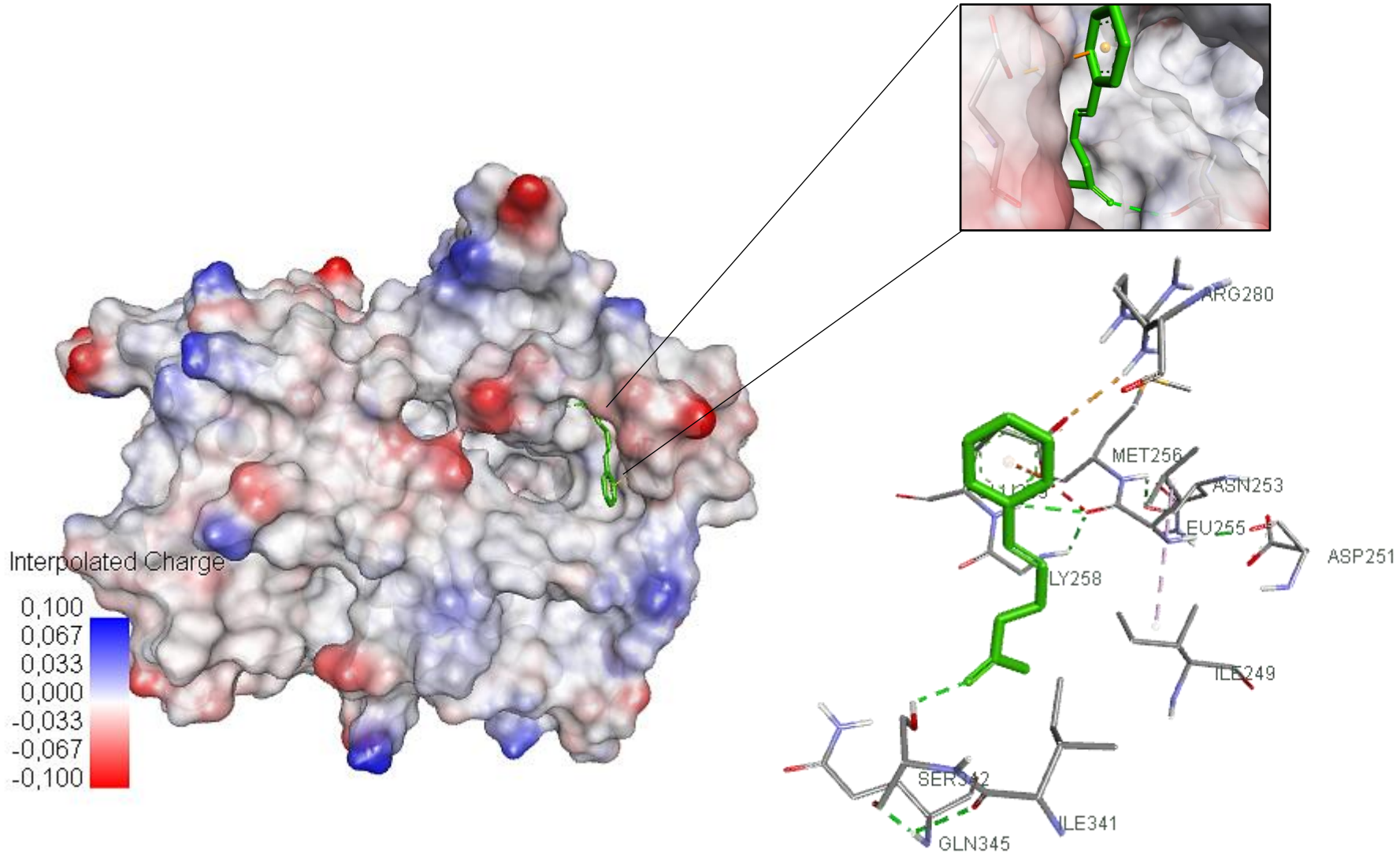


Figure 5.37. Molecular electrostatic potential map between (E)-Cinnamyl acetate and PPARα/γ

➢ ***Discussion :***

The therapeutic potential of many target proteins is now studied by structure determination **[221].** Computational methodologies have become a crucial component of many drug discovery programs for hit identification, lead optimisation followed by approaches such as ligand or structure-based virtual screening techniques **[222].** PPARα/γ is a target protein, which is associated with intervening lipid digestion, furthermore, glucose homoeostasis, and it has been generally utilise for the treatment of dyslipidemia and diabetes **[223].**

In this study, The attempting of ligands to dock against the target protein (PPARα/γ) seems obvious. Totally 7 compounds were subjected for the docking against PPARα/γ (PDB ID : 3G9E ; Figures 5.25-5.31).

These 7 compounds present in cinnamon essential oil were checked by analysis of the Molecular surface of PPARα/γ; this analysis is divided into two sections : hydrogen bonding and Interpolated charge.

Hydrogen bonds are the most crucial bonds which need to be considered while searching for active sites, mainly because H bonds are the most essential contributors in the stability of the protein structure. This is due to the fact that proteins are made of NH and OH groups that can donate H bonds and other groups that will accept them. Thus, H bonds help in the specificity of protein-ligand interactions stabilizing the ligand in the binding pockets **[217].**

Initially, we observed that the residue of amino-acid of target protein forming Hydrophobic bonds interaction with the ligands (α-Pinene, Camphene, DL-Limonene) represented in pink dotted lines (Fig 5.25-5.27), the Cinnamaldehyde interacted with the residue of PPARα/γ forming three hydrogen bonds (Fig 5.30). As for Benzenepropanal, it has formed two hydrogen bonds that were all conventional which are represented in green dotted lines (Fig 5.29). On the other side, (E)-Cinnamyl acetate and 2- methylbenzofuran have formed one hydrogen bond that was conventional bonds (Fig 5.31, 5.28) respectively.

The molecular electrostatic potential (MEP) is used to predict the relative reactivity positions of a molecule for electrophilic and nucleophilic attack. The electrostatic potential is considered predictive of chemical reactivity because regions of negative potential are expected to be sites of protonation and nucleophilic attack, while regions of positive potential may indicate electrophilic attack **[225].**

The electrostatic potential (electron density) surface of the 7 ligands except Camphene component is shown in Fig 5.32-5.37. The color code of the compound lies in the range of -0.100 to +0.100. Red and blue color in the MEP surface point to more electron rich (negative) and electron poor region (positive) respectively. Nitrogen, oxygen and fluorine positioned surfaces are colored red indicating electron rich area and the other region are slightly blue indicating positivity area **[225].**

We note that the surface of the electrostatic potential around the ligands is colored red indicating that the region is rich in electrons, these large negatively charged patches is formed due to the mediation of polar interactions in the formation of the complex ligand-PPARα/γ **[226].**

In silico screening and docking studies proved that the compounds studied have better binding affinity and also bind to the functionally important active site of PPARα/γ as predicted by BIOVIA DS program.

From this study, we understood that the cinnamon essential oil has the ability to interact with PPARα/γ, thereby it enhances insulin secretion and reducing the risk of diabetes. So, the 7 ligands studied may be the reason for the anti-diabetic potential of Cinnamon, thus validating the in vitro results. Hence, further studies may be warranted.

General conclusion

The purpose of our thesis is to figure out the physico-chemical, and antibacterial features of a Laurasia plant, and its antioxidant activity, as well as an assessment of its hypoglycemic impact. We chose the Vietnamese cinnamon plant for the study.

Organoleptic properties show that cinnamon is a mildly viscous liquid, hydrophobic and yellowish. It has a sweet, spicy, woody aroma. The physical properties of cinnamon indicate that it has a density greater than water (1,022) ; it also has the ability to reflect light with a refraction value of 1.615. pH of our oil is 5.86, which indicates its purity. Cinnamon represents a low water content (11.3%) and low minerals (3.55%). According to Chemical properties results, the cinnamon essential oil has a low free acid but is rich in esters and FFA where SV =61.71 mgKOH/g.

Analysis of the extracts and the essential oil by TLC chromatography allowed us to identify seven compounds otherwise. The qualitative and quantitative analysis carried out by GC/MS shows that there are many kinds of compounds identified, including aldehydes, esters, alcohols, terpenes and aromatics with a total of 27 compounds. The major abundant component of CEO was cinnamaldehyde 93.96%.

CEO showed significantly higher antioxidant activity than the extract, where the IC_{50} value for both is 12.89 µg/ml and 41.68 µg/ml respectively. The influence of the high content of phenolic compounds in our essential oil and the low it in the extract is the reason that explains these results.

The evaluation of the antibacterial activity of essential oil and the three extracts was carried out in vitro on three bacterial strains by two different methods (*Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus*) which where obtained from the medicals biologicals analysis laboratory of Dr. A.Zibouche.

The two methods revealed that our Essential Oil has a high inhibitory power against the three strains. Nevertheless, the ethanol extract is the only extract that has an inhibitory capacity for all three strains.

The evaluation of the anti-hypoglycemic effect of Cinnamon essential oil has been studied by in vitro and in silico approach with two proteins related to diabetes mellitus which are α-amylase and Peroxisome proliferators activated receptor gamma (PPARα/γ).

In vitro study was carried out at the level of the Bio-Ressources Naturelles Locales BORN at Chlef University. Cinnamon EO (at concentrations of 50 µg/ml) showed 71.93% inhibitory effects against the α-amylase activity with an IC_{50} value of 22.8 µg/ml; in addition to in silico analysis, out of 27 compounds of cinnamon essential oil, just 7 have good glide score and also potentially interacted with PPARα/γ where the attempting of ligands to dock against the target protein seems obvious using BIOVIA DS program. The residue of amino-acid of target protein formed different bonds with the studied ligands, the most important of which was the hydrogen bonds, which were formed with only four ligands (2-methylbenzofuran, Benzenepropanal, Cinnamaldehyde, (E)-Cinnamyl acetate).

According to in vitro and in silico results, volatile cinnamon oil and some of its components have anti-diabetic potential.

This study demonstrated that cinnamon has various biologically active compounds, mainly cinnamaldehyde. These bioactive compounds have a wide range of effects on the human body and can be used to treat a variety of diseases and metabolic disorders due to their antioxidant, antimicrobial and activities antidiabetic.

Appendices

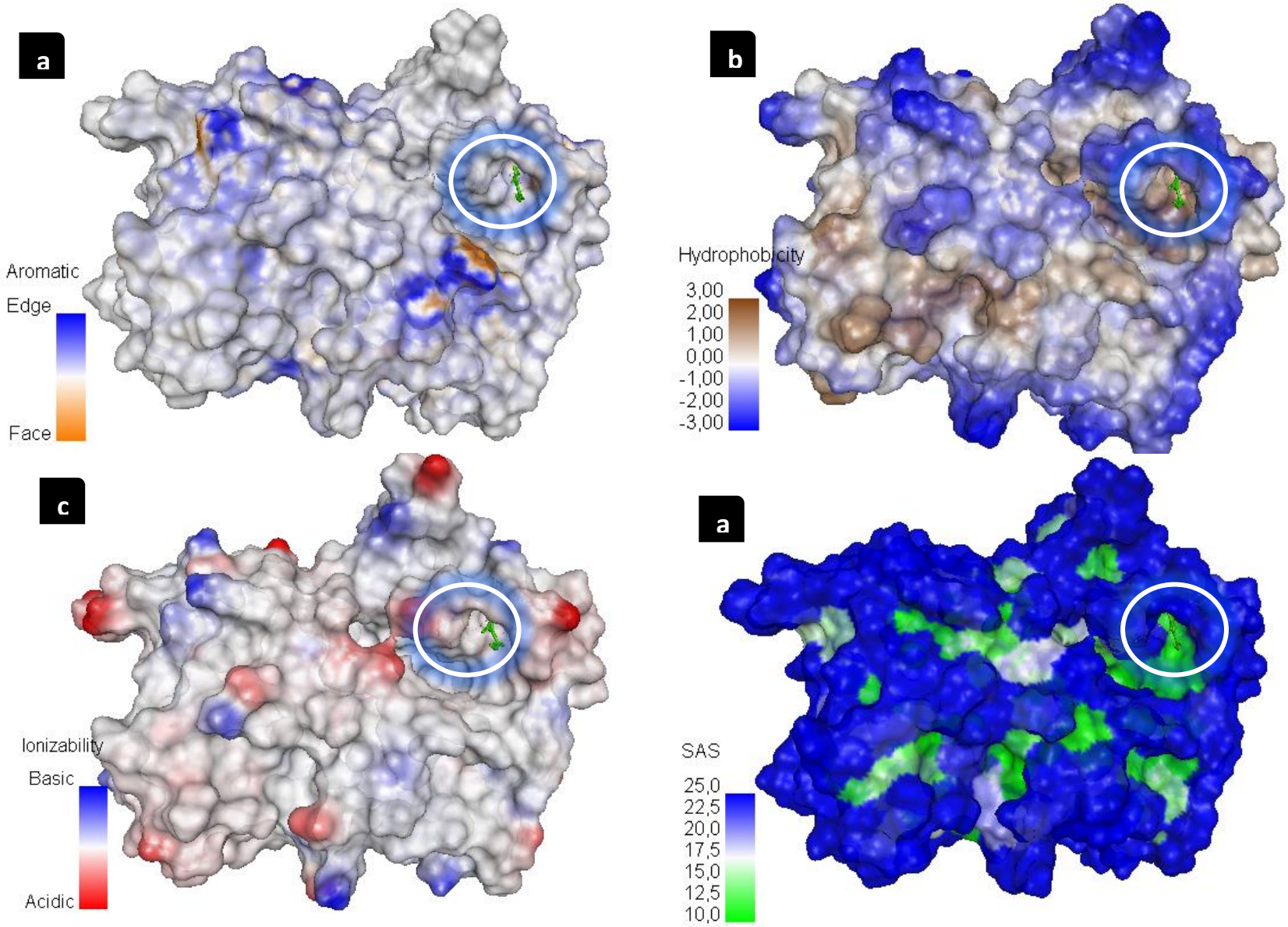


Figure 1. The schematic representation of (a) aromatic (b) hydrophobicity(c)ionizability (d) solvent accessibility surface of of 6654 around 3G9E target.

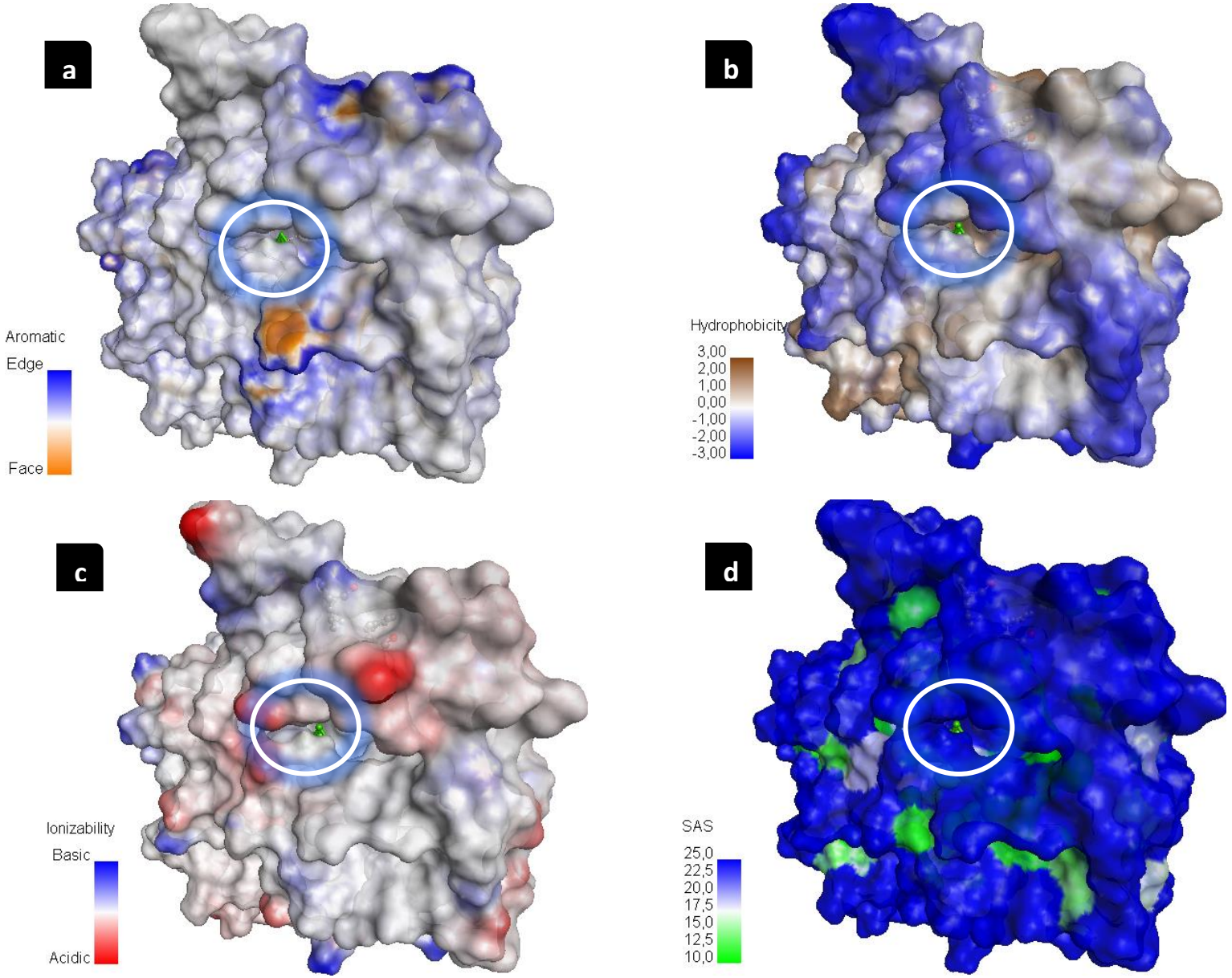


Figure 2. The schematic representation of (a) aromatic (b) hydrophobicity(c)ionizability (d) solvent accessibility surface of of 22311 around 3G9E target.

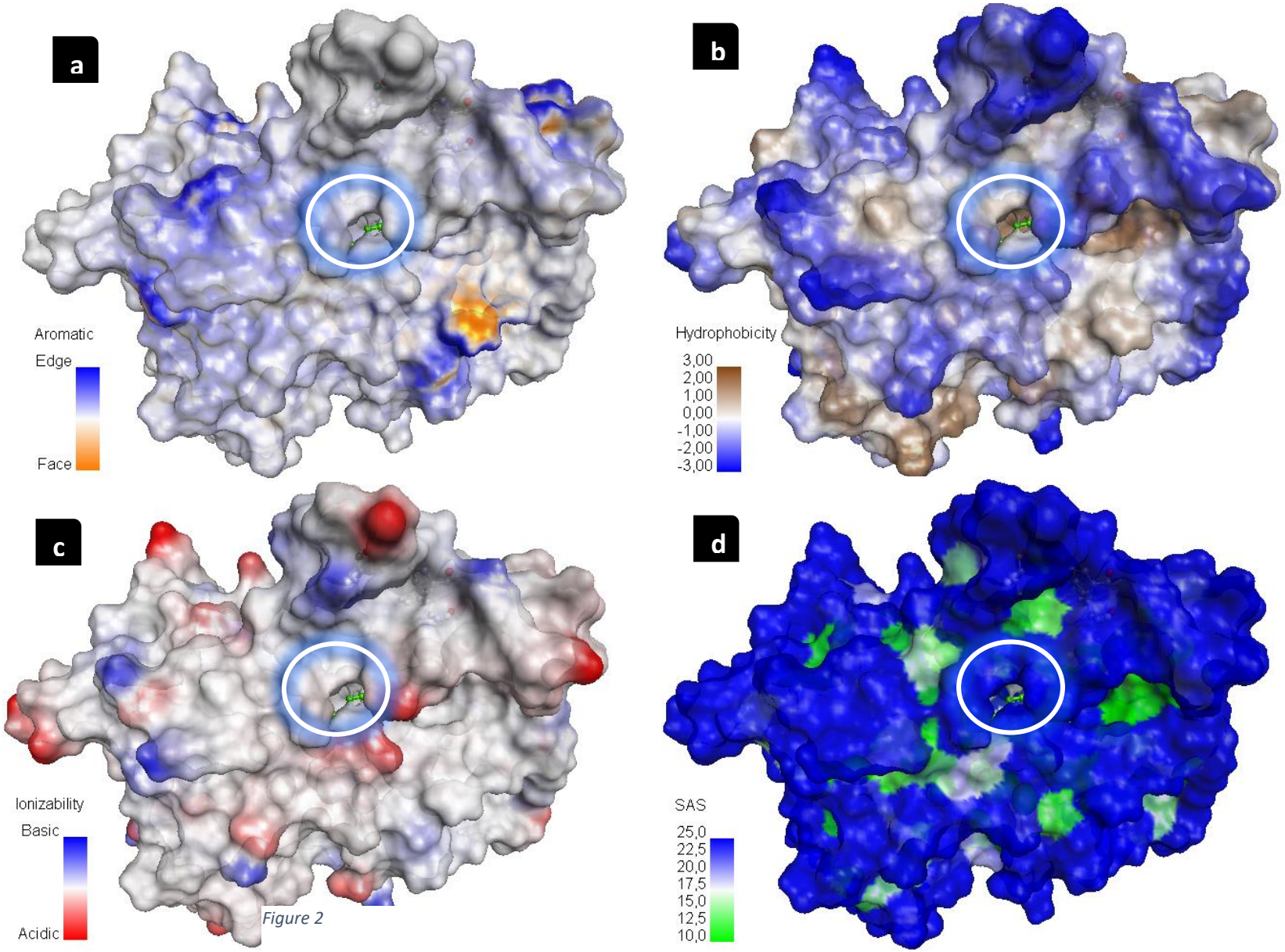


Figure 3. The schematic representation of (a) aromatic (b) hydrophobicity(c)ionizability (d) solvent accessibility surface of of 20263 around 3G9E target.

Figure 2

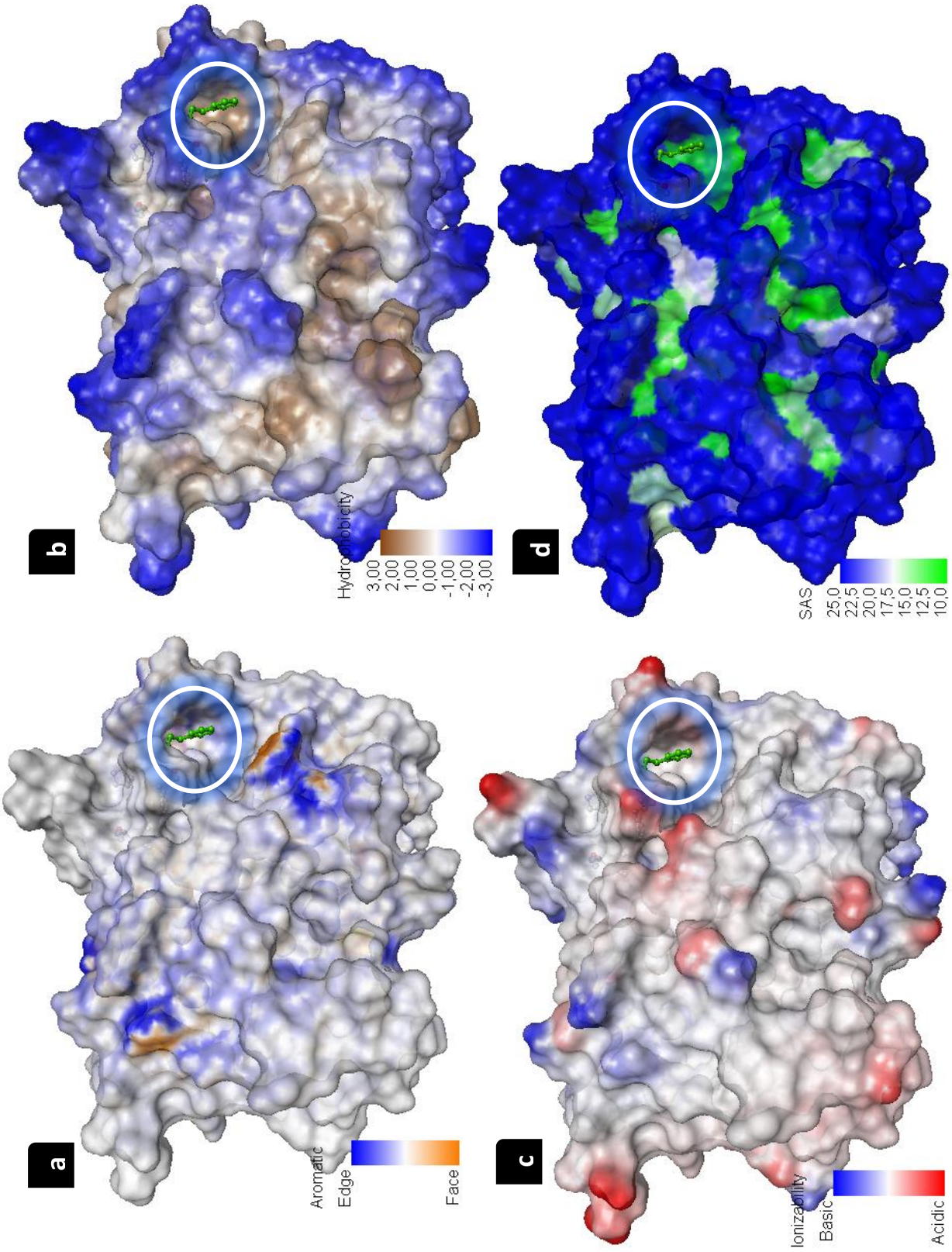


Figure 4. The schematic representation of (a) aromatic (b) hydrophobicity(c)ionizability (d) solvent accessibility surface of of 7707 around 3G9E target.

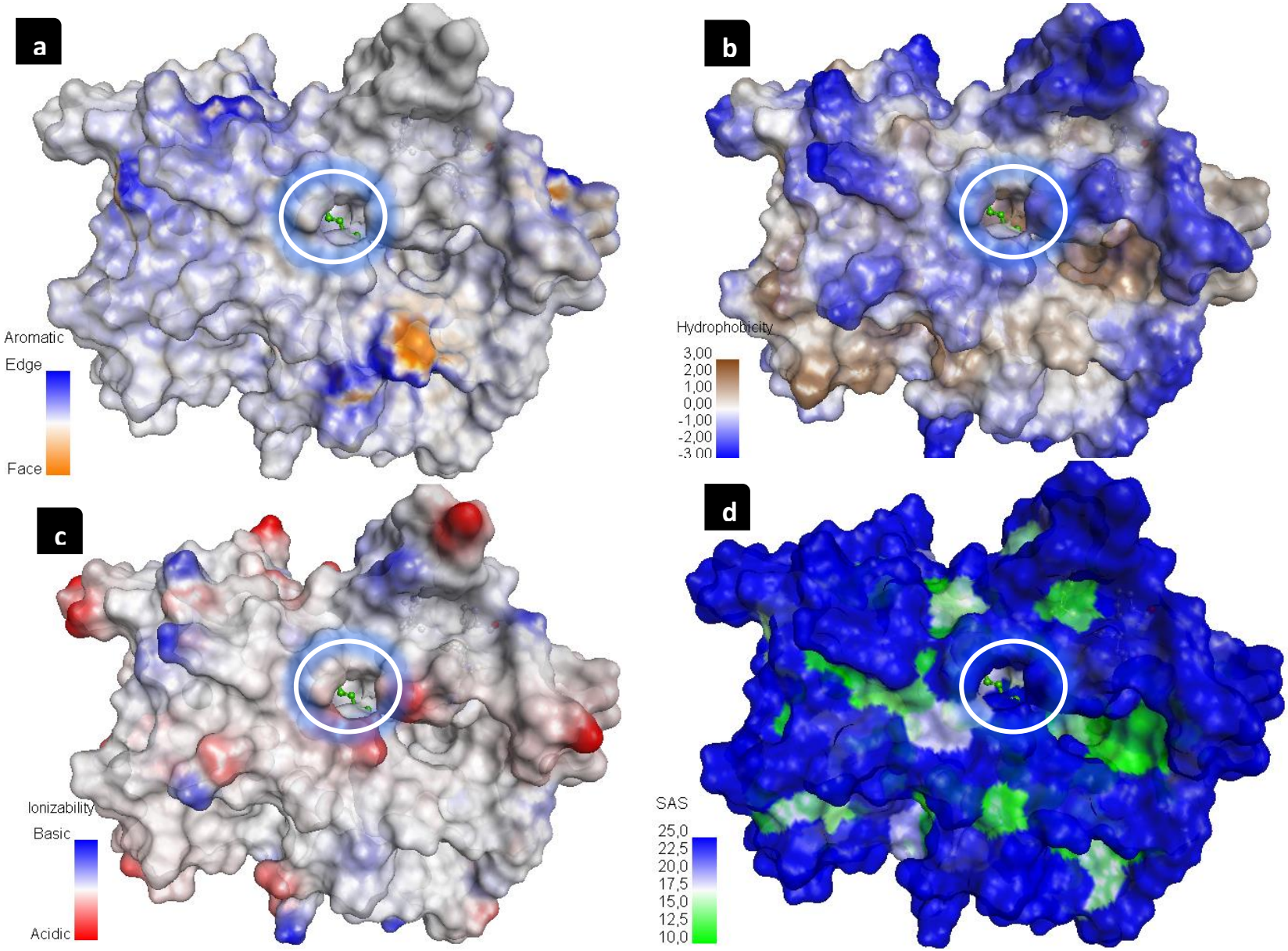


Figure 5. The schematic representation of (a) aromatic (b) hydrophobicity(c)ionizability (d) solvent accessibility surface of of 637511 around 3G9E target.

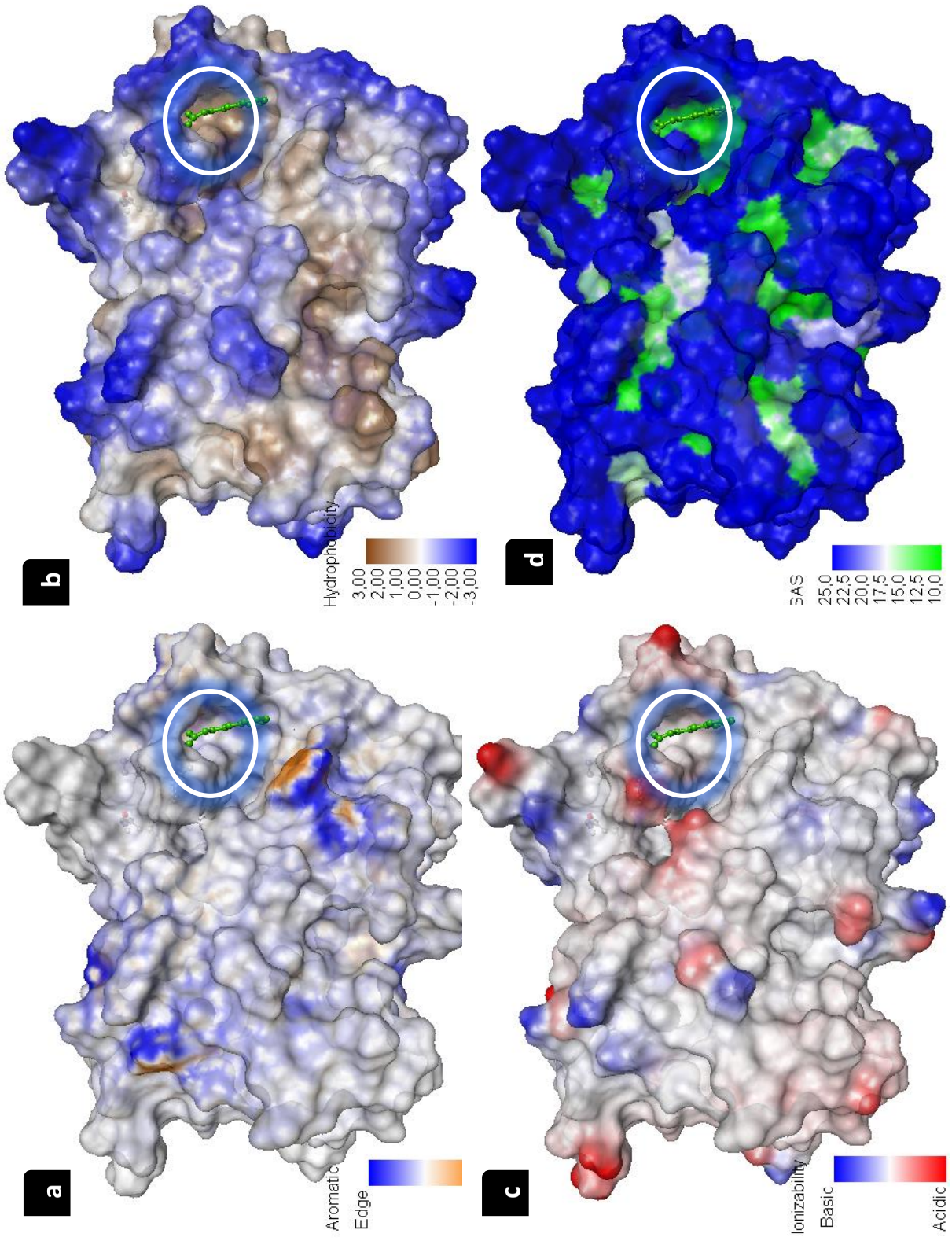


Figure 6. The schematic representation of (a) aromatic (b) hydrophobicity(c)ionizability (d) solvent accessibility surface of of 5282110 around 3G9E target.

Bibliographic references

[1] ***D. S. N. B. K. Prasanth, M. Murahari, V. Chandramohan, S. P. Panda, L. R. Atmakuri, & C. Guntupalli***, « In silico identification of potential inhibitors from Cinnamon against main protease and spike glycoprotein of SARS CoV-2 », Journal of Biomolecular Structure and Dynamics, (2020) :1–15.

[2] ***S. Deyno, K. Eneyew, S. Seyfe, N. Tuyiringire, E. L. Peter, R. Ashebir Muluye, … P. Engeu Ogwang,*** « Efficacy and safety of Cinnamon in Type 2 Diabetes Mellitus and Pre-diabetes patients: A Meta-analysis and Meta-regression », Diabetes Research and Clinical Practice 156, (2019) : 107815.

[3] ***N. Abirami, & R. Arulmozhi,*** « In-Silico Approach Towards Protein Targets Related to Diabetes Mellitus-An Overview », Oriental Journal of Chemistry, 33(04), (2017) :1614–1622.

[4] ***B. Chandrasekaran, S. N. Abed, O. Al-Attraqchi, K. Kuche, & R. K. Tekade,*** « Computer-Aided Prediction of Pharmacokinetic (ADMET) Properties », Dosage Form Design Parameters, (2018) :731–755.

[5] ***N. Stevens, K. Allred,*** « Antidiabetic Potential of Volatile Cinnamon Oil: A Review and Exploration of Mechanisms Using In Silico Molecular Docking Simulations », Molecules 27.3, (2022) : 853.

[6] ***P. V. Rao, S.H. Gan,*** « Cinnamon: a multifaceted medicinal plant », Evidence-Based Complementary and Alternative Medicine 2014 (2014).

[7] ***A. Sangal,*** « Role of cinnamon as beneficial antidiabetic food adjunct: A review », Advances in Applied Science Research 2.4 (2011): 440-450.

[8] ***M. Vangalapati, N. Sree Satya, D. Surya Prakash, and S. Avanigadda,*** « A review on pharmacological activities and clinical effects of cinnamon species », Research Journal of pharmaceutical, biological and chemical sciences 3.1 (2012): 653-663.

[9] ***P. N. Ravindran, K. Nirmal-Babu, and M. Shylaja,*** « Cinnamon and cassia: the genus Cinnamomum », CRC press, 2003.

[10] ***T. C. Huang, H. Y. Fu, C. T. Ho, D. Tan, Y. T. Huang, and M. H. Pan,*** « Induction of apoptosis by cinnamaldehyde from indigenous cinnamon Cinnamomum osmophloeum Kaneh

through reactive oxygen species production, glutathione depletion, and caspase activation in human leukemia K562 cells », Food chemistry 103.2 (2007) : 434-443.

[11] ***C. Orwa, A. Mutua, R. Kindt, R. Jamnadass, S. Anthony***, « Agroforestree Database: a tree reference and selection guide. Version », Agroforestree Database: a tree reference and selection guide. Version 4. (2009)

[12] ***P.N. Ravindran, K. N. Babu***, « Introduction, in: Ravindran, P.N., Babu, N.K., Shylaja, 634 M. (Eds.), Cinnamon and Cassia », CRC PRESS, Florida, USA, (2004), pp. 1–13.

[13] ***G. S. Chatoor,*** « The Spic(e)y story » Saboor Chatoor (Pvt) Ltd. (2017) : p. 47.

[14] ***J. Thomas, and P. P. Duethi***, « Handbook of herbs and spices », In KV Peter (Ed.), (2001) : 143-153.

[15] ***D. G. Barceloux***, « Cinnamon (cinnamomum species) », Disease-a-month 6(55), (2009): 327-335.

[16] ***S. Subasinghe, C. S. Hettiarachchi, and N. Iddagoda,*** « In-vitro propagation of cinnamon (Cinnamomum verum Presl.) using embryos and in vitro axillary buds », Journal of Advanced Agricultural Technologies Vol 3.3 (2016).

[17] ***B. Krishnamoorthy, J. Rema,*** « Nursery techniques in tree spices », Indian Cocoa, Arecanut and Spice J 11(3), (1987) :83–84

[18] ***R. O. B. Wijesekera and C. O. Chichester,*** « The chemistry and technology of cinnamon », Critical Reviews in Food Science & Nutrition 10.1 (1978): 1-30.

[19] ***Anonymous,*** « Cinnamon cultivation and processing (Technical Bulletin 5) », Department of Export Agricultur of Sri Lanka, (2015) :41 pp

[20] ***C. Alimentarius,*** « Food and Agriculture Organization of the United Nations » World Heath Organisation. Viale delle Terme di Caracalla 100 (2003).

[21] ***S. Kirkham, R. Akilen, S. Sharma, & A. Tsiami***, « The potential of cinnamon to reduce blood glucose levels in patients with type 2 diabetes and insulin resistance », Diabetes, obesity and metabolism 11.12 (2009): 1100-1113.

[22] ***D. W. Peterson, R. C. George, R. C, F. Scaramozzino, N. E. LaPointe, R. A. Anderson, D. J. Graves, & J. Lew,*** « Cinnamon extract inhibits tau aggregation associated with Alzheimer's disease in vitro », Journal of Alzheimer's Disease 17.3 (2009) : 585-597.

[23] ***M. S. Shihabudeen, H. Priscilla, and K. Thirumurugan***, « Cinnamon extract inhibits α-glucosidase activity and dampens postprandial glucose excursion in diabetic rats », Nutrition & metabolism 8.1 (2011): 1-11.

[24] ***P. A. Paranagama, G. S. Jayathilake, A. L. Jayawardene, A. M. Mubarak, U. M. Senanayeke, S. Wimalasena*** « A comparison of essential oil constituents of bark, leaf, root and fruit of cinnamon (Cinnamomum zeylanicum Blum) grown in Sri Lanka », Journal of the National Science Foundation of Sri Lanka 29, (2001) :3-4

[25] ***H. S. Kusuma, & M. Mahfud,*** « Preliminary study: Kinetics of oil extraction from sandalwood by microwave-assisted hydrodistillation », ASEAN Journal of Chemical Engineering, 15(2), (2015) : 62–69

[26] ***N. Bousbia, M. A. Vian, M. A. Ferhat, B. Y. Meklati, & F. Chemat***, « A new process for extraction of essential oil from Citrus peels: Microwave hydrodiffusion and gravity », Journal of food Engineering 90.3, (2009): 409-413.

[27] ***S. Chanthaphon, S. Chanthachum, & T. Hongpattarakere,*** « Antimicrobial activities of essential oils and crude extracts from tropical Citrus spp. against food-related microorganisms », Songklanakarin Journal of Science & Technology 30, (2008).

[28] ***M. D. Anjazi, S. K., & S. M. Sauid***, « Effects Of Ultrasonic Pretreatment And Hydrodistillation Time On The Extraction Of Essentials Oil From Dried Kaffir Lime Leaves », Universiti Teknologi MARA, (2015).

[29] ***M. S. Suhaila, A. A. Faris,*** « Extraction methods of essential oil from kaffir lime (Citrus hystrix): A review », Malaysian Journal of Chemical Engineering & Technology 1, (2018) :56–64.

[30] ***J. Y. Lee, W. Park,*** « Anti-inflammatory effect of myristicin on RAW 264.7 macrophages stimulated with polyinosinic-polycytidylic acid », Molecules 16.8, (2011) : 7132-7142.

[31] ***W. X. Du, C. W. Olsen, R. J. Avena-Bustillos, T. H. McHugh, C. E. Levin, M. Friedman,*** « Effects of allspice, cinnamon, and clove bud essential oils in edible apple films on physical properties and antimicrobial activities », Journal of Food Science 74.7, (2009): M372-M378.

[32] ***T. Nuryastuti, H. C. van der Mei , H. J. Busscher, S. Iravati, A. T. Aman, B. P. Krom,*** « Effect of cinnamon oil on icaA expression and biofilm formation by Staphylococcus epidermidis », Applied and environmental microbiology 75.21, (2009): 6850-6855 .

[33] ***G. Meades, R. L. Henken, G. L. Waldrop, M. M. Rahman, S. D. Gilman, G. P. Kamatou, A. M. Viljoen, S. Gibbon,*** « Constituents of cinnamon inhibit bacterial acetyl CoA carboxylase." Planta medica 76.14, (2010) : 1570-1575.

[34] ***F. Q. Guerra, J. M. Mendes, J. P. Sousa, M. F. Morais-Braga, B. H. Santos, H. D. Melo Coutinho, E. D. Lima,*** « Increasing antibiotic activity against a multidrug-resistant Acinetobacter spp by essential oils of Citrus limon and Cinnamomum zeylanicum », Natural product research, (2011).

[35] ***G. K. Jayaprakasha, L. J. Rao,*** « Chemistry, biogenesis, and biological activities of Cinnamomum zeylanicum », Crit Rev Food Sci Nutr 51(6), (2011) :547–562.

[36] ***S. Mandal, M. DebMandal, K. Saha, N. K. Pal,*** « In vitro antibacterial activity of three Indian spices against methicillin-resistant Staphylococcus aureus », Oman Medical Journal 26.5, (2011) : 319.

[37] ***L. Wang, F. Liu, Y. Jiang, Z. Chai, P. Li, Y. Cheng, H. Jing, X. Leng,*** « Synergistic antimicrobial activities of natural essential oils with chitosan fi lms », J Agric Food Chem 59(23), (2011) :12411–12419.

[38] ***S. Duessel, R. M. Heuertz, U. R. Ezekiel,*** « Growth inhibition of human colon cancer cells by plant compounds », Clin Lab Sci 21(3), (2008) :151–157.

[39] ***M. Govindarajan,*** « Larvicidal and repellent properties of some essential oils against Culex tritaeniorhynchus Giles and Anopheles subpictus Grassi (Diptera: Culicidae) » Asian Pac J Trop Med 4, (2011) :106–111.

[40] ***H. Ping, G. Zhang, G. Ren***, « Antidiabetic effects of cinnamon oil in diabetic KK-Ay mice », Food Chem Toxicol 48, (2010) :2344–2349.

[41] ***Q. Jia, X. Liu, X. Wu, R. Wang, X. Hu, Y. Li, C. Huang***, « Hypoglycemic activity of a polyphenolic oligomer-rich extract of Cinnamomum parthenoxylon bark in normal and streptozotocininduced diabetic rats », Phytomedicine 16(8), (2009) :744–750.

[42] ***Y. Shen, M. Fukushima, Y. Ito, E. Muraki, T. Hosono, T. Seki, T. Ariga,*** « Verification of the antidiabetic effects of cinnamon (Cinnamomum zeylanicum) using insulin-uncontr using insulin-uncontrolled type 1 diabetic rats and cultured adipocytes », Bioscience, biotechnology, and biochemistry 74.12 ,(2010): 2418-2425.

[43] ***M. Z. Shahid, H. Saima, A. Yasmin, M. T. Nadeem, M. Imran, M. Afzaal***, « Antioxidant capacity of cinnamon extract for palm oil stability », Lipids in health and disease 17.1 (2018) : 1-8.

[44] ***W. A. N. M. Wariyapperuma, S. Kannangara, S. Yasanandana,*** « In vitro anti-diabetic effects and phytochemical profiling of novel varieties of Cinnamomum zeylanicum (L.) extracts », PeerJ 8, (2020), 8, 1–23.

[45] ***J. Lin, A. R. Opoku, M. Geheeb-Keller, A. D. Hutchings, S. F. Terblanche, A. K. Jäger, & J. Van Staden,*** « Preliminary screening of some traditional zulu medicinal plants for anti-inflammatory and anti-microbial activities », Journal of Ethnopharmacology 68.1-3, (1999) : 267-274.

[46] ***E. N. Matu and J. van Staden,*** « Antibacterial and anti-inflammatory activities of some plants used for medicinal purposes in Kenya », Journal of Ethnopharmacology 87.1, (2003) : 35-41.

[47] ***L. K. Chao, K. F. Hua, H. Y. Hsu, S. S. Cheng, J. Y. Liu, and S. T. Chang,*** « Study on the antiinflammatory activity of essential oil from leaves of Cinnamomum osmophloeum », Journal of Agricultural and Food Chemistry 53.18, (2005) : 7274-7278.

[48] ***Y. T. Tung, P. L. Yen, C. Y. Lin, and S. T. Chang,*** « Antiinflammatory activities of essential oils and their constituents from different provenances of indigenous cinnamon (Cinnamomum osmophloeum) leaves », Pharmaceutical Biology, vol. 48, no. 10, (2010), pp. 1130–1136.

[49] ***A. Garc´ıa-Lafuente, E. Guillamon, A. Villares, M. A. Rostagno, ´and J. A. Mart´ınez,*** « Flavonoids as anti-inflammatory agents: implications in cancer and cardiovascular disease », Inflammation Research, vol. 58, no. 9, (2009), pp. 537–552.

[50] ***N. Cho, K. Y. Lee, J. Huh, J. H. Choi, H. Yang, E. J. Jeong, & S. H. Sung,*** « Cognitive-enhancing effects of Rhus verniciflua bark extract and its active flavonoids with neuroprotective and anti-inflammatory activities », Food and Chemical Toxicology, vol. 58, (2013), pp. 355–361.

[51] ***L. B. Gende, I. Floris, R. Fritz, and M. J. Eguaras,*** « Antimicrobial activity of cinnamon (Cinnamomum zeylanicum) essential oil and its main components against paenibacillus larvae from argentine », Bulletin of Insectology, vol. 61, no. 1, (2008), pp. 1–4.

[52] ***S. Prabuseenivasan, M. Jayakumar, and S. Ignacimuthu***, « In vitro antibacterial activity of some plant essential oils », BMC Complementary and Alternative Medicine, vol. 6, (2006).

[53] ***R. Becerril, R. Gomez-Lus, P. Go ni, P. L opez, and C. Nerın,*** « Combination of analytical and microbiological techniques to study the antimicrobial activity of a new active food packaging containing cinnamon or oregano against E. coli and S. aureus », Analytical and Bioanalytical Chemistry, vol. 388, no. 5-6, (2007), pp. 1003–1011.

[54] ***N. Matan, H. Rimkeeree, A. J. Mawson, P. Chompreeda, V. Haruthaithanasan, and M. Parker***, « Antimicrobial activity of cinnamon and clove oils under modified atmosphere conditions », International Journal of Food Microbiology, vol. 107, no. 2, (2006). pp. 180–185.

[55] ***T. Bandara, I. Uluwaduge, E. R. Jansz,*** « Bioactivity of cinnamon with special emphasis on diabetes mellitus: a review », Int J Food Sci Nutr 63(3), (2012) :380–386

[56] ***S. C. Ho, P. W. Chang,*** « Inhibitory effects of several spices on infammation caused by advanced glycation end products », Am J Plant Sci 3, (2012) :995–1002.

[57] ***P. Ranasinghe,*** « Evaluation of Cinnamomum zeylanicum (Ceylon cinnamon) as a pharmaceutical agent for diabetes mellitus », PhD Thesis, Faculty of Medicine, University of Colombo, Sri Lanka, (2019).

[58] ***J. J. Dugoua, D. Seely, D. Perri, K. Cooley, T. Forelli, E. Mills, & G. Koren,*** « From type 2 diabetes to antioxidant activity: a systematic review of the safety and efficacy of common and cassia cinnamon bark », Canadian journal of physiology and pharmacology 85.9, (2007): 837-847.

[59] ***C. A. Newall, L. A. Anderson, & J. D. Phillipson,*** « Herbal medicines. A guide for health-care professionals », The pharmaceutical press, (1996).

[60] ***A. H. Shah, A. H. Al-Shareef, A. M. Ageel, & S. Qureshi,*** « Toxicity studies in mice of common spices, Cinnamomum zeylanicum bark and Piper longum fruits », Plant Foods for Human Nutrition (Formerly Qualitas Plantarum), 52(3), (1998) : 231-239.

[61] ***J. J. Dugoua, D. Seely, D. Perri, K. Cooley, T. Forelli, E. Mills, & G. Koren,*** « From type 2 diabetes to antioxidant activity : a systematic review of the safety and efficacy of common and cassia cinnamon bark », Canadian journal of physiology and pharmacology 85.9, (2007) : 837-847.

[62] ***A. H. Shah, A. H. Al-Shareef, A. M. Ageel, & S. Qureshi,*** « Toxicity studies in mice of common spices, Cinnamomum zeylanicum bark and Piper longum fruits », Plant Foods for Human Nutrition 52.3, (1998): 231-239.

[63] ***H. Cao, J. F. Jr. Urban, & R. A. Anderson,*** « Cinnamon polyphenol extract affects immune responses by regulating anti-and proinflammatory and glucose transporter gene expression in mouse macrophages », The Journal of nutrition 138.5 (2008): 833-840.

[64] ***R. A. Anderson,*** « Chromium and polyphenols from cinnamon improve insulin sensitivity: plenary lecture », Proceedings of the Nutrition Society 67.1 (2008): 48-53

[65] ***R. Sicree, J. Shaw J and P. Zimmet P,*** « The Global Burden. Diabetes and Impaired Glucose Tolerance. Prevalence and Projections », International Diabetes Federation, pp. 16–103.

[66] ***R. W. Shillitoe,*** « Psychology and diabetes: psycholocial factors in management and control », Chapman and Hall, (1988).

[67] ***J. J. Caro, A.J. Ward, J. A. O'Brien,*** « Lifetime costs of complications resulting from type 2 diabetes in the U.S », Diabetes Care, (2002) :476–481.

[68] gamma-dynacare medical laboratories (2011).

[69] ***M. Patlak,*** « New weapons to combat an ancient disease: treating diabetes », The FASEB Journal 16.14 (2002): 1853e-1853e.

[70] ***A. American Diabetes,*** « Diagnosis and classification of diabetes mellitus », Diabetes care, (2014) ; 37 Suppl 1: S81-90.

[71] Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. National Diabetes Data Group. Diabetes 28(12), (1979) :1039-57.

[72] ***D. Daneman,*** « Type 1 diabetes », The Lancet 367.9513 (2006) : 847-858.

[73] ***American Diabetes Association,*** « Diagnosis and classification of diabetes mellitus », Diabetes care 29.1, (2006): S43.

[74] ***R. Hanas,*** « Type 1 diabetes in children, adolescents, and young adults : how to become an expert on your own diabetes », Class Publishing Ltd, (2007).

[75] ***K. G. Alberti, P. Z. Zimmet,*** « Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report

of a WHO consultation », Diabetic medicine : a journal of the British Diabetic Association 15(7), (1998) :539-53.

[76] ***S. E. Kahn, M. E. Cooper, S. del Prato,*** « Pathophysiology and treatment of type 2 diabetes: perspectives on the past, present, and future », The Lancet 383.9922, (2014): 1068-1083.

[77] ***UK Prospective Diabetes Study Group,*** « UK Prospective Diabetes Study 16: overview of 6 years' therapy of type II diabetes: a progressive disease », Diabetes 44.11, (1995): 1249-1258.

[78] ***P. Zimmet, K. G. Alberti, J. Shaw,*** « Global and societal implications of the diabetes epidemic », Nature 414.6865, (2001) : 782-787.

[79] ***P. Zimmet, P, « K. J. Alberti, J. Shaw »,*** « Global and societal implications of the diabetes epidemic », Nature 414, (2001) : 782-787.

[80] ***C. G. Schalkwijk, C. D. Stehouwer, «*** Vascular complications in diabetes mellitus: the role of endothelial dysfunction », Clinical science 109.2, (2005): 143-159.

[81] ***J. Tuomilehto, K. Borch-Johnsen, A. Molarius, T. Forsen, D. Rastenyte, C. Sarti, & A. Reunanen,*** « Incidence of cardiovascular disease in Type 1 (insulin-dependent) diabetic subjects with and without diabetic nephropathy in Finland », Diabetologia 41.7, (1998): 784-790.

[82] ***J. Stamler, O. Vaccaro, J. D. Neaton, & D. Wentworth,*** « Diabetes, other risk factors, and 12-yr cardiovascular mortality for men screened in the multiple risk factor intervention trial », Diabetes Care 16, (1993) :434–444.

[83] ***S. M. Haffner, S. Lehto, T. Rönnemaa, K. Pyörälä, & M. Laakso, M,*** « Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction », New England journal of medicine 339.4, (1998): 229-234.

[84] ***H. E. Mulnier, H. E. Seaman, V. S. Raleigh, S. S. Soedamah-Muthu, H. M. Colhoun, R. A. Lawrenson, & C. S. De Vries,*** « Risk of stroke in people with type 2 diabetes in the UK: a study using the General Practice Research Database », Diabetologia 49.12, (2006): 2859-2865.

[85] ***N. J. Morrish, S. L. Wang, L. K. Stevens, J. H. Fuller, & H. Keen,*** « Mortality and causes of death in the WHO Multinational Study of Vascular Disease in Diabetes », Diabetologia 44.2, (2001): S14-S21

[86] ***A. D. Deshpande, M. Harris-Hayes, & M. Schootman,*** « Epidemiology of diabetes and diabetes-related complications », Phys. Ther. 88, (2008) :1254–1264.

[87] ***T. Cundy, A. Holden, & E. Stallworthy***, « Early worsening of diabetic nephropathy in type 2 diabetes after rapid improvement in chronic severe hyperglycemia », Diabetes Care 44.3 ,(2021): e55-e56.

[88] ***P. Romero-Aroca, S. De La Riva-Fernandez, A. Valls-Mateu, R. Sagarra-Alamo, A. Moreno-Ribas, & N. Soler,*** « Changes observed in diabetic retinopathy: eight-year follow-up of a Spanish population », British Journal of Ophthalmology 100.10, (2016): 1366-1371

[89] ***G. Sloan, D. Selvarajah, S. Tesfaye***, « Pathogenesis, diagnosis and clinical management of diabetic sensorimotor peripheral neuropathy », Nature Reviews Endocrinology 17.7, (2021) : 400-420.

[90] ***M. C. Perez-Matos, M. C. Morales-Alvarez, & C. O. Mendivil***, « Lipids: a suitable therapeutic target in diabetic neuropathy? », Journal of diabetes research 2017 (2017).

[91] ***R. S. Stiegler, P. Z. Zimmet, A. J. Cameron and J. E. Shaw,*** « Lifestyle management: preventing Type 2 diabetes and cardiovascular complications », Clinical Practice 6.4, (2009): 489 .

[92] ***M. N. Piero,*** « Hypoglycemic effects of some Kenyan plants traditionally used in management of diabetes mellitus in eastern province », Doctoral dissertation, Msc thesis, Kenyatta University, (2006).

[93] ***S. E. Inzucchi,*** « Oral antihyperglycemic therapy for type 2 diabetes: scientific review », Jama 287.3, (2002): 360-372.

[94] ***L. T. Curtis LT,*** « New technologies and therapies in the management of diabetes », American journal of managed care, 13(2 suppl), (2007): S47-S54.

[95] ***L. M. Njagi, C. M. Kibiti, J. J. N. Ngeranwa, A. N. M. Njagi, W. M. Njue and P. K. Gathumbi,*** « Herbal management of diabetes mellitus: a rapidly expanding research avenue », Int J Curr Pharm Res, 4 (2), (2012):1-4.

[96] ***R. B. Colquitt, D. A. Colquhoun, & R. H. Thiele,*** « In silico modelling of physiologic systems », Best practice & research Clinical anaesthesiology 25.4, (2011): 499-510.

[97] ***S. Ekins, J. Mestres, B. Testa,*** « In silico pharmacology for drug discovery: methods for virtual ligand screening and profiling », British journal of pharmacology 152.1, (2007): 9-20.

[98] ***H. B. Sieburg,*** « Physiological studies in silico », Studies in the Sciences of Complexity 12, (1990): 321–342.

[99] ***A. Danchin, C. Medigue, O. Gascuel, H. Soldano, A. Henaut,*** « From data banks to data bases », Res Microbiol 142, (1991): 913–916.

[100] ***A. Danchin***, « The Delphic Boat – What Genomes Tell Us », Harvard University Press: Cambridge, MA, (2002).

[101] ***R. B. Colquitt, D. A. Colquhoun, & R. H. Thiele,*** « In silico modelling of physiologic systems », Best Practice & Research Clinical Anaesthesiology, 25(4), (2011) :499–510.

[102] ***A. Van Soom, L. Vandaele, L. J. Peelman, K. Goossens, & A. Fazeli,*** « Modeling the interaction of gametes and embryos with the maternal genital tract: From in vivo to in silico », Theriogenology, 73(6), (2010) :828–837.

[103] ***Z. S. Khachaturian, J. Lombardo,*** « In silico modeling system: a national research resource for simulation of complex brain disorders », Alzheimer's & Dementia: the Journal of the Alzheimer's Association 5.1, (2009): 1-4.

[104] ***L. Huynh, R. Masereeuw, T. Friedberg, M. Ingelman-Sundberg, P. Manivet P,*** « In silico platform for xenobiotics ADME-T pharmacological properties modeling and prediction. Part I: beyond the reduction of animal model use », Drug discovery today 14.7-8, (2009): 401-405.

[105] ***J. C. Madden, S. J. Enoch, A. Paini, & M. T. D. Cronin, M. T. D,*** « A review of in silico tools as alternatives to animal testing: principles, resources and applications », Alternatives to Laboratory Animals 48.4, (2020): 146-172.

[106] ***E. Carnesecchi, C. Toma, A. Roncaglioni, N. Kramer, E. Benfenati, & J. L. C. Dorne,*** « "Integrating QSAR models predicting acute contact toxicity and mode of action profiling in honey bees (A. mellifera): Data curation using open source databases, performance testing and validation », Science of the Total Environment 735, (2020): 139243 .

[107] ***G. Piir, I. Kahn, A. T. Garc´ıa-Sosa, S. Sild, P. Ahte, & U. Maran,*** « Best practices for QSAR model reporting: physical and chemical properties, ecotoxicity, environmental fate, human health, and toxicokinetics endpoints », Environmental health perspectives 126.12, (2018): 126001.

[108] ***B. Testa, S. D. Kra¨mer,*** « The biochemistry of drug metabolism – an introduction. Part 1: principles and overview », Chem Biodivers 3, (2006): 1053–1101.

[109] ***A. Alber,*** « Relations between molecular structure and biological activity: stages in the evolution of current concepts », Ann Rev Pharmacol 11, (1971): 13–36.

[110] ***A. Albert,*** « Selective Toxicity. The Physcico-Chemical Basis of Therapy », Chapman and Hall: London, ‘1985.

[111] ***H. Meyer,*** « Zur Theorie der Alkoholnarkose », Arch Expl Pathol Pharmakol 42, (1899): 110–118.

[112] ***C. E. Overton*** « Studien über die Narkose », zugleich ein Beitrag zur allgemeinen Pharmakologie. G. Fischer, (1901).

[113] ***C. Hansch, T. Fujita,*** « p-σ-π Analysis. A Method for the Correlation of Biological Activity and Chemical Structure », Journal of the American Chemical Society 86.8, (1964): 1616-1626.

[114] ***C. Hansch,*** « Quantitative relationships between lipophilic character and drug metabolism », Drug Metab Rev 1, (1972): 1–14.

[115] E. J. Arı¨ens, « Receptors: from fiction to fact », Trends Pharmacol Sci 1, (1979): 11–15.

[116] ***J. Parascandola***, « Origins of the receptor theory », Trends Pharmacol Sci 1, (1980): 189–192.

[117] ***B. Shaker, S. Ahmad, J. Lee, C, Jung, & D. Na,*** « In silico methods and tools for drug discovery », Computers in Biology and Medicine 137, 104851, (2021).

[118] ***P. E. Brandish, C. S. Chiu, J. Schneeweis, N. J. Brandon, C. L. Leech, O. Kornienko, E. M. Scolnick, B. Strulovici, W. Zheng,*** « A cell-based ultra-high-throughput screening assay for identifying inhibitors of D-amino acid oxidase », SLAS Discovery 11.5, (2006): 481-487

[119] ***A. Tropsha,*** « QSAR in drug discovery », Drug design: Structure-and ligand-based approaches 1, (2010).

[120] ***A. C. Nascimento, R. B. Prudˆencio, I. G. Costa,*** « A multiple kernel learning algorithm for drug-target interaction prediction », BMC Bioinf. 17, (2016) : 1–16.

[121] ***W. Wang, M. T. Kim, A. Sedykh, H. Zhu,*** « Developing enhanced blood–brain barrier permeability models: integrating external bio-assay data in QSAR modeling, Pharm », Res. 32, (2015) 3055–3065.

[122] ***P. Schyman, R. Liu, V. Desai, A. Wallqvist,*** « vNN web server for ADMET predictions », Front. Pharmacol 8, (2017) 889.

[123] ***R. Liu, G. Tawa, A. Wallqvist,*** « Locally weighted learning methods for predicting dose-dependent toxicity with application to the human maximum recommended daily dose », Chemical Research in Toxicology 25.10, (2012): 2216-2226.

[124] ***T. Katsila, G.A. Spyroulias, G.P. Patrinos, M.-T. Matsoukas,*** « Computational approaches in target identification and drug discovery », Computational and structural biotechnology journal 14, (2016): 177-184.

[125] ***J. L. Jenkins, A. Bender, J. W. Davies,*** « In silico target fishing: predicting biological targets from chemical structure », Drug Discov, Today Technol 3, (2006) 413–421.

[126] ***J. Drews,*** « Drug discovery: a historical perspective », Science 287, (2000) 1960–1964.

[127] ***R. Reddy, R. Mutyala, P. Aparoy, P. Reddanna, M. R. Reddy,*** « Computer aided drug design approaches to develop cyclooxygenase based novel anti-inflammatory and anti-cancer drugs », urrent pharmaceutical design 13.34, (2007): 3505-3517

[128] ***M. Cordeiro, A. Speck-Planche,*** « Computer-aided drug design, synthesis and evaluation of new anti-cancer drugs », Curr. Topics Med. Chem. 12, (2012) 2703.

[129] ***R. Balamurugan, A. Stalin, S. Ignacimuthu,*** « Molecular docking of γ-sitosterol with some targets related to diabetes », European journal of medicinal chemistry 47, (2012): 38-43

[130] ***H. L. Sham, D. J. Kempf, A. Molla, K. C. Marsh, G .N. Kumar, C. M. Chen, W. Kati, K. Stewart, R. Lal, A. Hsu,*** « ABT-378, a highly potent inhibitor of the human immunodeficiency virus protease », Antimicrobial agents and chemotherapy 42.12, (1998): 3218-3224

[131] ***L. Doyon, S. Tremblay, L. Bourgon, E. Wardrop, M. G. Cordingley***, « Selection and characterization of HIV-1 showing reduced susceptibility to the non-peptidic protease inhibitor tipranavir », Antiviral research 68.1, (2005): 27-35.

[132] ***M. Batool, B. Ahmad, S. Choi,*** « A structure-based drug discovery paradigm », International journal of molecular sciences 20.11, (2019): 2783.

[133] ***P. M. Njogu, E. M. Guantai, E. Pavadai, K. Chibale,*** « Computer-aided drug discovery approaches against the tropical infectious diseases malaria, tuberculosis, trypanosomiasis, and leishmaniasis », ACS infectious diseases 2.1, (2016): 8-3.

[134] ***A. Singh, S. Zahra, & S. Kumar,*** « In-silico Tools in Phytochemical Research », Phytochemistry: An in-silico and in-vitro Update. Springer, Singapore, (2019) : 351-372.

[135] ***I. Davidson-Hunt,*** « Ecological ethnobotany: stumbling toward new practices and paradigms », MASA J 16.1, (2000): 1-3.

[136] ***A. A. Lagunin, R. K. Goel, D. Y. Gawande, P. Pahwa, T. A. Gloriozova, A. V. Dmitriev, & V. V. Poroikov,*** « Chemo-and bioinformatics resources for in silico drug discovery from medicinal plants beyond their traditional use: a critical review », Natural product reports 31.11, (2014): 1585-1611 .

[137] ***M. Ekor, M,*** « The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety », Front. Neurol. 4, (2013) :1–10**.**

[138] ***S. Kar, & K. Roy, «*** QSAR of phytochemicals for the design of better drugs », Expert opinion on drug discovery 7.10, (2012) : 877-902.

[139] ***C. D. Selassie,*** « History of quantitative structure-activity relationships », In: Abraham DJ, editor. Burger's medicinal chemistry and drug discovery. John Wiley ; New York, USA, (2003), p. 1-48.

[140] ***E. A. Coats,*** « The CoMFA steroids as a benchmark dataset for development of 3D QSAR methods », 3D QSAR in drug design. Springer, Dordrecht, (1998) :199-213.

[141] ***S. Vilar, G. Cozza, S. Moro,*** « Medicinal chemistry and the molecular operating environment (MOE): application of QSAR and molecular docking to drug discovery », Current topics in medicinal chemistry 8.18, (2008): 1555-1572.

[142] ***Y. C. Wong, M. Ahmad-Mudzaqqir, & W. Wan-Nurdiyana,*** « Extraction of essential oil from cinnamon (Cinnamomum zeylanicum) », Oriental journal of chemistry 30.1, (2014): 37 .

[143] ***L. Ban, B. Narasimhamoorthy, L. Zhao, J. A. Greaves, and W. D. Schroeder,*** « Antioxidant activities from different rosemary clonal lines », Food chemistry 201, (2016): 259-263.

[144] ***D. Naviglio, P. Scarano, M. Ciaravolo and M. Gallo***, « Rapid Solid-Liquid Dynamic Extraction (RSLDE): A powerful and greener alternative to the latest solid-liquid extraction techniques », Foods 8.7, (2019): 245.

[145] ***AFNOR (Association Française de Normalisation),*** « Épices et aromates », Paris, NF V00 0001, (1978).

[146] ***ISO279,*** « In: Essential Oils—Determination of Relative Density at 20 Degrees C—Reference Method. ISO », editor. Multiple Distributed through American National Standards Institute (ANSI); Gèneve, Switzerland, (1998).

[147] ***ISO 280,*** « Essential oils: Determination of refractive index », 2nd ed. International Organization for Standardization, Geneva, (1998).

[148] ***A. F. Khalil, H. O. Elkatry, H. F. El Mehairy,*** « Protective effect of peppermint and parsley leaves oils against hepatotoxicity on experimental rats », Ann Agric Sci. (2015) ; 60 : 353-359.

[149]* L. I. Spirling, I. R. Daniels,*** « Botanical perspectives on health peppermint: more than just an after-dinner mint », The journal of the Royal Society for the Promotion of Health 121.1 (2001): 62-63.

[150] ***W. B. Jensen***, « The symbol for pH », Journal of Chemical Education 81.1, (2004): 21.

[151] ***OWAC (Association of Official Agricultural Chemists),*** « Official Methods of Analysis », (1997).

[152] ***Pharmacopée Européenne***, 8ème édition, Service Européen de la Qualité du Médicament-Conseil de L'Europe, (2001).

[153] ***H. B. W. Patterson***, « Quality and control », Hydrogenation of fats and oils. AOCS Press, (2011) : 329-350.

[154] ***R. Barret,*** « Importance and evaluation of lipophilicity », Therapeutical Chemistry, (2018): 53-78.

[155] ***L. Cai,*** « Thin layer chromatography », Current Protocols Essential Laboratory Techniques 8.1, (2014): 6-3.

[156] ***D. A. Skoog, F. J. Holler & S. R. Crouch, S. R,*** « Principles of instrumental analysis. thomson brooks », Cole, Canada, (2007).

[157] ***R. P. Adams,*** « Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry », Vol. 456. Carol Stream: Allured publishing corporation, (2007).

[158] ***A.V. Badarinath, K. Mallikarjuna Rao, C. Madhu Sudhana Chetty, S. Ramkanth, T.V.S. Rajan, K. Gnanaprakash,*** « A review on in-vitro antioxidant methods: comparisions, correlations and considerations », International Journal of PharmTech Research 2.2, (2010): 1276-1285

[159] ***B. Bozin, N. Mimica-Dukic, I. Samojlik, E. Jovinj,*** « Antimicrobial and antioxidant properties of rosemary and sage (Rosmarinus officinalis L. and Salvia officinalis L., Lamiaceae) essential oils », Journal of agricultural and food chemistry 55.19, (2007): 7879-7885.

[160] ***M. Viuda-Martos, Y. R. Navajas, E. S. Sapata, J. Fernandez-´ Lopez, J. A. Pérez-Álvarez,*** « Antioxidant activity of essential oils of five spice plants widely used in a Mediterranean die », Flavour and Fragrance Journal 25.1, (2010): 13-19.

[161] ***M. Mkaddem, J. Bouajila, M. Ennajar, A. Lebrihi, F. Mathieu, & M. Romdhane,M,*** « Chemical composition and antimicrobial and antioxidant activities of Mentha (longifolia L. and viridis) essential oils», Journal of food science 74.7, (2009): M358-M363.

[162] ***R. Amorati, M. C. Foti, & L. Valgimigli,*** « Antioxidant Activity of Essential Oils », Journal of Agricultural and Food Chemistry, 61(46), (2013), 10835–10847.

[163] ***W. Brand-Williams, M. E. Cuvelier, C. L. W. T. Berset,*** « Use of a free radical method to evaluate antioxidant activity », LWT-Food science and Technology 28.1, (1995): 25-30 .

[164] ***C. Gardeli, V. Papageorgiou, A. Mallouchos, K. Theodosis, M. Kamaitis,*** « Essential oil composition of Pistacia lentiscus L. and Myrtus communis L.: Evaluation of antioxidant capacity of methanolic extracts », Food chemistry 107.3, (2008): 1120-1130.

[165] ***F. Candan, M. Unlu, B. Tepe B,*** « Antioxidant and antimicrobial activity of the essential oil and methanol extracts of Achillea millefolium subsp », Millefolium Afan. (Asteraceae) Journal of Ethnopharmacology ; 87(2-3), (2003) :215–220.

[166] ***A. Oubihi, L. Ouryemchi, I. Nounah, K. Tarfaoui, H. Harhar, M. Ouhssine, & Z. Guessous,*** « Chemical composition, antibacterial and antifungal activities of Thymus leptobotrys Murb essential oil », Advances in Traditional Medicine 20.4, (2020) : 673-679.

[167] ***A. M. El-Mahmood, J. H. Doughari,*** « Bacteriological examination of some diluted disinfectants routinely used in the Specialist Hospital Yola, Nigeria », African Journal of Pharmacy and Pharmacology 3.5, (2009): 185-190.

[168] ***L. Nedorostova, P. Kloucek, L. Kokoska, M. Stolcova, & J. Pulkrabek,*** « Antimicrobial properties of selected essential oils in vapour phase against foodborne bacteria », Food control 20.2, (2009): 157-160.

[169] ***P. J. Kumar, and M. Clark,*** « Textbook of clinical medicine », London, UK : Saunders, (2002) :1099–1121

[170] ***M. J. Meneses, B. M. Silva, M. Sousa, R. Sa, P. F. Oliveira, & M. G Alves,*** « Antidiabetic drugs: mechanisms of action and potential outcomes on cellular metabolism », Current pharmaceutical design 21.25, (2015): 3606-3620.

[171] ***T. C. Huang, H. Y. Fu, C. T. Ho, D. Tan, Y. T. Huang, & M. H. Pan,*** « Induction of apoptosis by cinnamaldehyde from indigenous cinnamon Cinnamomum osmophloeum Kaneh through reactive oxygen species production, glutathione depletion, and caspase activation in human leukemia K562 cells », Food chemistry 103.2, (2007): 434-443.

[172] ***O. L. Franco, D. J. Rigden, F. R. Melo, M. F. Grossi-de,*** « Plant α-amylase inhibitors and their interaction with insect α-amylases: Structure, function and potential for crop protection », European journal of biochemistry 269.2, (2002): 397-412 .

[173] ***S. Forli, R. Huey, M. E. Pique, M. F. Sanner, D. S. Goodsell, & A. J. Olson,*** « Computational protein–ligand docking and virtual drug screening with the AutoDock suite », Nature protocols 11.5, (2016) : 905-919 .

[174] ***B. Svensson, J. M. Autry, & D. D. Thomas,*** « Molecular modeling of fluorescent SERCA biosensors », P-Type ATPases. Humana Press, New York, NY, (2016) : 503-522.

[175] ***S. Yuan, H. C. S. Chan, H, & Z. Hu,*** « Using PyMOL as a platform for computational drug design », Wiley Interdisciplinary Reviews : Computational Molecular Science 7.2, (2017) : e1298.

[176] ***H. M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T. N. Bhat, H. Weissig, ... & P. E. Bourne,*** « The protein data bank », Nucleic acids research 28.1, (2000) : 235-242.

[177] ***Y. Wang, J. Xiao, T. O. Suzek, J. Zhang, J. Wang, & S. H. Bryant,*** « PubChem : a public information system for analyzing bioactivities of small molecules », Nucleic acids research 37.suppl_2, (2009): W623-W633.

[178] ***C. A. Lipinski, F. Lombardo, B. W. Dominy, P. J. Feeney,*** « Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings », Advanced drug delivery reviews 23.1-3, (1997): 3-25.

[179] ***Books of Dante – novembre*** 2014.

[180] ***Pharmacopie européennne***, (2011).

[181] ***B. Poaty, J. Lahlah, F. Porqueres, & H. Bouafif,*** « Composition, antimicrobial and antioxidant activities of seven essential oils from the North American boreal forest », World Journal of Microbiology and Biotechnology 31.6, (2015): 907-919 .

[182] ***(Pharmacopée Européenne*** : 8 ème édition, Service Européen de la Qualité du Médicament-Conseil de L'Europe, (2001).

[183] ***pharmacopie européennne*** (2005).

[184] ***F. Couic-Marinier, and A. Lobstein,*** « Mode d'utilisation des huiles essentielles », Actualités pharmaceutiques 52.525, (2013): 26-30.

[185*] ***AFNOR ; NF T-75 111, 112*** « association française de normalisation, Norme Huile essentielle » Paris, France, (2000).

[186] ***H. Wagner, S. Bladt, E. M. gainski,*** « Plant Drug Analysis (Translated by A. Scott) », Berlin, itd: Springer Verlag, (1984).

[187] ***W. C. Still, M. Kahn, A. Mitra,*** « Rapid chromatographic technique for preparative separations with moderate resolution », The Journal of Organic Chemistry 43.14, (1978): 2923-2925.

[188] ***G. J. E. Nychas,*** « Natural antimicrobials from plants », New methods of food preservation. Springer, Boston, MA, (1995) :58-89.

[189] ***V. A. Parthasarathy, B. Chempakam, T. J. Zachariah,*** « Chemistry of Spices », Cabi, (2008).

[190] ***H. Türkez, E. Aydın,*** « In vitro assessment of cytogeneticand oxidative effects of α-pinene », Toxicol Ind Health 32, (2016) ; 32:168-76.

[191] ***B. Salehi, S.Upadhyay, I. Erdogan Orhan, A. Kumar Jugran, S. L. D. Jayaweera, D. A. Dias, ... & J. Sharifi-Rad,*** « Therapeutic potential of α-and β-pinene: A miracle gift of nature », Biomolecules 9.11, (2019): 738.

[192] ***A. B. Cutillas, A. Carrasco, R. Martinez-Gutierrez, V. Tomas, J. Tudela,*** « Rosmarinus officinalis L. essential oils from Spain: Composition, antioxidant capacity, lipoxygenase and acetylcholinesterase inhibitory capacities, and antimicrobial activities », Plant Biosystems-An International Journal Dealing with All Aspects of Plant Biology 152.6, (2018): 1282-1292.

[193] ***C. Mishra, M. A. Khalid, D. Tripathi, A. A. Mahdi,*** « Comparative anti-diabetic study of three phytochemicals on high-fat diet and streptozotocin-induced diabetic dyslipidemic rats », International Journal of Biomedical and Advance Research 9.8, (2018): 8.

[194] ***G. I. K. Marei, M. A. Abdel Rasoul, S. A. M. Abdelgaleil***, « Comparative antifungal activities and biochemical effects of monoterpenes on plant pathogenic fungi », Pesticide biochemistry and physiology 103.1, (2012): 56-61.

[195] ***I. Ullah, A. L. Khan, L. Ali, A. R. Khan, M. Waqas, J. Hussain, … J. H. Shin,*** « Benzaldehyde as an insecticidal, antimicrobial, and antioxidant compound produced by Photorhabdus temperata M1021 », Journal of Microbiology 53.2, (2015): 127-133.

[196] ***J. O. Nogueira,*** « Ação antimicrobiana de diferentes terpenos e fenilpropanoides em Escherichia coli e Staphylococcus aureus », Master dissertation, Universidade Federal de Lavras, Lavras, Brazil, (2019).

[197] ***P. Anandakumar, S. Kamaraj, & M. K. Vanitha,*** « D-limonene: A multifunctional compound with potent therapeutic effects », Journal of Food Biochemistry 45.1, (2021): e13566.

[198] ***Z. M. Cai, J. Q. Peng, Y. Chen, L. Tao, Y. Y. Zhang, L. Y. Fu, … X. C. Shen, X.-C.*** « 1, 8-Cineole: a review of source, biological activities, and application », Journal of Asian natural products research 23.10, (2021): 938-954.

[199] ***S. Mokenapelli, M. Gutam, J. R. Yerrabelli, V. K. Irlapati, N. Gorityala, S. R. Sagurthi, & P. R. Chitneni***, « Design and Synthesis of Novel 2-Substituted-Benzyl-5-(2-Methylbenzofuran-3-yl)-2H-Tetrazoles: Anti-Proliferative Activity », Russian Journal of Bioorganic Chemistry 46.5, (2020): 845-855.

[200] ***J. Liu, Q. Zhang, R. Li, S. Wei, C. Huang, Y. Gao, & X. Pu,*** « The traditional uses, phytochemistry, pharmacology and toxicology of Cinnamomi ramulus: a review »,Journal of Pharmacy and Pharmacology, (2019): 319-342.

[201] ***A .S. Sokolova, O. I. Yarovaya, A. A. Shtro, M. S. Borisova, E. A. Morozova, T. G. Tolstikova, … N. F. Salakhutdinov,*** « Synthesis and biological activity of heterocyclic borneol derivatives », Chemistry of Heterocyclic Compounds 53.3, (2017): 371-377.

[202] ***C. Khaleel, N. Tabanca, & G. Buchbauer,*** « α-Terpineol, a natural monoterpene: A review of its biological properties », Open Chemistry, 16(1), (2018) :349–361.

[203] ***R. Ilijeva, & G. Buchbauer,*** « Biological Properties of Some Volatile Phenylpropanoids », Natural Product Communications, 11(10), (2016).

[204] ***H. Türkez, K. Çelik, & B. Toğar,*** « Effects of copaene, a tricyclic sesquiterpene, on human lymphocytes cells in vitro », Cytotechnology, 66(4), (2013) :597–603.

[205] ***G. K. Jayaprakasha, & L. J. M. Rao***, « Chemistry, Biogenesis, and Biological Activities ofCinnamomum zeylanicum », Critical Reviews in Food Science and Nutrition, 51(6), (2011) :547–562.

[206] ***G. H. Ma, K. X. Chen, L. Q. Zhang, & Y. M. Li***, « Advance in biological activities of natural guaiane-type sesquiterpenes », Medicinal Chemistry Research, (2019).

[207] ***B. Ivanescu, A. Miron, & A. Corciova***, « Sesquiterpene Lactones fromArtemisiaGenus: Biological Activities and Methods of Analysis » Journal of Analytical Methods in Chemistry, 2015, (2015) :1–21.

[208] ***K. C. Wang, J. S. Chang, L. C. Chiang, & C. C. Lin***, « 4-Methoxycinnamaldehyde inhibited human respiratory syncytial virus in a human larynx carcinoma cell line », Phytomedicine, 16(9), (2009) :882–886.

[209] ***M. C. Garcia-Parrilla,*** « Radical scavenging ability of polyphenolic compounds towards DPPH free radical », Talanta, (2007) :230– 235.

[210] ***S. Robet-Dernet,*** « antibiotique et antibiogramme » Ed . Vigot. Paris. P233. 1995.996.

[211] ***A. G. Ponce, R. Fritz, C. Del Valle, & S. I. Roura,*** « Antimicrobial activity of essential oils on the native microflora of organic Swiss chard », LWT-Food Science and Technology 36.7, (2003): 679-684.

[212] ***H. Zhu, M. Du, L. Fox, M. J. Zhu***, « Bactericidal effects of Cinnamon cassia oil against bovine mastitis bacterial pathogens », Food Control 66, (2016): 291–299.

[213] ***W. A. Rutala, D. J. Webe,*** « Healthcare Infection Control Practices Advisory Committee (HICPAC). Guideline for disinfection and sterilization in healthcare facilities », Center for disease control, (2008).

[214] ***A. Sabir, B. El Khalfi, F. Errachidi, I. Chemsi, A. Serrano, & A. Soukri,*** « Evaluation of the potential of some essential oils in biological control against phytopathogenic agent Pseudomonas syringae pv. tomato DC3000 responsible for the tomatoes speck », Journal of Plant Pathology & Microbiology, 08(09), (2017).

[215] ***X. Li, X. Wu and L. Huang,*** « Correlation between antioxidant activities and phenolic contents of radix Angelicae sinensis (Danggui) », Molecules 14.12, (2009): 5349-5361

[216] ***M. Ogata, M. Hoshi, S. Urano, et T. Endo,*** « Activité antioxydantede Eugénol et composés monomères et dimères apparentés », Chimique et bulletin pharmaceutique, 48 (10), (2000): 1467-1469.

[217] ***Hydrogen Bond Elsevier Publications;*** https://www.sciencedirect.com/topic/ biochemistry-genetics-and-molecular-biology/hydrogen-bond, (2018).

[218] ***A. B. Medagama,*** « The glycaemic outcomes of Cinnamon, a review of the experimental evidence and clinical trials » Nutrition journal 14.1, (2015): 1-12.

[219] ***N. J. Hayward, G. J. McDougall, S. Farag, J. W. Allwood, C. Austin, F. Campbell, … V. Ranawana,*** « Cinnamon Shows Antidiabetic Properties that Are Species-Specific: Effects on Enzyme Activity Inhibition and Starch Digestion », Plant Foods for Human Nutrition, 74(4), (2019) :544–552.

[220] ***M. Bhutkar, S. Bhise,*** « In vitro assay of alpha amylase inhibitory activity of some indigenous plants », International journal of chemical sciences, 10(1), (2012) : 457-462.

[221] ***G. Klebe,*** « Recent developments in structure-based drug design », Journal of molecular medicine, 78 (5), (2000) :269–281.

[222] ***S. P. Leelananda, and S. Lindert***, « Computational methods in drug discovery », Beilstein journal of organic chemistry 12, (2016), 2694–2718.

[223] ***American Diabetes Association,*** « Economic costs of diabetes in US in 2002 », Diabetes care, 26 (3), (2003) :917–932.

[224] ***M. Friedman, P. R. Henika, C. E. Levin, and R. E. Mandrell R. E,*** « Antibacterial activities of plant essential oils and their components against Escherichia coli O157: H7 and Salmonella enterica in apple juice », Journal of agricultural and food chemistry 52.19, (2004): 6042-6048 .

[225] ***J. Hossen, T. K. Pal,*** « DFT Study of Efinaconazole, an Antifungal Drug and Its Molecular Docking against a Holoenzyme (pdb ID : 3IDB) », J. Sci. Res. 13 (2), (2021) :679-694.

[226] ***H. Striegl, Y. Roske, D. Ku¨mmel, U. Heinemann,*** « Unusual Armadillo Fold in the Human General Vesicular Transport Factor p115 », PLoS ONE 4(2), (2009) :e4656.

References of practical part

« Results and discussion »

C

Chouitah, 2012 : ***O. Chouitah,*** « Composition chimique et activité antibactérienne des huiles essentielles des feuilles de Glycyrrhiza glabra », Diss. Université d'Oran1-Ahmed Ben Bella, (2012).

F

Friedman et al. (2004) : ***M. Friedman, P. R. Henika, C. E. Levin, and R. E. Mandrell R. E,*** « Antibacterial activities of plant essential oils and their components against Escherichia coli O157: H7 and Salmonella enterica in apple juice », Journal of agricultural and food chemistry 52.19, (2004): 6042-6048 .

H

Hellal, 2011 : ***Z. Hellal,*** « Contribution à l'étude des propriétés antibactériennes et antioxydantes de certaines huiles essentielles extraites des Citrus. Application sur la sardine (Sardina pilchardus) », Diss. Université Mouloud Mammeri, (2011).

P

Poaty et al., 2015 : ***B. Poaty, J. ahlah, F. Porqueres, & H. ouafif,*** « Composition, antimicrobial and antioxidant activities of seven essential oils from the North American boreal forest », World Journal of Microbiology and Biotechnology 31.6, (2015): 907-919.

S

Still et al., 1978 : ***W. C. Still, M. Kahn, & A. Mitra,*** « Rapid chromatographic technique for preparative separations with moderate resolution." The Journal of Organic Chemistry 43.14 (1978): 2923-2925.

Seu-Saberno et Blakeway, (1984) *: K. P. Svoboda, & J. B. Hampson,* « Bioactivity of essential oils of selected temperate aromatic plants: antibacterial, antioxidant, antiinflammatory and other related pharmacological activities », Plant Biology Department, SAC Auchincruive, Ayr, Scotland, UK., KA6 5HW 16 , (1999): 1-7

Seu-Saberno and Blakeway, (1984) ***:M. Seu-Saberno and J. Blakeway,*** « La mouse de chêne, une base de la parfumerie », Pour la science, Edition Française de Scientific American, (1984).

- **ADME/T :** Absorption, distribution, metabolism, excretion, and toxicity
- **AV :** Acid value
- **ADT :** AutoDockTools
- **CVD :** Cardiovascular disease
- **CE :** Cinnamon extract
- **CEO :** Cinnamon essential oil
- **CVCs :** Cinnamon volatile compounds
- **CMD :** Command Prompt
- **ºC :** Degree centigradeCHD coronary heart disease
- **DM :** Diabetes mellitus
- **DR :** Diabetic retinopathy
- **DF :** Diabetic foot
- **DPN :** diabetic peripheral neuropathy
- **DPPH :** 2,2-diphenyl-1-picrylhydrazyl
- **DMSO :** Dimethyl sulfoxide
- **2D :** two-dimensional
- **3D :** three-dimensional
- **DS :** Discovery Studio
- **E .coli :** Escherichia coli.
- **EIMS :** Electron Ionization Mass Spectroscopy
- **EV :** Ester value
- **EO :** Essential oil
- FFA : Free faty acide
- **HDL :** High-density lipoprotein
- **HbA1c :** Glycated haemoglobin
- **HTS :** High-throughput screening
- **IDDM :** Insulin-dependent Diabetes Mellitus
- **IC50 :** 50% of inhibitory concentration
- **IR :** Infrared radiation
- **ID :** Identity document

- **GC-MS :** Gas Chromatography Mass Spectrometer
- **KOH :** Potassium hydroxide
- **LDL :** Low-density lipoprotein
- **MEP :** Molecular electrostatic potential
- **Max :** Maximum
- **M :** Molarity
- **NIDDM :** Non Insulin-dependent Diabetes Mellitus
- **N :** Normality
- **PPARα/γ :** Peroxisome proliferator-activated receptors α/γ
- **PD :** pharmacodynamic
- **PK :** Pharmacokenitics
- **pH :** Potential of hydrogen
- **POV :** Peroxide Value
- **PPARα/γ :** Peroxisome proliferators activated receptor gamma
- **PDB :** Protein Data Bank
- **QSARs :** Quantitative structure–activity relationships
- **R_f :** Retention factor
- **RH :** Relative humidity
- **RD :** Relative density
- **SD :** Steam distillation
- **SDF :** Spatial Data File
- **SV :** Saponification value
- **Si :** Silicon
- **T1DM :** Type 1 diabetes Mellitus
- **T2DM :** Type 2 diabetes Mellitus
- **TZD :** Thiazolidinedione
- **t :** Temperature
- **TLC :** Thin-layer chromatography
- **UV :** Ultraviolet
- **USFDA :** United States Food and Drug Administration
- **v/v :** Volume of solute / volume of solution
- **WHO :** World Health Organization

- **µl :** Microliter
- **λ :** Wavelength
- **% :** Percent sign

اللهم ارزقني التوفيق في هذا العمل، وزدني علمًا وعملًا صالحًا، واجعلني من المتقين المخلصين، اللهم إني توكلت عليك وسلمت أمري إليك، لا ملجأ ولا منجى منك إلا إليك. اللهم ارحم أمي واجعل هذا العمل وهذا التعب كله صدقة لها تضاف إلى حسناتها يا رب.