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Subject

In silico assessment of insecticidal actvity of essential oil compounds of two north african endemic species *thymus algeriensis* and *thymus numidicus*

Presented by:

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DEDICATION

this humble work would never have been possible without the support and love of the wonderful people I have the honor of calling my family, they are my core and my everything. First and foremost, I am deeply beholden to my parents who supported me in every possible aspect, have never once doubted me, and gave me the strength to continuously push myself further in my professional and personal development.

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ABSTRACT

Bioinformatics, or in silico biology, is a rapidly growing field that encompasses the theory and application of computational approaches to model, predict, and explain biological function at the molecular level. This information rich field requires new skills and new understanding of genome-scale studies in order to take advantage of the rapidly increasing amount of sequence, expression, and structure information in public and private databases.

Research in biology can not currently do without computer tools to process the data produced and optimize its advances. One of these tools is molecular modeling and more specifically molecular stowage (more commonly known as "docking"). The initial use of molecular "docking" was to predict and reproduce protein-ligand complexes. Docking is the basis for molecular recognition and the type of interaction.

Our work consists in studying the insecticidal activity of major compounds of the essential oil of *thymus algeriensis* and *thymus numidicus* ,by the bioinformatic approach for the study of protein-ligand interaction in silico of four proteins (Drosophila melanogaster acetylcholinesterase, Bombyx mori sigma-class glutathione transferase, Ostrinia furnacalis group I chitinase catalytic domain and Drosophila melanogastercentral nervous system glutamate receptor). The in silico approach and the in vitro studies are complementary, so major compounds of the essential oils of *thymus algeriensis* and *thymus numidicus* have a weak insecticidal activity.

Résumé

La bioinformatique, ou biologie in silico, est un domaine en pleine expansion qui englobe la théorie et l'application d'approches informatiques pour modéliser, prédire et expliquer la fonction biologique au niveau moléculaire. Ce domaine riche en informations nécessite de nouvelles compétences et une nouvelle compréhension des études à l'échelle du génome afin de tirer parti de la quantité rapidement croissante d'informations sur les séquences, l'expression et la structure dans les bases de données publiques et privées.

La recherche en biologie ne peut actuellement se passer d'outils informatiques pour traiter les données produites et optimiser ses avancées. L'un de ces outils est la modélisation moléculaire et plus précisément le stockage moléculaire (plus communément appelé "docking"). L'utilisation initiale du "docking" moléculaire était de prédire et de reproduire des complexes protéine-ligand. L'arrimage est à la base de la reconnaissance moléculaire et du type d'interaction.

Notre travail consiste à étudier l'activité insecticide des principaux composés de l'huile essentielle de *thymus algeriensis* et de *thymus numidicus*, par l'approche bioinformatique pour l'étude de l'interaction protéine-ligand in silico de quatre protéines (Drosophila melanogaster acétylcholinestérase, Bombyx mori sigma-classe glutathione transférase, Ostrinia furnacalis groupe I domaine catalytique de la chitinase et Drosophila melanogasterrécepteur du glutamate du système nerveux central). L'approche in silico et les études in vitro sont complémentaires, de sorte que les principaux composés des huiles essentielles de *thymus algeriensis* et de *thymus numidicus* ont une faible activité insecticide.

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ملخص

تعد المعلوماتية الحيوية ، أو في بيولوجيا السيليكو ، مجالًا متناميًا يشمل نظرية وتطبيق الأساليب الحسابية لنمذجة الوظيفة البيولوجية والتنبؤ بها وشرحها على المستوى الجزيئي. يتطلب هذا المجال الغني بالمعلومات مهارات جديدة وفهمًا جديدًا للدراسات على مستوى الجينوم من أجل الاستفادة من الكمية المتزايدة بسرعة من معلومات التسلسل والتعبير والهيكل في قواعد البيانات العامة و نشر.

لا يمكن لبحوث الأحياء حاليًا الاستغناء عن أدوات الكمبيوتر لمعالجة البيانات المنتجة وتحسين تقدمها. إحدى هذه الأدوات هي النمذجة الجزيئية والتخزين الجزيئي بشكل أكثر دقة (يطلق عليه أكثر شيوعًا "الالتحام"). كان الاستخدام الأولي للالتحام الجزيئي هو التنبؤ بمجمعات البروتين - الترابط وإعادة إنتاجها. الإرساء هو أساس التعرف الجزيئي ونوع التفاعل.

يتكون عملنا من دراسة نشاط المبيدات الحشرية للمركبات الرئيسية للزيت العطري من الغدة الصعترية *thymus algeriensis* و *thymus numidicus* ، من خلال نهج المعلومات الحيوية لدراسة تفاعل البروتين - الترابط في السيليكو لأربعة بروتينات (Drosophila melanogaster acetylcholinesterase, Bombyx mori sigma-class glutathione transferase, Ostrinia furnacalis group I chitinase catalytic domain and Drosophila melanogastercentral nervous system glutamate receptor) إن النهج في السيليكو والدراسات المختبرية مكملان لبعضهما البعض ، لذا فإن المركبات الرئيسية للزيوت الأساسية من *thymus algeriensis* و *thymus numidicus* لها نشاط مبيد حشري منخفض

Introduction

For thousands of years, humans have been using various crop protection products to control insects, diseases, and weeds that harm or destroy food crops (Dent 2000). Empirical at the beginning, the crop protection knew enormous progress with the discovery of the pesticides of synthesis. However, their massive and irrational use in the fight against the devastating insects of the cultures generated perverse effects such as the development of phenomena of resistance at the treated devastating insects (Lee 2002), undesirable effects on non-target organisms, such as important insect predators (Papachristos et Milonas 2008) and parasitoids (Borgemeister et al. 1993), even other beneficial organisms such as earthworms (Reddy et Rao 2008) and pollinators have also been affected by application of synthetic pesticides to the detriment of crop species (Nderitu et al. 2007). Besides, there is also the pollution of the biosphere, the presence of residues of pesticides in the treated agricultural produce reaching inadmissible contents. In front of the extent of the problem, the methods of fights must be reconsidered (Bughio et Wilkins 2004).

These last years, work was oriented towards the search for biopesticides biodegradable to replace the conventional pesticides, the use of the plants like source of pesticides was the several work object (Regnault-Roger et al. 1993, Owusu 2000). Among all natural products, essential oils showed pharmacological activities. They have been recognized for their antibacterial, antifungal, antioxidant and insecticidal properties (Ayvaz et al. 2010). For instance, newly emerging pathogens (Schafer et Wink 2009) and decreased efficacy and resistance of pathogens to antibiotics has necessitated development of new alternatives, and consequently the interest in the use of medicinal plants, as well as their essential oils, has grown in leaps and bounds (Bakkalia et al. 2008). The combination of essential oil and antibiotics showed substantial antimicrobial effects (Janssen et al. 1987). In foods systems, higher concentrations of essential oils are needed to have similar antimicrobial effects as those obtained in vitro. The use of essential oils and their isolated components are new approaches to increase their efficacy, taking advantage of their synergistic and additive effects (Bassolé et Rodolfo-Juliani 2012).

The genus *Thymus* is one of the largest and economically most important genera within the Lamiaceae (= Labiatae) family. *Thymus* species are distributed throughout the arid,

temperate and cold regions of the Old World north of the equator, and on the coasts of Greenland (Morales 1989). The number of species within this genus is assumed to be approximately 350 species (Mabberley 1997).

Many *Thymus* species are extensively used, dry or fresh, as culinary herbs. Also, essential oils obtained from these species were utilized as flavor ingredients in a wide variety of foods, beverages and confectionery products, as well as in perfumery for the scenting of soaps and lotions (Giweli et al. 2013). Throughout the history, the aerial parts and the volatile constituents of *Thymus* species have been highly recommended; they were commonly used as herbal teas, condiments and spices, so as for various medicinal purposes (Stahl-Biskup et Saez 2002). Furthermore, this plant is also widely used in folk medicine against illnesses of the digestive tube and antiabortion (Le Floc'h, 1983). Also, they are employed in popular medicinal for its expectorant, antitussive, analgesic, antibroncholitic, antispasmodic, carminative and diuretic effects (Dob et al., 2006). In Algeria, they have been used as astringent, expectorant and cicatrising agents (Baba Aïssa, 1991).

The species of this genus are rich in essential oils and were characterized by a great variability of both morphology and chemotypes (Stahl-Biskup 1991). Thyme oil is among the world's top ten essential oils regarding its use as a food preservative (Stahl-Biskup et Saez 2002). So in recent years, several reports have been published concerning the composition and the biological properties of the essential oils and extracts of this genus (Hazzit et al. 2009). Recent researches was shown that many essential oils from different *Thymus* plants exhibited promising biological properties like antioxidant (Hazzit et al. 2006), antimicrobial (Karman et al. 2001) and anti-inflammatory activities (Isamili et al. 2002).

For the Algerian area, Quezel and Santa (1963) describe 12 *Thymus* species of which eight are endemic in Algeria or in North Africa. Between this eight endemic species we find *Thymus algeriensis* Boiss and *T. numidicus* (Poiret).

T. algeriensis is the most widespread North African species, endemic to Algeria, Libya, Tunisia and Marocco. Fresh or dried, it is largely used only as a culinary herb (Chaieb et Boukhriss 1998). Its chemical composition has been already studied (Giordiani et al. 2008, Giweli et al. 2013), although results of its biological activity are still scarce. *T. algeriensis* is also used in traditional medicine, as a fresh or dry seasoning, in respiratory and digestive tube disorders and against abortion (Giweli et al. 2013). Moreover, *T. algeriensis* is a short lived, diploid (2n=2x = 30) and gynodioecious shrub (Morales 1986). It reproduces by seeds and can reach 20–50 cm in height. The leaves are opposite and linear/ lanceolate (6–12 mm). The

flowers, with ovate bracts and pink purplish or whitish purple corolla, are small (5–7 mm). Flowering takes place between April and June (Ben El Hadj et al. 2010).

On the other hand, *T. numidicus* (Arabic 'Zaatar') is an endemic species to northern Algeria and Tunisia (Quezel et Santa 1962), where it is present in two varieties differentiated by the color of the flowers, either purplish-white or violet. Three studies on the composition of its essential oil have been previously reported in the literature (Hadef 2004, Hazzit et al. 2006). *T. numidicus* is a short lived and outcrossing shrub predominantly bee-pollinated. It is a hermaphrodite species and possesses a capacity for asexual reproduction by either vegetative propagation. It can reach 10–15 cm in height. Leaves are opposite and linear/lanceolate (4–15 mm) (Maire et Quézel 1987). Flowers are hermaphrodite, large (15 mm) and grouped in dense terminal heads with an uneven calyx (3 mm) and a pink corolla (6 mm). Flowering takes place between April and June (Pottier-Alapetite, 1981).

Several works have been reported in the field of the insecticides where the use of computational approaches has served as strong support for the better understanding of physicochemical properties (Aschi et al. 2007), insecticidal activity (Sparks et al. 2008) and toxicological profiles (Eldred et al. 1999). These 'virtual' experiments are usually used to complement traditional 'wet' procedures and are particularly useful when the latter have an invasive nature that may alter the properties of the specimens being investigated. The key element in this context is predictability, that is, a computer model can be used to accelerate the hypothesis generation and validation cycles of research as long as it can provide predictable outputs (Ventura et al. 2006).

Emerging challenges of managing and interpreting large amounts of complex biological data have given rise to a quickly growing field of computational biology, or bioinformatics. While this field is not new, it is rapidly becoming indispensable to life scientists as biology evolves toward high throughput approaches to whole genome molecular analyses. Bioinformatics can be defined as 'a scientific discipline that encompasses all aspects of biological information acquisition, processing, storage, distribution, analysis and interpretation' (Benton 1996). This interdisciplinary field attempts to link the theory and application of mathematics, biology and computer science in order to understand the significance and relationships between quantitative and qualitative biological data at the cellular, biochemical, and molecular level. To this end, numerous public, commercial, and proprietary software applications and databases have been developed to allow researchers to analyze physical and

genetic data to infer functionality in a variety of model organisms, as well as humans (Blake 2000).

Computational, computer simulations or *in silico* analyses lend substantial predictive power in addition to traditional methods of experimental biology. *In silico* analyses can provide alternative and efficient means to generate new hypotheses, aid in designing appropriate experiments, and in interpreting large amounts of information from genome-scale studies. As a result, computational biology has the potential to influence all levels of biologically based research and increase the scope and efficiency of both basic and applied science (Norris et al. 2000). Currently, *in silico* experiments are being increasingly employed to predict the behaviour of plants under different environmental conditions (Hammer et al. 2004). The rationale behind *in silico* approaches are the relatively lower cost and the time factor involved, when compared to standard experimental approaches (Darvas et al. 2002). As an example, it takes a minute in an *in silico* model to screen 20,000 molecules, but takes 20 weeks in the "wet" laboratory to do the same exercise (Hodgson 2001).

Thus, this work aims to assess the effectiveness of the *Thymus algeriensis* and *T. numidicus*'s chemical composition on *in silico* system.

Materials and Methods

This work aims to study the interactions between two molecules: a receptor and a ligand by molecular docking in order to elucidate that they are the most active molecules (essential oils component)?

To do this, we used the following material:

I. Materials

I.1. microcomputer:

We used a microcomputer with 2 GB of RAM, Intel(R) Core I3 CPU M370 @ 2.40 GHz and Intel HD Graphics card. All the programs used are installed under the Windows XP operating system.

I.2 Programs:

In this study, several programs were used to carry out the practical part:

- The MOE (Molecular Operating Environment) package, version 2014.0901 was used in our study. MOE is a set of several applications (suite package) intended for the discovery and computer-aided design of biologically active molecules. It allows several tasks to be carried out in a very short time.

It allows to design the molecules, to minimize them in order to have the best conformations and positions of these molecules. It also makes it possible to anchor several ligands gathered in a database in the active site of a protein in a successive manner.

Figure 1: The MOE suite under Windows.

I.3 Data Banks:

I.3.1 "PDB" (Protein Data Bank):

The Protein Database (PDB) was created as the first open access digital data resource in the field of biology and medicine. Today it is a leading global resource for experimental data essential for scientific discovery with 167780 Biological Macromolecular Structures.

Through a web-based information portal and downloadable data archives, the PDB provides access to 3D structural data for large biological molecules (proteins, DNA and RNA). These are the molecules of life, which are found in all organisms on the planet.

Knowledge of the 3D structure of a biological macromolecule is essential for understanding its role in human and animal health and disease, its function in plants and food and energy production, and its importance for other topics related to global prosperity and sustainability.

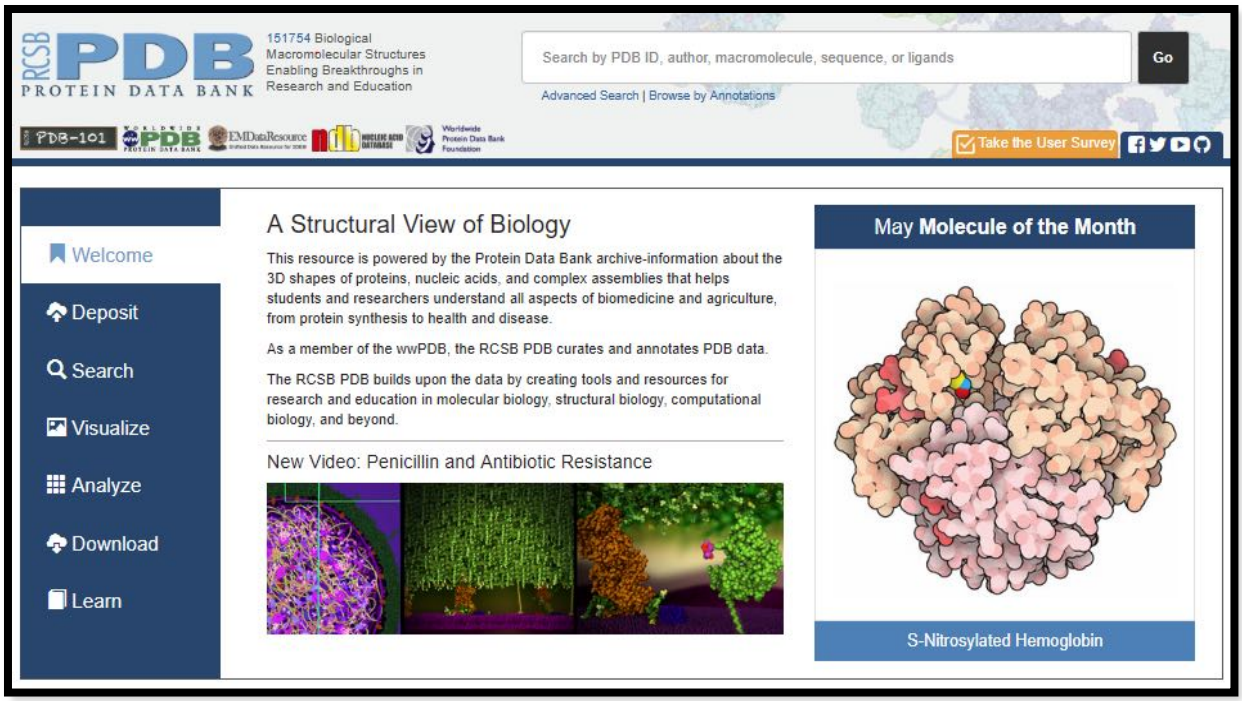

Figure 2: PDB (Protein Data Bank) homepage.

I.3.2 "Pubchem":

Pubchem is an American database of chemical molecules managed by the National Center for Biotechnology Information (NCBI), it contains small molecules but also large molecules such as nucleotides, carbohydrates, lipid peptides and chemically modified macromolecules.

Pubchem lists several million compounds by putting online, for each substance, a large amount of data of various types: chemical, biochemical production, toxicology, environmental...

Their consultation is free and can be done directly from the website "https://pubchem.ncbi.nlm.nih.gov/".

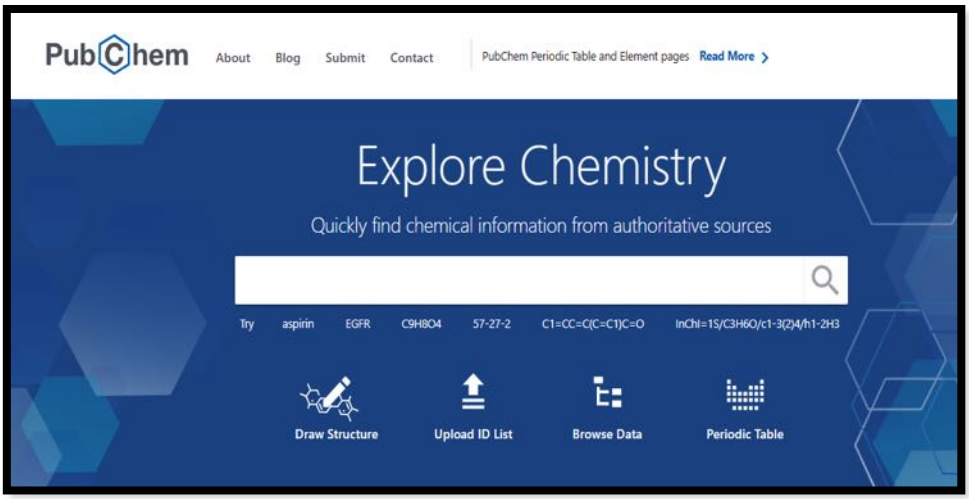

Figure 3: PubChem's homepage.

II. Methods

II.1 Choice of proteins:

One of the major limiting factors of docking techniques is the flexibility of the target protein, in particular the conformational rearrangements at the active site induced by ligand binding (induced fit), which often leads, during docking, to the generation of false negatives (biologically active molecules wrongly predicted with low or no in silico activity). The generation of false negatives corresponds to a loss of relevant information during screening. The generation of false positives, which is inevitable during screening, is less disabling, as it only corresponds to a loss of time in the framework of a multi-step protocol.

For this reason, we have chosen a target that does not present any rearrangement of the so-called active (rigid structure). The choice of the code of the structure used has the following characters:

- A good resolution (generally lower than 2Å).

-Presence of a co-crystallized ligand.

II.2 Protein preparation

A typical PDB structure file is not suitable for immediate use in molecular modeling. A typical PDB file contains only heavy atoms, and may include a co-crystallized ligand, water molecules, metal ions, and co-factors. Some structures are multimeric, which may require their reduction to a single unit, and may possibly lack information on connectivity, which must be assigned, as well as bond order and formal charges.

Table 1 : The crystallographic data for the four proteins.

Code PDB	Classification	Resolution	Chaine	Ligand
1QON	HYDROLASE	2.72 Å	A	I40 : **9-(3-IODOBENZYLAMINO)-1,2,3,4-TETRAHYDROACRIDINE** C_{20} H_{19} I N_2 ZUCWQTWGZGIYPV-UHFFFAOYSA-N
3VPQ	TRANSFERASE	1.70 Å	A	GSH : **GLUTATHIONE** C_{10} H_{17} N_3 O_6 S RWSXRVCMGQZWBV-WDSKDSINSA-N
3WQW	HYDROLASE	2.00 Å	A	NAG : **2-acetamido-2-deoxy-beta-D-glucopyranose** C_8 H_{15} N O_6 OVRNDRQMDRJTHS-FMDGEEDCSA-N
5DT6	MEMBRANE PROTEIN	1.60 Å	A	GLU : **GLUTAMIC ACID** C_5 H_9 N O_4 WHUUTDBJXJRKMK-VKHMYHEASA-N

III. The process to be followed:

III. 1 Preparation of the protein:

The proteins Drosophila melanogaster acetylcholinesterase, Bombyx mori sigma-class glutathione transferase, Ostrinia furnacalis group I chitinase catalytic domain and Drosophila melanogastercentral nervous system glutamate receptor have been downloaded from the PDB database "Protein Data Bank" (https://www.rcsb.org/).

Simplification of proteins by removing water molecules, co-crystallized ligands, duplicate chains, metals and co-factors that are not involved in the active site of the protein, allows us to accelerate and obtain a simplified model of the enzyme.

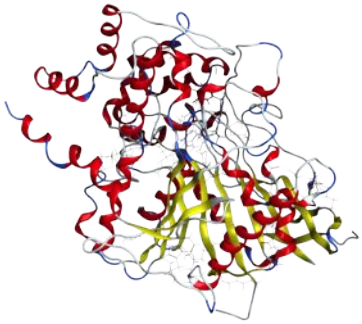

Figure 4-: protein 1 access code 1QON.

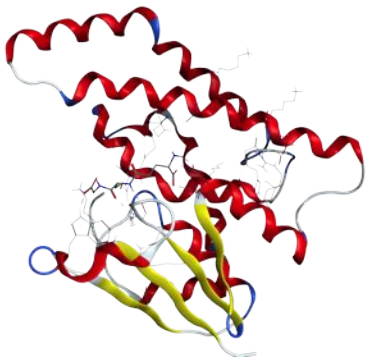

Figure 5: protein 2 access code 3VPQ.

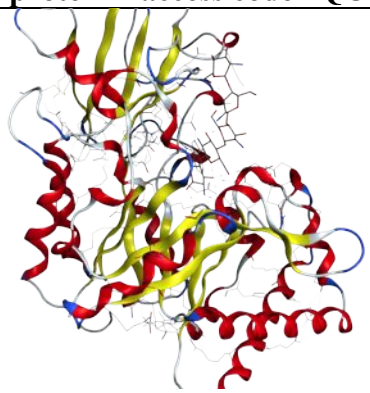

Figure 6: protein 3 access code 3WQW.

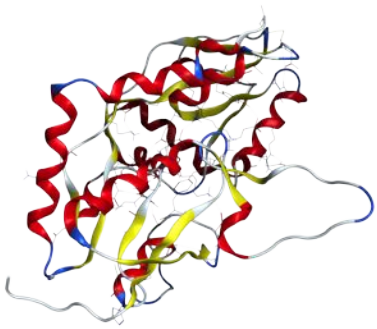

Figure 7: protein 4 access code 5DT6.

III. 2. Preparation of the database:

Compounds are obtained from several articles which we have listed in the tables in the Annexe. We chosed the major 7 compounds, and downloaded them from the PubChem website (https://pubchem.ncbi.nlm.nih.gov/).

Table 2 : Compound structures and their codes downloaded from PubChem.

Code PubChem	Composés
CID 17868	α-Thujene
CID 6654	α-Pinene
CID 6616	Camphene
CID 79035	Tricyclene
CID 6427476	Verbenene
CID 18818	Sabinene
CID 14896	β-Pinene

III. 3 Molecular docking :

The study of the interaction between the active site of the protein and the substrate to form a stable complex is carried out using MOE software. The steps are summarized in figure -8-.

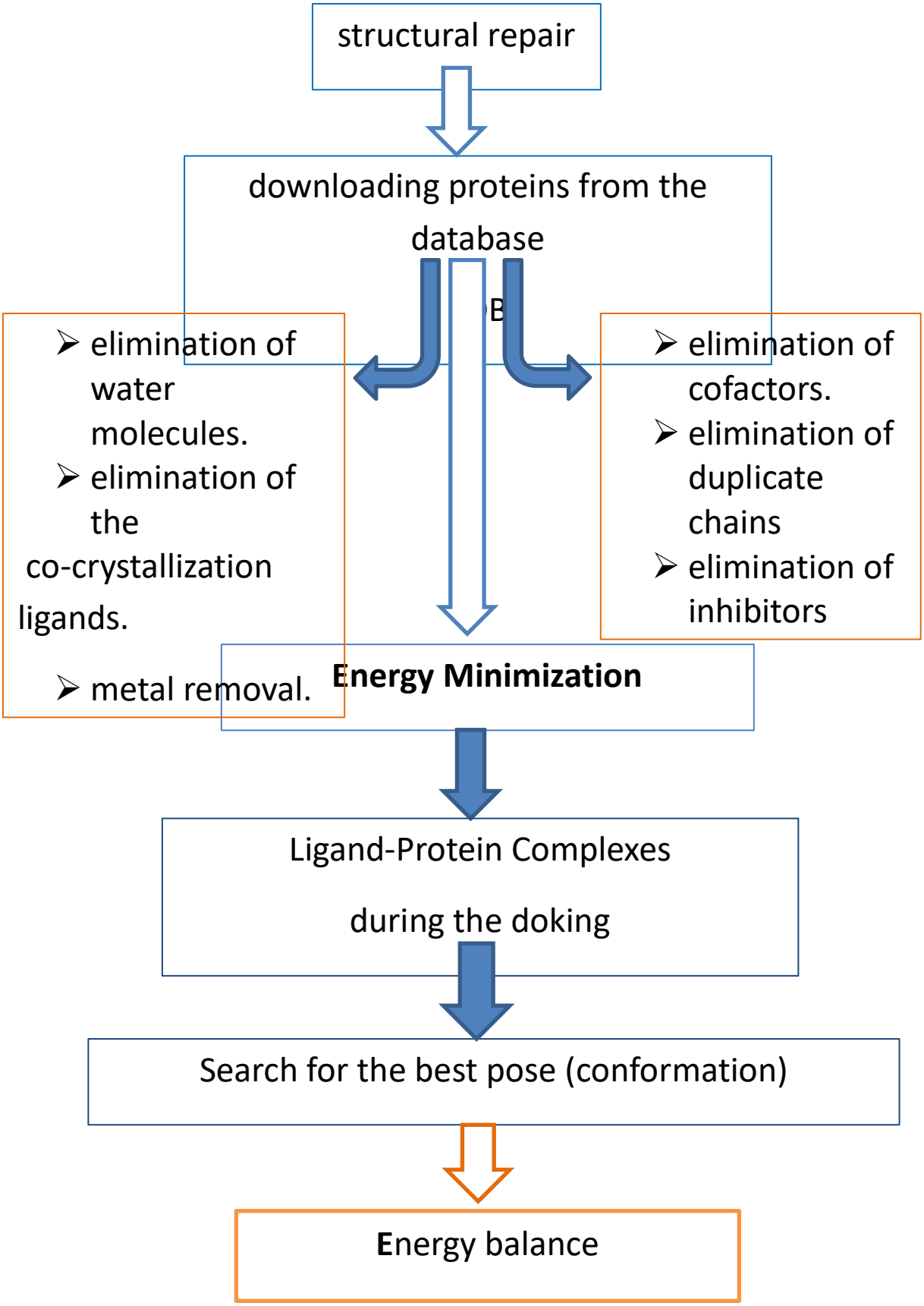

Figure 8: Diagram showing the Molecular Operating Environment (MOE) protocol.

Results and discussion

I. The reliability of the MOE Suite program:

Before approaching the study of the interaction between protein and ligand by various compounds (database of essential oils of Thymus algeriensis and Thymus numidicus.), we tried to evaluate the reliability of the MOE Suite program used. To do this, we proceeded to docking the co-crystallized ligand and calculated the pose deviation generated after docking and placement of the co-crystallized ligand (RMSD: root mean square deviation).

I.1 Calculation of the RMSD:

The prediction of the interaction mode consists in determining the correct positioning of the ligand in relation to its receptor. The ability of a program to do this is usually judged by the root mean square deviation or RMSD (root-mean-square deviation) of the ligand position calculated by the software relative to the reference ligand existing at the PDB. The prediction is acceptable if its value does not exceed 2 angstroms.

In our case, the RMSD values between co-crystallized ligand and stowed ligand were calculated by the Super-imposition application implemented in Schrödinger Suite. The results of the RMSD calculation obtained in our case are presented in Table 05.

Table 3 : GMDR values and their scores.

Protein	Code PDB	Ligand	Score	RMSD (Å)	RMSD max (Å)
Drosophila melanogaster acetylcholinesterase	1QON	**I40**	**-8.6348**	**0.1575**	**6.5115**
Bombyx mori sigma-class glutathione transferase	3VPQ	**GSH**	**-6.4600**	**0.5880**	**8.5388**
Ostrinia furnacalis group I chitinase catalytic domain	3WQW	**NAG**	**-9.7301**	**3.7501**	**16.8119**
Drosophila melanogastercentral nervous system glutamate receptor	5DT6	**GLU**	**-8.6883**	**0.6454**	**4.3356**

The table shows that the different RMSD values are consistent with the results of Chikhi and Bensegueni (2008), and Gabb et al (1997) who showed that any docking program only performs well when the RMSD is less than or equal to 2 Å.

I.2 Visual analysis:

Visual analysis is an essential step in confirming the results of the RMSD calculation. It is used to determine if a simulated ligand overlaps with the co-crystallized ligand.

Visualization of the molecular docking results performed with the five proteins in Table 3 shows that the EOM simulated ligand models are correctly placed in the active site of each protein. They present spatial conformations perfectly superimposable to those determined experimentally by crystallography found in the PDB.

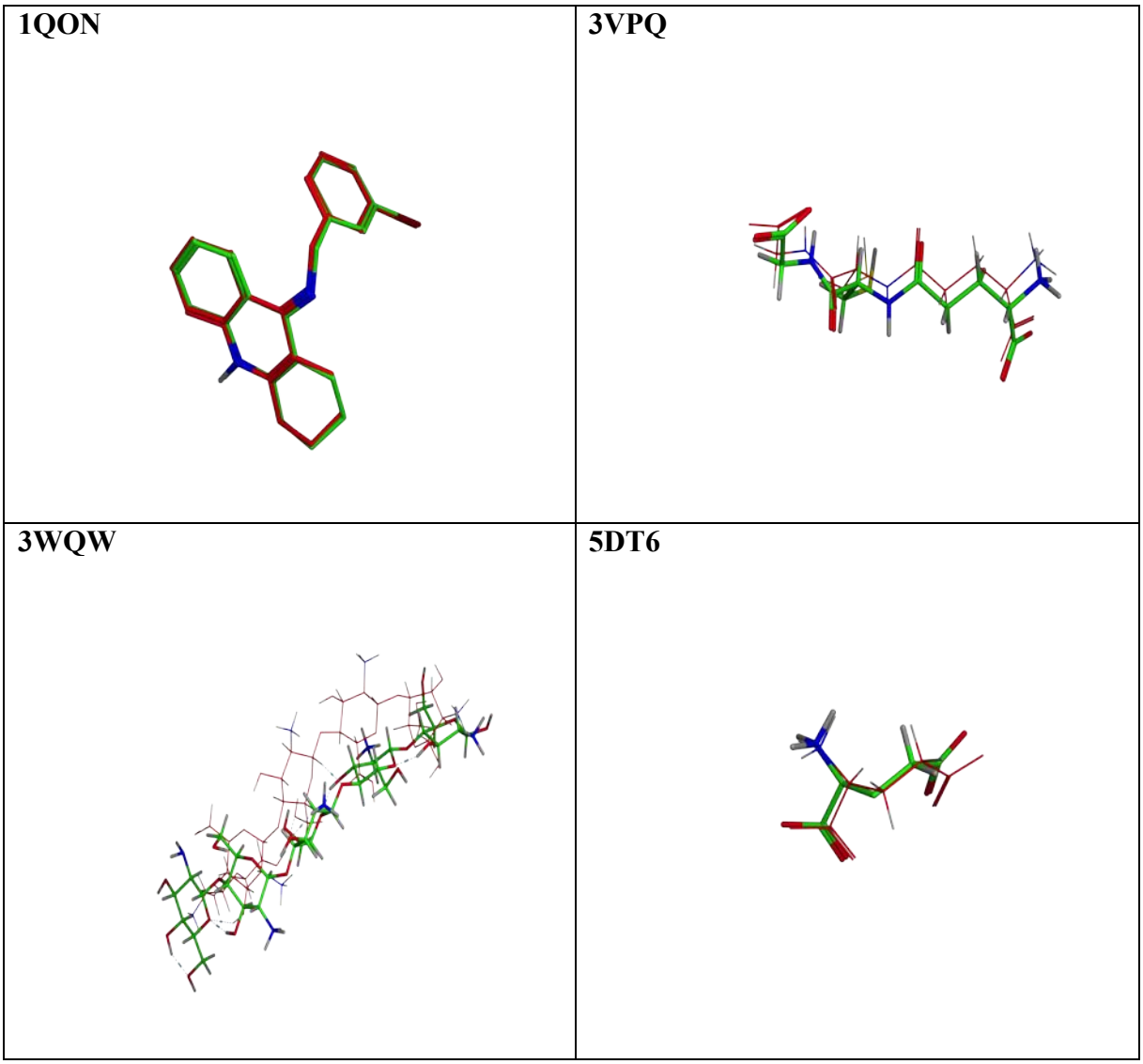

Figure 9-: Superposition of the geometry of the ligands obtained by crystallography (coloured green) and that calculated by molecular docking with MOE (coloured red).

I.3 Conclusion:

In the light of the results obtained with the RMSD calculation and the visual analysis, we can conclude that the MOE program is highly efficient and can be used without too much risk of errors, to generate complexes by in silico simulation.

II. Molecular docking results of essential oil compounds of *Thymus algeriensis* and *Thymus numidicus* with respect to the chosen targets:

II.1 Study of the interactions "Protein - Ligand co-crystallized" by MOE Continued

Molecular docking allows the ligands to be positioned within the active site of the receptor of the different enzymes.

The ligand-receptor complex formed, will adopt the most stable conformation, generating an

lowest energy level. The search for the most favourable conformation between the ligand (flexible) and the receptor (rigid). For each ligand, a number of conformations called poses.

Our approach consists first of all in studying the interaction between proteins - ligands. Using the MOE Suite programme, we have identified the best score, i.e. the one with the lowest energy corresponds to the best interactions between the ligand and the active site of the enzyme. The results are grouped in table N° 4.

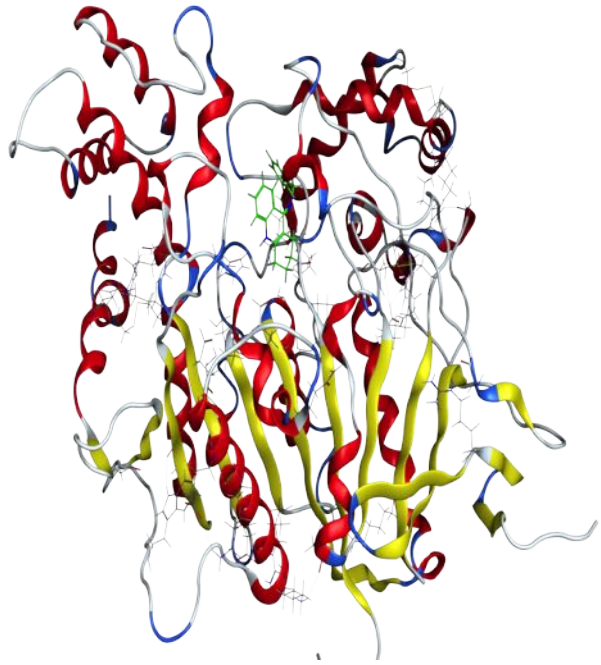

Figure a: Complexe 1(Drosophila melanogaster acetylcholinesterase + **I40**)

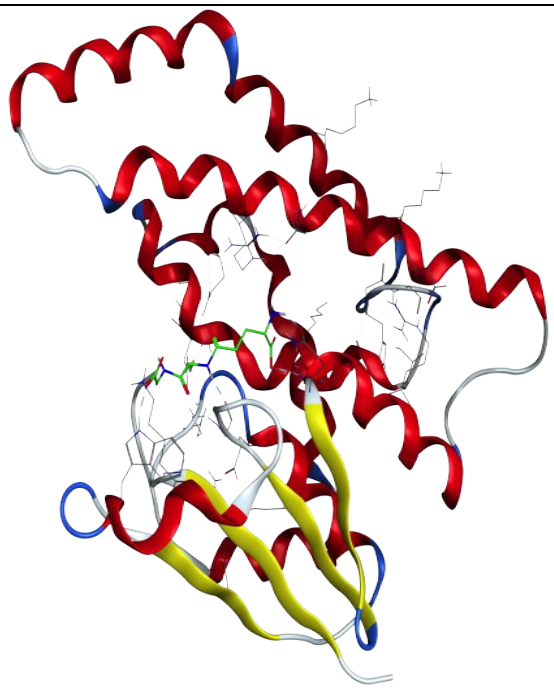

Figure b: Complexe 2(Bombyx mori sigma-class glutathione transferase + **GSH**)

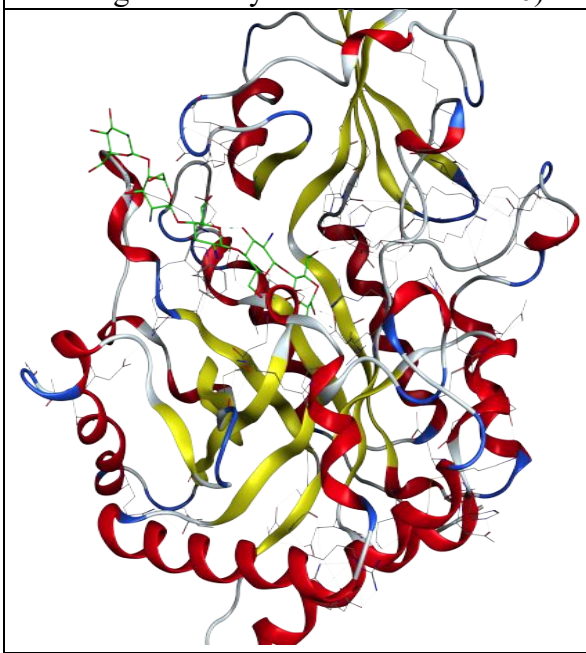

Figure c: Ostrinia furnacalis group I chitinase catalytic domain + **NAG**)

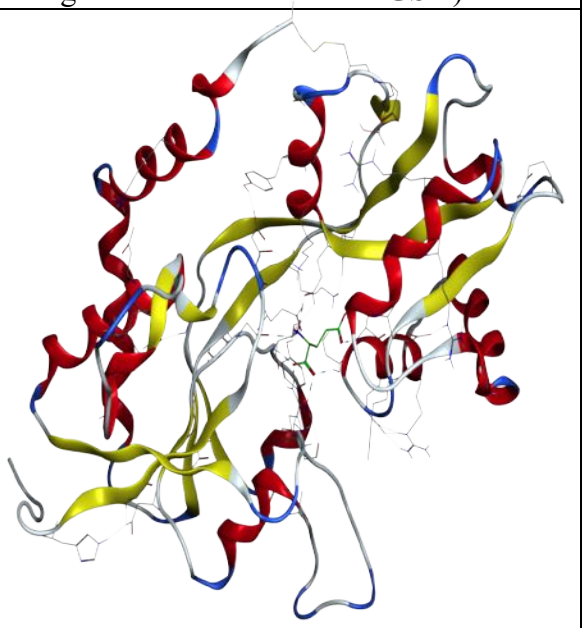

Figure d: Complexe 4(Drosophila melanogastercentral nervous system glutamate receptor + **GLU)**

Figure 10-: The position of the co-crystallized ligands in the 4 complexes.

Table 4 : The energy balance of the 4 complexes.

Molecules	**S**
Complexe 1	-8.6348
Complexe 2	-6.4600
Complexe 3	-9.7301
Complexe 4	-8.6883

Table 5 : The results of the different compounds with the different targets.

	CID	1QON	3VPQ	3WQW	5DT6
alpha-Thujene	17868	-4.7791	-3.6675	-4.2438	0.3160
alpha-Pinene	6654	-4.4318	-3.6674	-4.3363	1.6396
Camphene	6616	-4.5979	-3.6346	-4.2675	3.7014
Tricyclene	79035	-4.1508	-3.6620	-4.1353	2.5372
Verbenene	6427476	-4.2834	-3.5745	-4.1839	0.5653
Sabinene	18818	-4.7391	-3.5350	-4.1359	1.2161
beta-Pinene	14896	-4.3973	-3.5906	-4.1400	1.7168

II.2 Discussion:

The docking procedure allows the generation of a list of complexes representing the favourable association modes between the ligand and the macromolecular receptor. The next step is to evaluate these complexes, in order to find the one or those most likely to best reproduce the actual association mode. The association between proteins and ligands is governed by several thermodynamic parameters: hydrophobic interactions, electrostatic interactions and hydrogen interactions.

A. Electrostatic interactions:

When they concern dipole/permanent dipole interactions on the one hand and salt bridges on the other hand. The latter correspond to interactions between charged regions determined by the laws of electrostatics. At physiological pH, the positively charged residues (blue colour) are the basic amino acids and the N-terminal end of the polypeptide chain, the negatively charged residues (red colour) are the acidic amino acids and the C-terminal end and the neutral residues (white colour) are the neutral amino acids.

Complex 01: Drosophila melanogaster-acetylcholinesterase

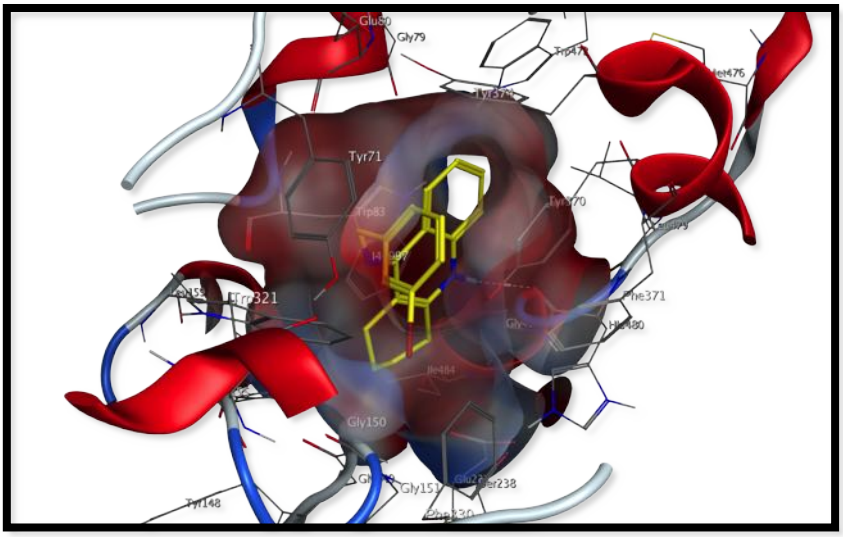

Figure 11 : Graphical representation in surface mode of the electrostatic interactions of the active site with I40.

Figure 11: Shows that the Drosophila melanogaster acetylcholinesterase receptor and their ligand (I40) in the electrostatic field; have observed regions in the receptor in blue i.e. having positively charged atoms, regions in red i.e. having negatively charged atoms and regions in white i.e. having neutral atoms.

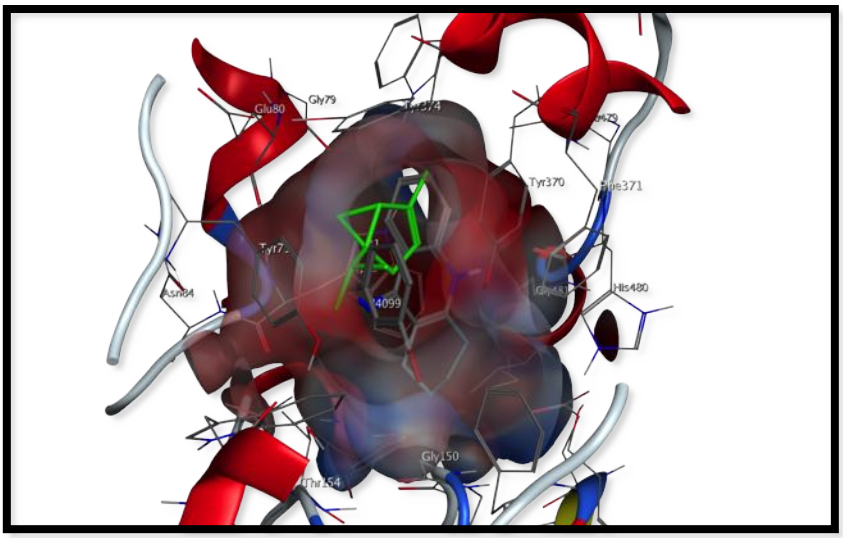

Figure 12: Graphical representation in surface mode of the electrostatic interactions of the active site with alpha-Thujene.

Figure 12: Shows that the Drosophila melanogaster acetylcholinesterase receptor and the ligand alpha-Thujene in the electrostatic field; have observed regions in the receptor in blue i.e. having positively charged atoms, regions in red i.e. having negatively charged atoms.

Complex 02: Bombyx mori sigma-class glutathione transferase

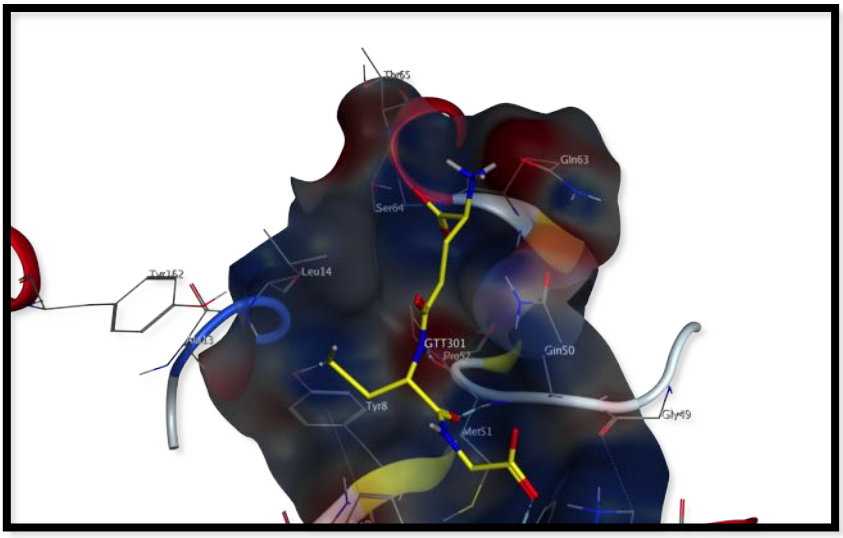

Figure 13 : Surface mode graphical representation of the electrostatic interactions of the active site with GSH.

Figure 13: Shows that the Bombyx mori sigma-class glutathione transferase receptor and its ligand (GSH) in the electrostatic field; have observed regions in the receptor in blue i.e. having positively charged atoms.

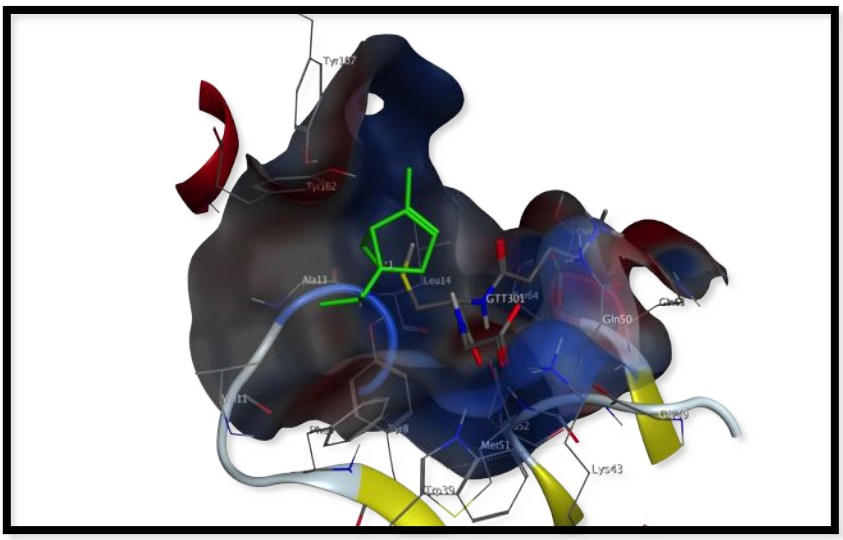

Figure 14: Surface mode graphical representation of the electrostatic interactions of the active site with alpha-Thujene.

Figure 14: Shows that the Bombyx mori sigma-class glutathione transferase receptor and the ligand alpha-Thujene in the electrostatic field; have observed most of the receptor regions in positively charged blue i.e. has basic atoms.

Complex 03: Ostrinia furnacalis group I chitinase catalytic domain

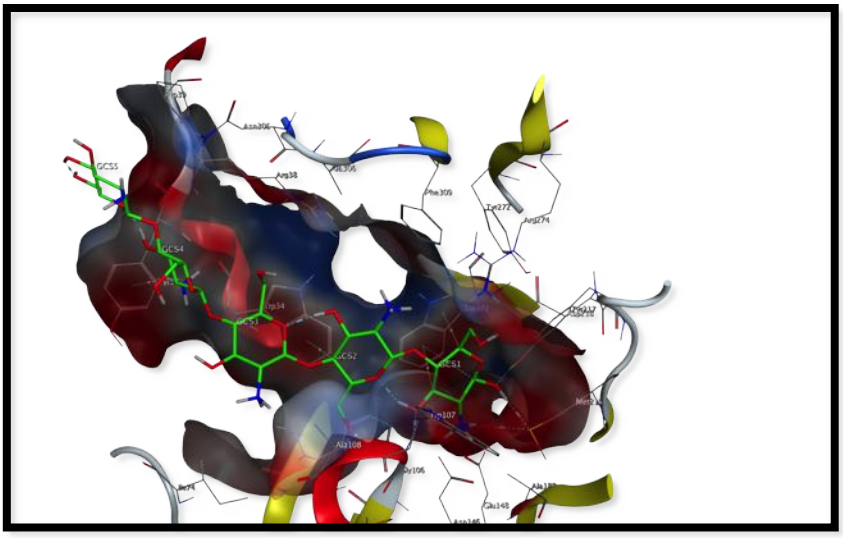

Figure 15 : Surface mode graphical representation of the electrostatic interactions of the active site with NAG.

Figure 15: Shows that the Ostrinia furnacalis group I chitinase catalytic domain receptor and their ligand (NAG) in the electrostatic field; have observed most of the receptor regions in red i.e. having negatively charged atoms,and regions in the receptor in blue i.e. having positively charged atoms.

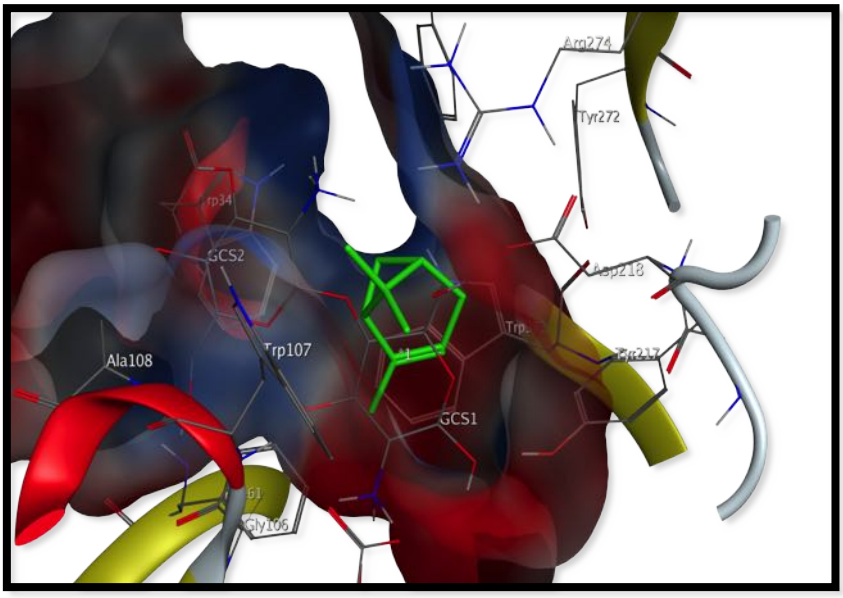

Figure 16 : Surface mode graphical representation of the electrostatic interactions of the active site with alpha-Pinene.

Figure 16: Shows that the Ostrinia furnacalis group I chitinase catalytic domain receptor and the ligand alpha-Pinene in the electrostatic field; have observed regions in the receptor in blue i.e. possess positively charged atoms, regions in red i.e. possess negatively charged atoms.

Complex 04: Drosophila melanogastercentral nervous system glutamate receptor

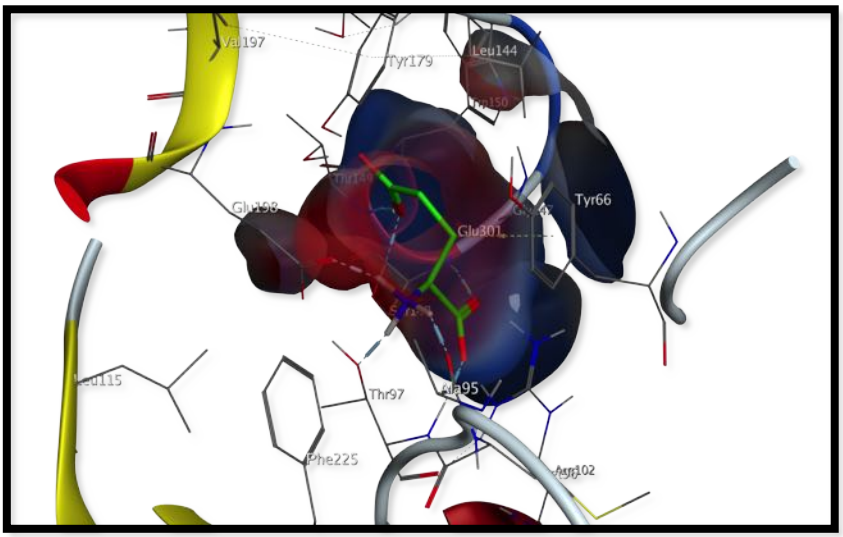

Figure 17 : Surface mode graphical representation of the electrostatic interactions of the active site with GLU.

Figure 17: Shows that the Drosophila melanogastercentral nervous system glutamate receptor and their ligand (GLU) in the electrostatic field; have observed regions in the receptor in blue i.e. have positively charged atoms, regions in red i.e. have negatively charged atoms and regions in white i.e. having neutral atoms.

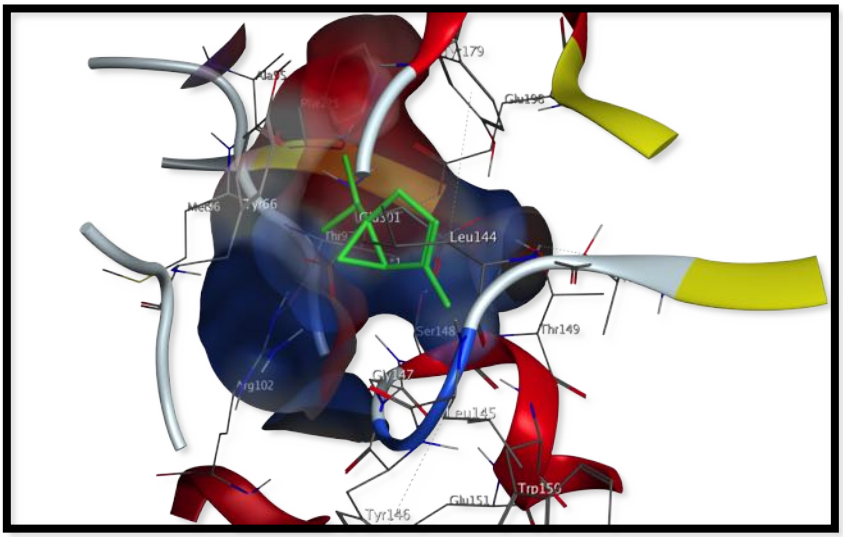

Figure 18 : Surface mode graphical representation of the electrostatic interactions of the active site with alpha-Thujene.

Figure 18: Shows that the Drosophila melanogastercentral nervous system glutamate receptor and the ligand alpha-Thujene in the electrostatic field; have observed regions in the receptor in blue i.e. have positively charged atoms, regions in red i.e. have negatively charged atoms.

B. Hydrophobic interactions (Lipophilicity):

In order to account more finely for protein-ligand interactions, other criteria based on energetics can be taken into account:

the hydrophobic effect: several hydrophobic amino acids (forming a hydrophobic "patch", green color) and also some hydrophilic amino acids (forming a hydrophilic "patch", purple color example the figures (19,20…26) in contact at the interface "hydrophobic complementarity" lead to a stabilizing interaction for many complexes.

Complex 01: Drosophila melanogaster acetylcholinesterase

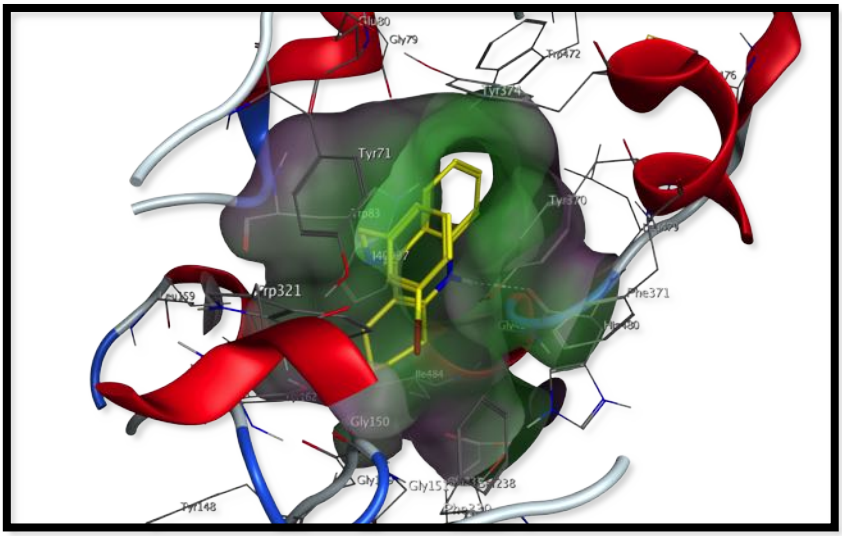

Figure 19 : Surface mode graphical representation of the hydrophobic interactions of the active site with I40.

Figure 19: Shows that the Drosophila melanogaster acetylcholinesterase receptor and their ligand (I40) in the Hydrophobic patch and Hydrophilic patch observed most of the receptor regions are green in colour i.e. has lipophilic atoms.

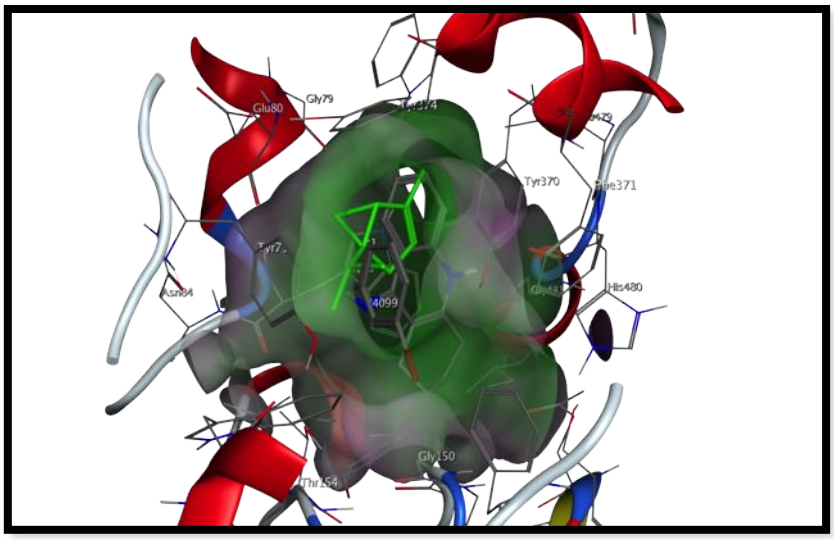

Figure 20 : Surface mode graphical representation of the hydrophobic interactions of the active site with alpha-Thujene.

Figure 20: Shows that the Drosophila melanogaster acetylcholinesterase receptor and the ligand alpha-Thujene in the Hydrophobic patch and Hydrophilic patch observed most of the receptor regions are green in colour i.e. has lipophilic atoms.

Complex 02: Bombyx mori sigma-class glutathione transferase

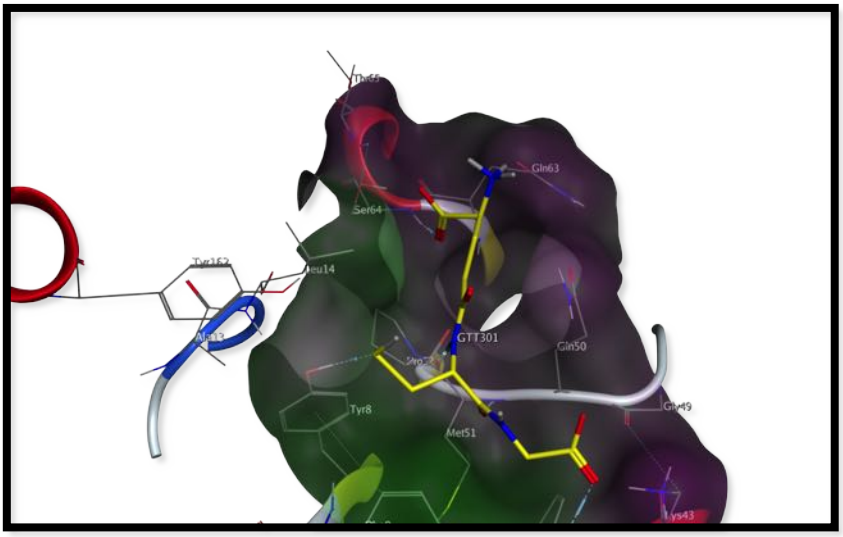

Figure 21: Surface mode graphical representation of the hydrophobic interactions of the active site with GSH.

Figure 21: Shows that the Bombyx mori sigma-class glutathione transferase receptor and its ligand (GSH) in the "Hydrophobic patch" and "Hydrophilic patch" have observed that most of the receptor regions are green in color i.e. have lipophilic atoms and some purple regions i.e. have hydrophilic atoms.

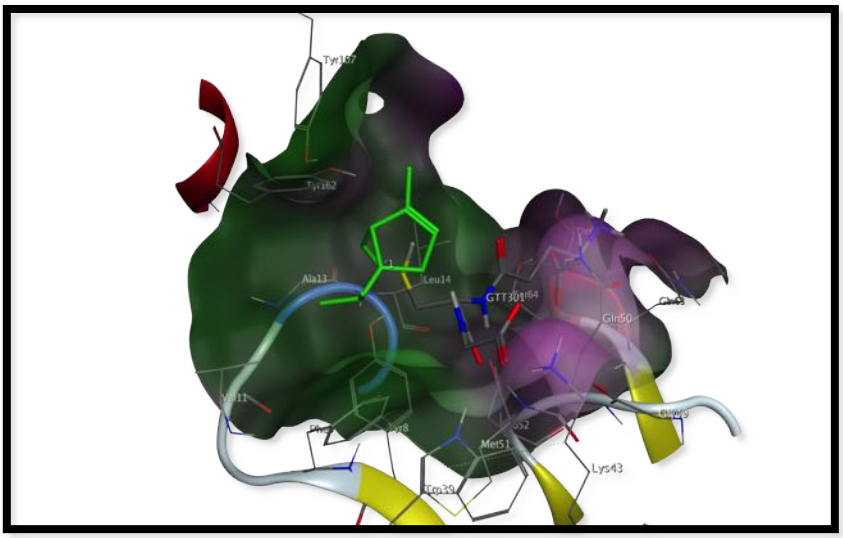

Figure 22 : Surface mode graphical representation of the hydrophobic interactions of the active site with alpha-Thujene.

Figure 22: Shows that the Bombyx mori sigma-class glutathione transferase receptor and the ligand alpha-Thujene in the "Hydrophobic patch" and "Hydrophilic patch" have observed that most of the receptor regions are green in color i.e. have lipophilic atoms and some purple regions i.e. had hydrophilic atoms.

Complex 03: Ostrinia furnacalis group I chitinase catalytic domain

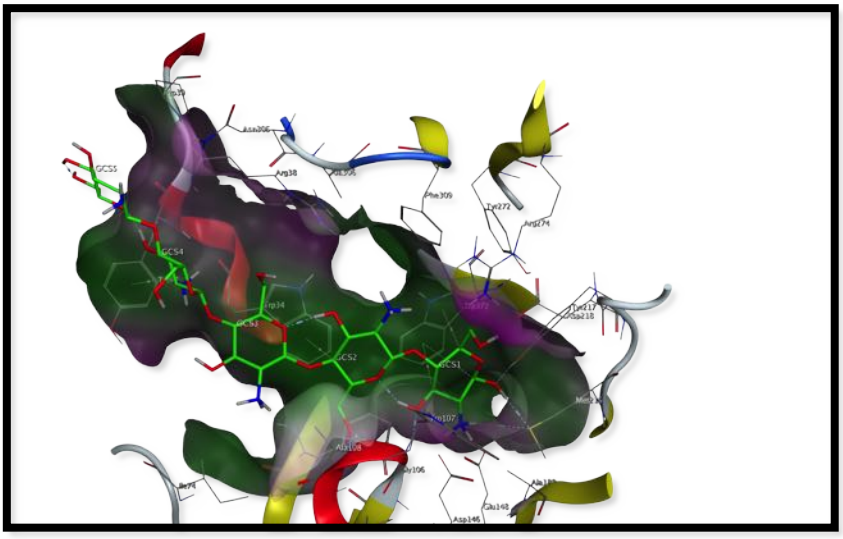

Figure 23 : Graphical representation in surface mode of the hydrophobic interactions of the active site with NAG.

Figure 23: Shows that the Ostrinia furnacalis group I chitinase catalytic domain receptor and their ligand (NAG) in the "Hydrophobic patch" and "Hydrophilic patch" have observed that most of the receptor regions are green in color i.e. have lipophilic atoms and some regions are purple in color i.e. have hydrophilic atoms.

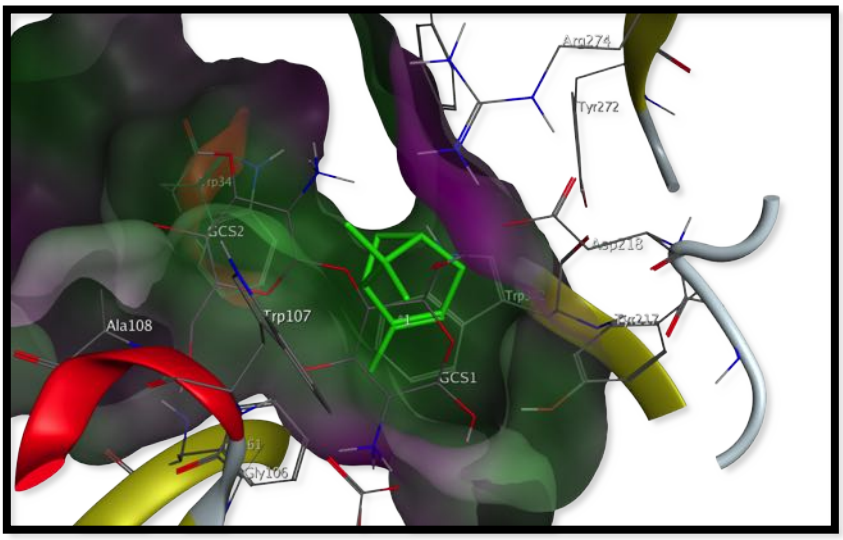

Figure 24 : Graphical representation in surface mode of the hydrophobic interactions of the active site with alpha-Pinene.

Figure 24: Shows that the Ostrinia furnacalis group I chitinase catalytic domain receptor and the ligand alpha-Pinene in the "Hydrophobic patch" and "Hydrophilic patch" have observed that most of the receptor regions are green in color i.e. have lipophilic atoms and some regions are purple in color i.e. have hydrophilic atoms.

Complex 04: Drosophila melanogastercentral nervous system glutamate receptor

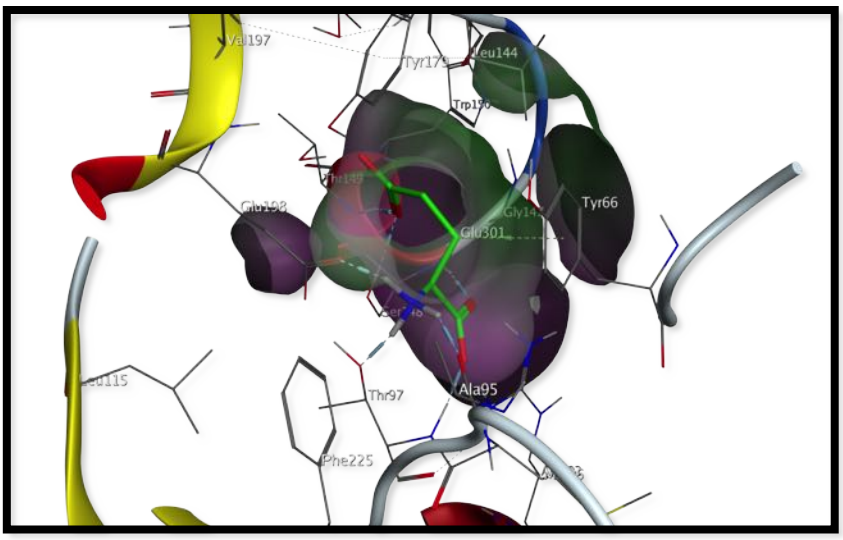

Figure 25 : Surface mode graphical representation of the hydrophobic interactions of the active site with GLU.

Figure 25: Shows that the Drosophila melanogastercentral nervous system glutamate receptor and their ligand (GLU) in the "Hydrophobic patch" and "Hydrophilic patch" have observed that most of the receptor regions are purple in color i.e. have hydrophilic atoms and some regions are green in color i.e. have lipophilic atoms.

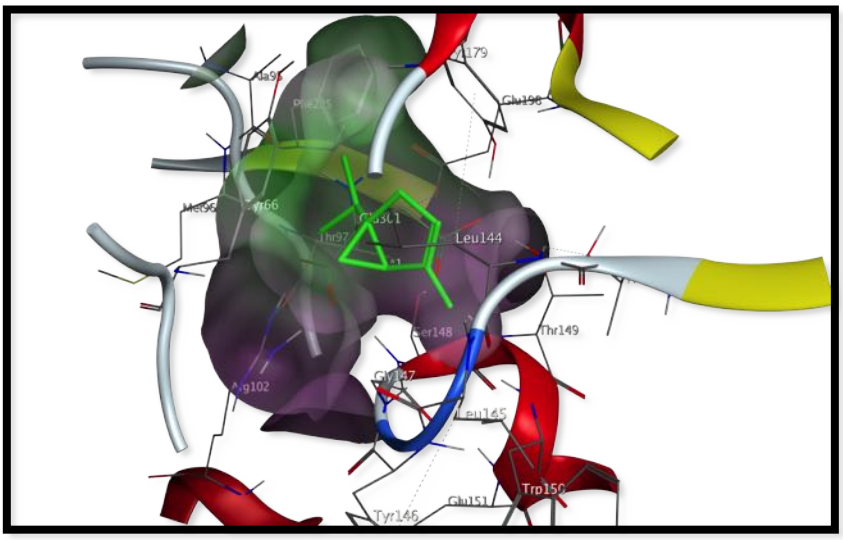

Figure 26 : Surface mode graphical representation of the hydrophobic interactions of the active site with alpha-Thujene.

Figure 26: Shows that the Drosophila melanogastercentral nervous system glutamate receptor and the ligand alpha-Thujene in the "Hydrophobic patch" and "Hydrophilic patch" have observed that most of the receptor regions are purple in color i.e. have hydrophilic atoms and some regions are green in color i.e. have lipophilic atoms.

II.3. Discussion:

As a result, approximate methods have been developed to distinguish (evaluate and classify) complexes among those generated by a docking procedure: score functions. (A. Jain 2006). (scoring) is the classification step, which consists of evaluating the affinity between the ligand and the protein and giving a score to the poses obtained during the docking phase. This score will make it possible to select the best pose among all those proposed. (G.L.Warrem 2006)

C- Amino acids interacting with the ligand:

The ligand is arranged and rendered using an enhanced version of the 3D layout representation, and protein residues are arranged around it to indicate spatial proximity links.

Interactions between 2.5 Å and 3.1 Å are considered strong and those between 3.1 Å and 3.55 Å are assumed to be medium. Interactions above 3.55 Å are weak.

The strength of a hydrogen bond can vary widely, but most hydrogen bonds in hydrogen bond interactions depend on a strong hydrogen bond acceptor and a hydrogen donor.

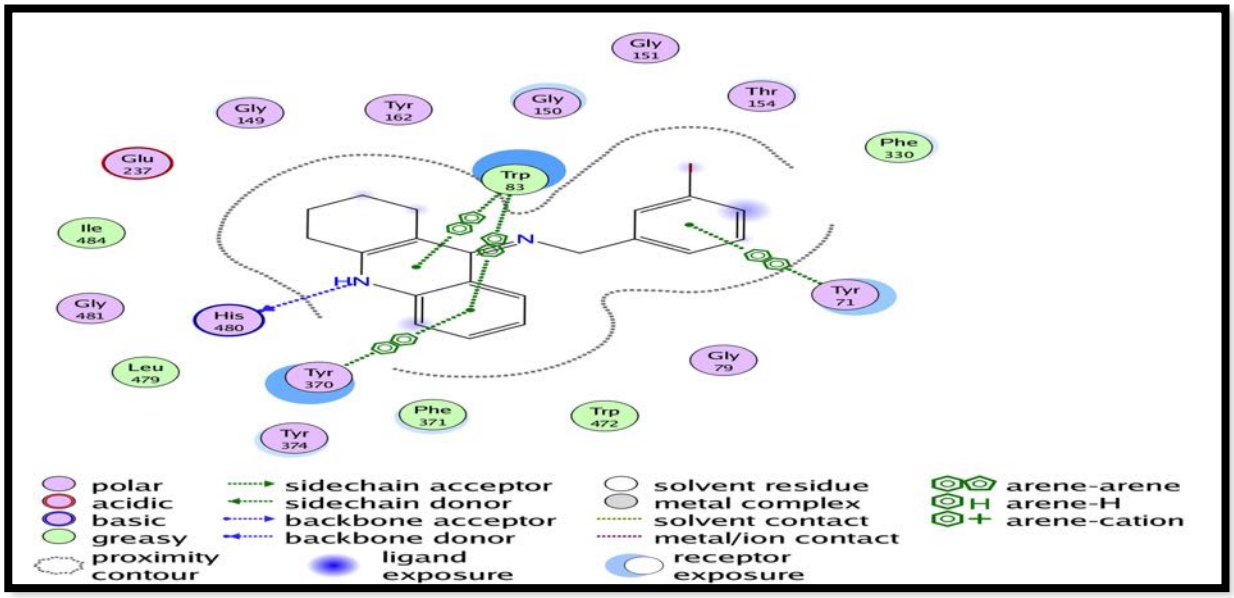

Figure 27 : Interaction Diagram of Complex 1 + I40.

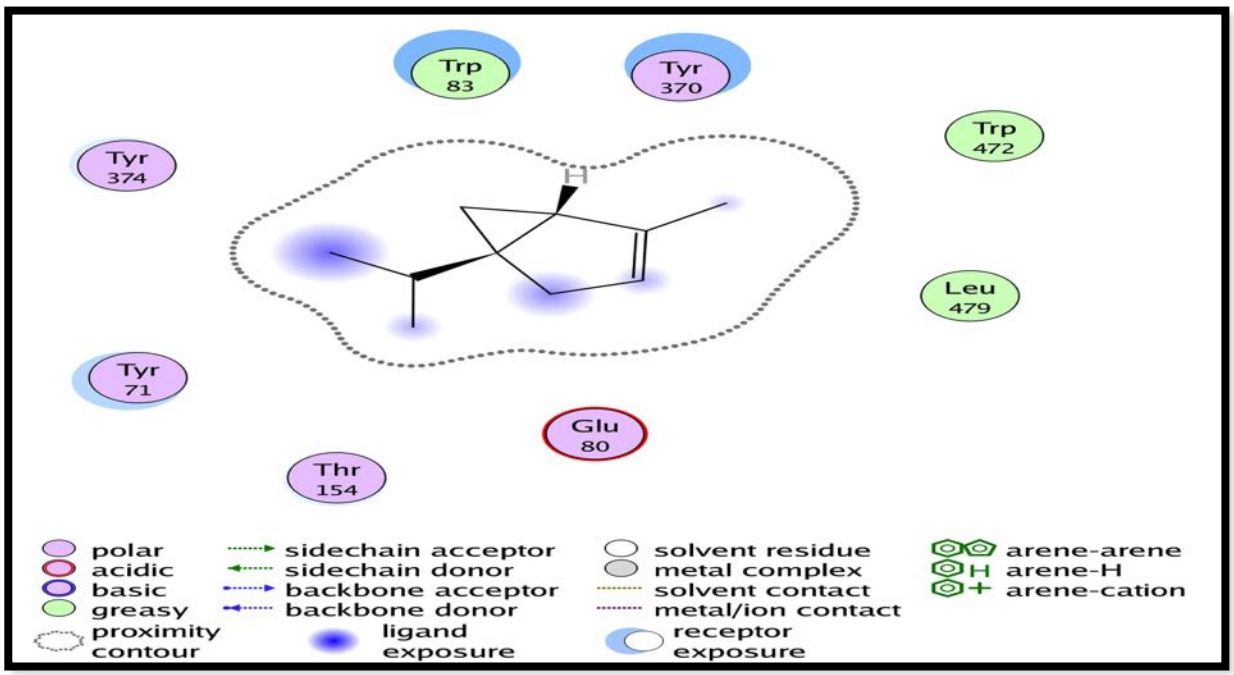

Figure 28 : Interaction Diagram of Complex 1 with alpha-Thujene.

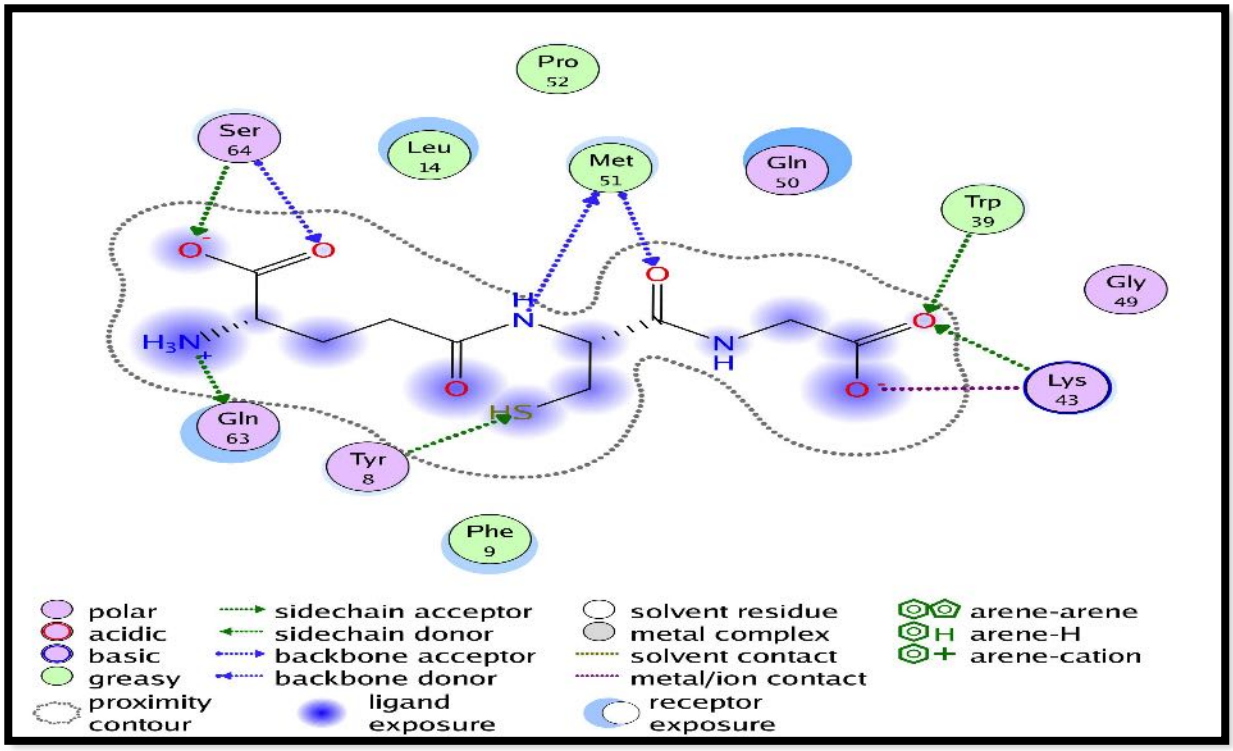

Figure 29 : Interaction diagram of complex 2+ GSH.

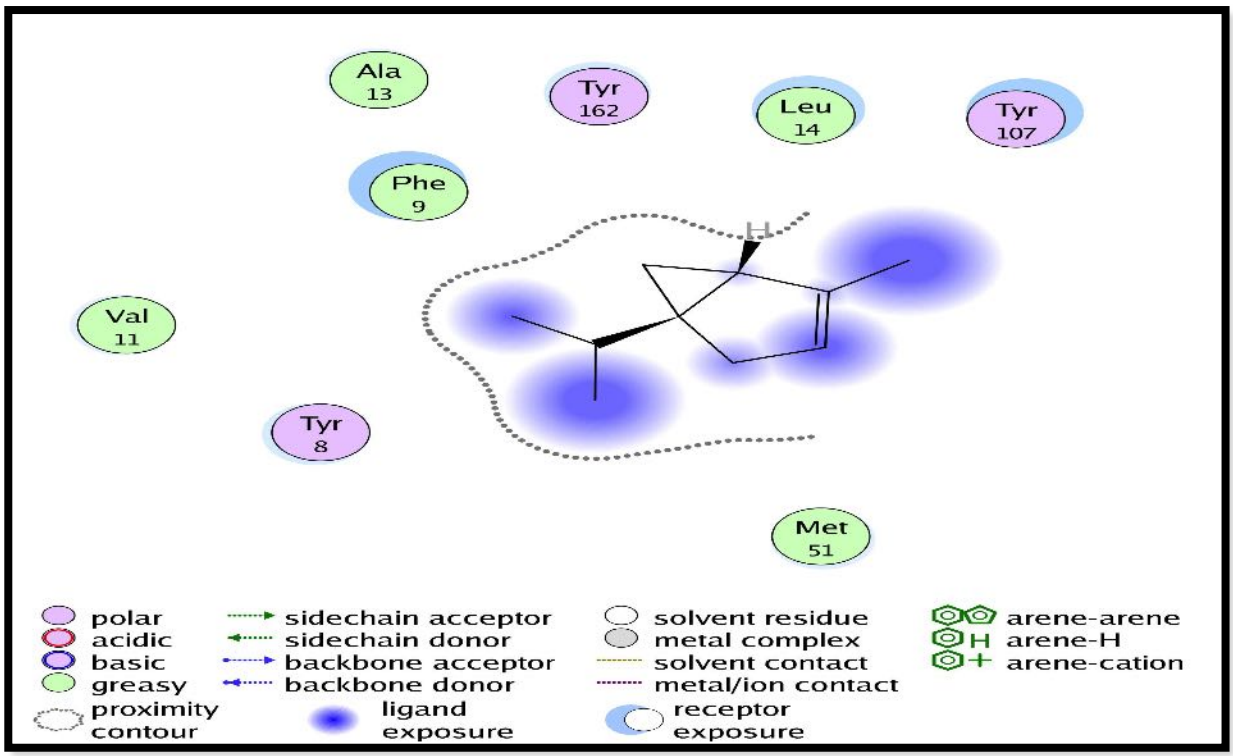

Figure 30 : Interaction diagram of complex 2 with alpha-Thujene.

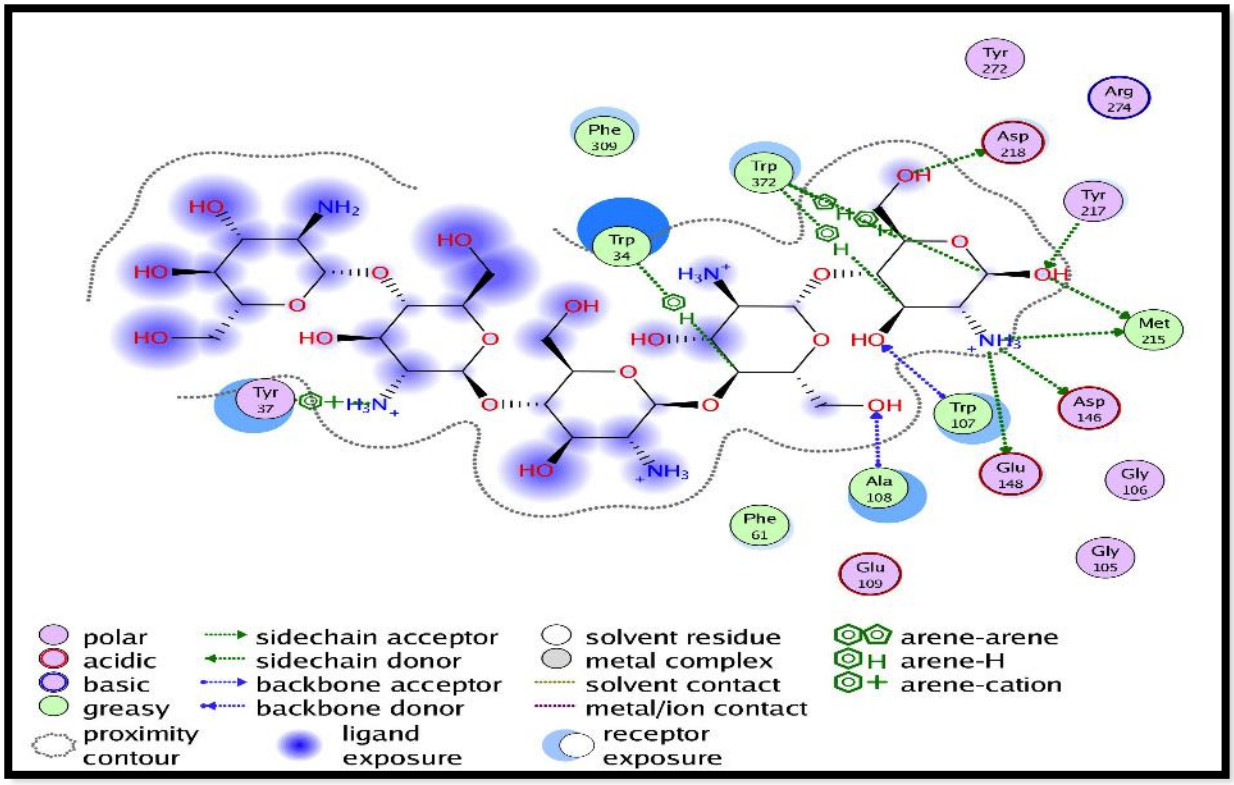

Figure 31 : Interaction diagram of complex 3 + NAG.

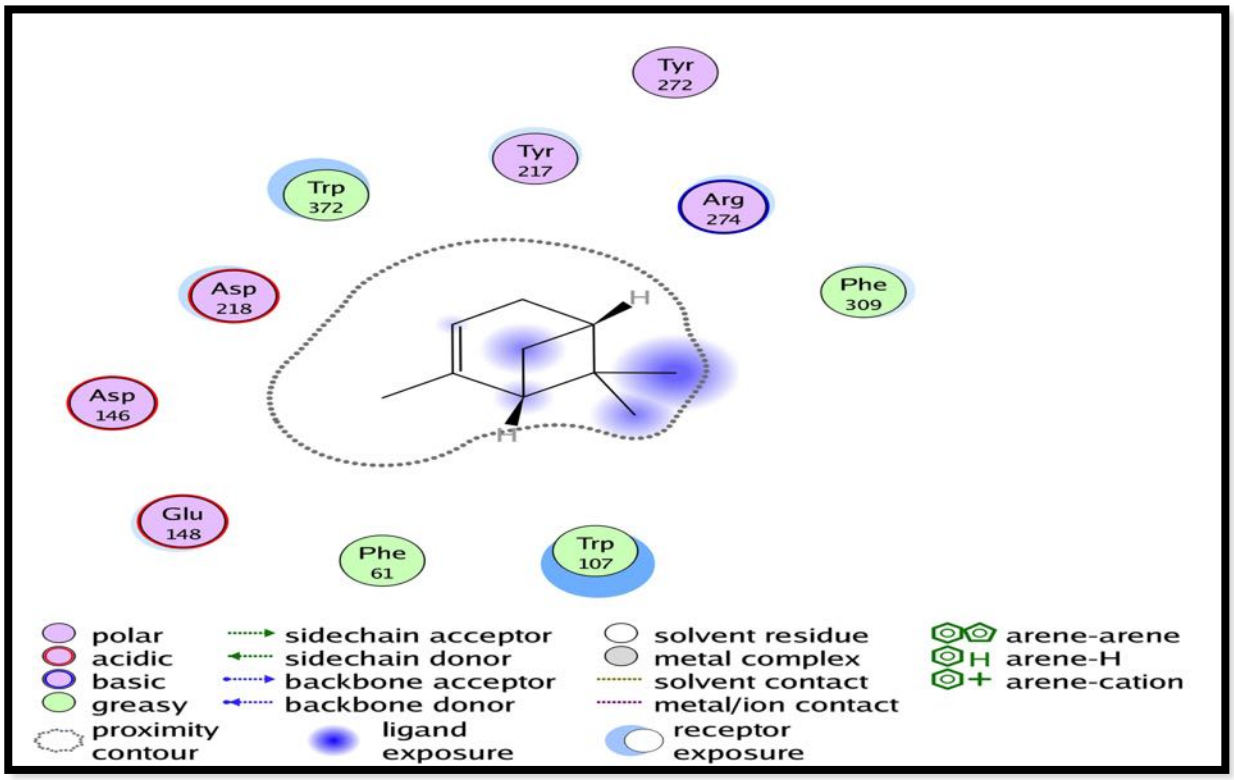

Figure 32 : Interaction Diagram of Complex 3 with alpha-Pinene.

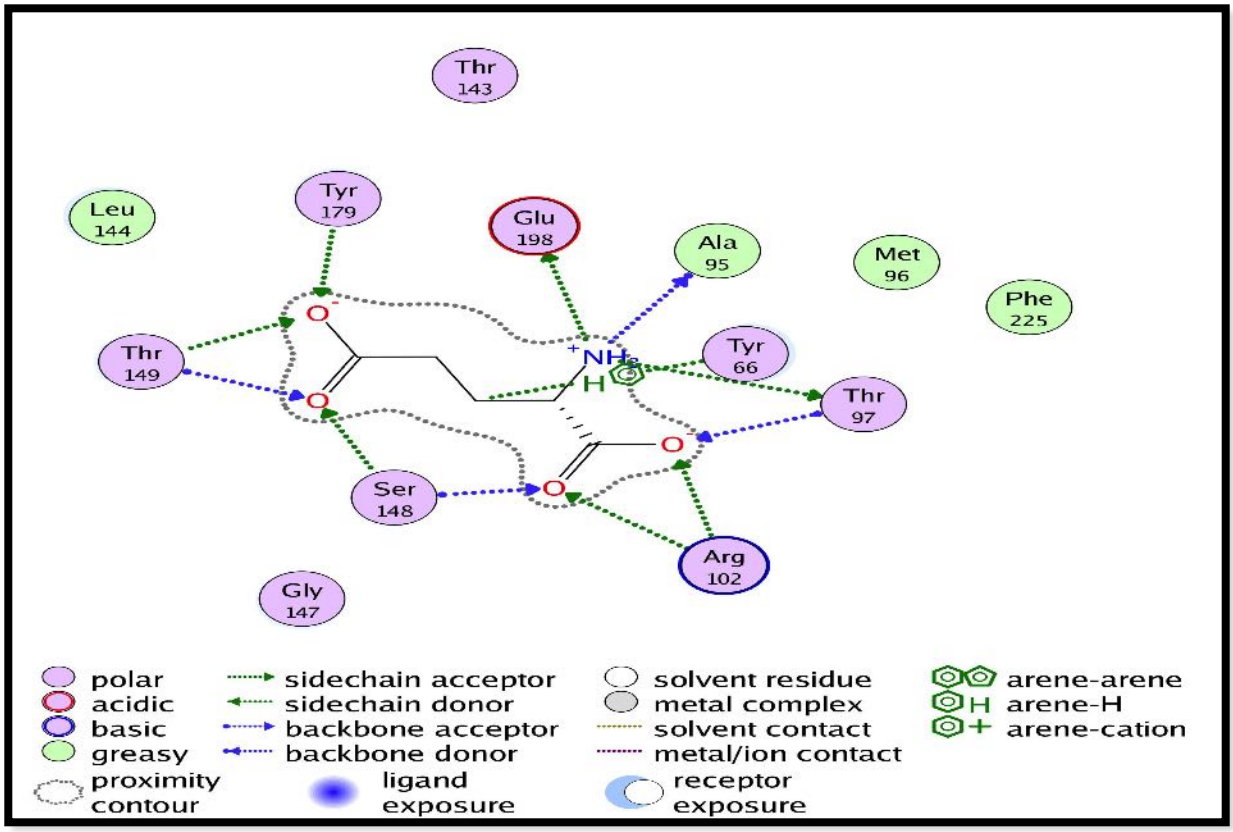

Figure 33 : Interaction diagram of complex 4+ GLU.

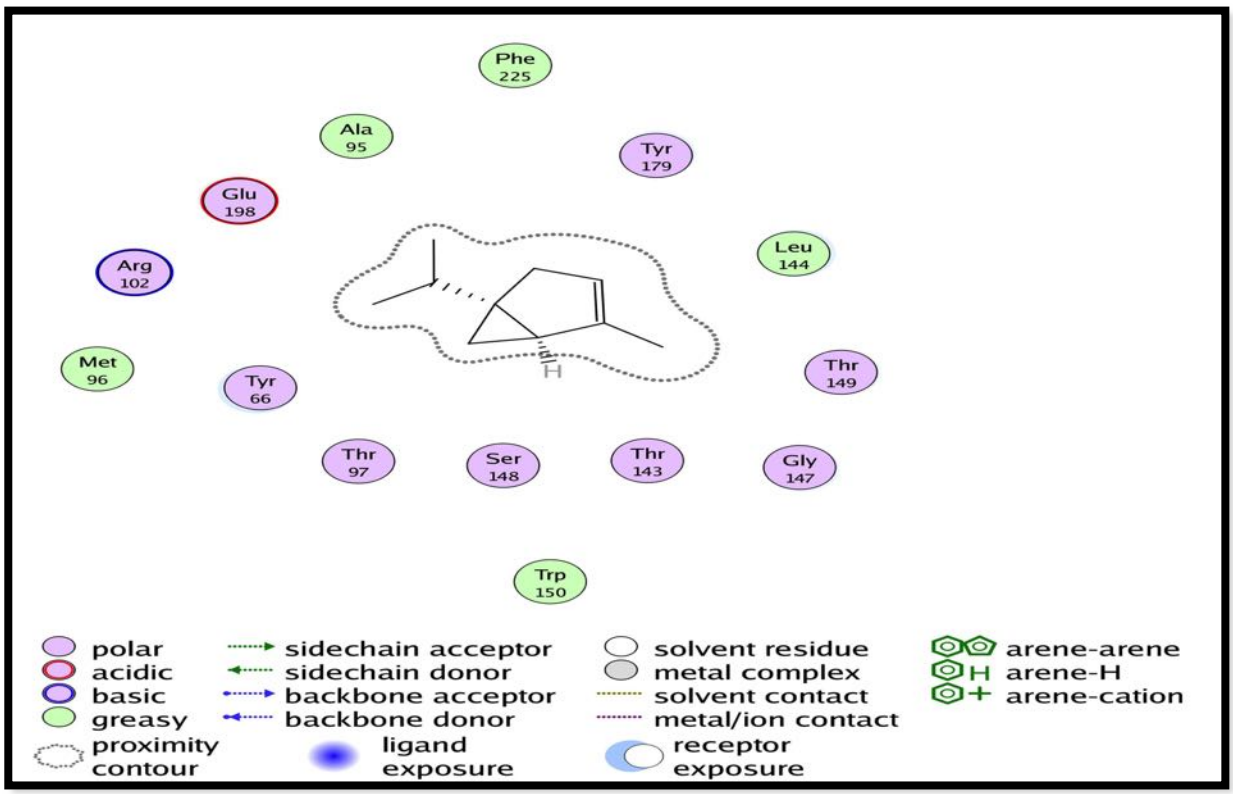

Figure 34 : Interaction diagram of complex 4 with alpha-Thujene.

III. Final conclusion in silico:

The interaction between a protein and its substrate is the first step in most biological reactions. To understand how it works and to define which residues are involved:

- The affinity between two molecules.

- The distances between the amino acids of the active site of the enzyme and the atoms of the ligand.

- The energy of interaction.

The flexibility of biological molecules results from the high dimensionality of the systems, but also from the intervention of a large number of weak interactions (hydrogen bonding, hydrophobic effect ...). It is known that it is an integral part of the dynamics of macromolecules and their proper functioning. The neglect of this parameter in the calculations introduces errors, it is therefore necessary to determine the intrinsic flexibility of an molecule but also to be able to introduce it, at least partially, into a procedure of docking (Carlson H.A, 2000; Carlson H.A, 2002.6; Carlson H.A, 2002.8; Teague S.J, 2003).

Docking software is therefore a very useful tool in biology, pharmacy and medicine, as most active ingredients are small molecules (ligands) that interact with a biological target of therapeutic interest, usually a protein (receptor), in order to influence the mechanism in which this protein is involved (Grosdidier A, 2007).

According to the results obtained, the calculation of the docking suite score energy was performed to form the complexes and identify the existing interactions. Finally, we note that complex 01 has the lowest energy with alpha-Thujene ligand (-4.7791),

The lowest energy compared to the other compounds means that alpha-thujene is the best one and Drosophila acetylcholinesterase is the most sensitive enzyme, but compared to the lowest energy cocrystalized ligand (-8.6348) means that alpha-Thujene have a weak affinity to 1QON wich means a weak insecticidal activty.

ANNEXES

Annexe 1: Chemical composition of essential oils of *Thymus numidicus*.

Composition	**references**
α-Thujene	Youcef Hadef et al. 2007, Hocine Laouer et al. 2009, Hocine Laouer et al. 2009
α-Pinene	Youcef Hadef et al. 2007, Kabouche a et al. 2005, Hocine Laouer et al. 2009
1,3,5-Cycloheptadiene	Youcef Hadef et al. 2007
Camphene	Youcef Hadef et al. 2007, Kabouche a et al. 2005, Hocine Laouer et al. 2009
Dimethyl bicyclo (3,1) diene (6,6-)	Youcef Hadef et al. 2007
Sabinene	Youcef Hadef et al. 2007, Hocine Laouer et al. 2009
β-Pinene	Youcef Hadef et al. 2007, Kabouche a et al. 2005, Hocine Laouer et al. 2009
α-Phellandrene	Youcef Hadef et al. 2007, Hocine Laouer et al. 2009
α-Terpinene	Youcef Hadef et al. 2007, Hocine Laouer et al. 2009

p-Cymene	Youcef Hadef et al. 2007, Kabouche a et al. 2005, Hocine Laouer et al. 2009
Trans 3-Carene-2-ol	Youcef Hadef et al. 2007
γ-Terpinene	Youcef Hadef et al. 2007, Kabouche a et al. 2005, Hocine Laouer et al. 2009
Sabinene hydrate trans	Youcef Hadef et al. 2007, Kabouche a et al. 2005
Linalool oxide cis	Youcef Hadef et al. 2007
α-Terpinolene	Youcef Hadef et al. 2007, Kabouche a et al. 2005
Linalool oxide trans	Youcef Hadef et al. 2007, Kabouche a et al. 2005
Linalool	Youcef Hadef et al. 2007, Kabouche a et al. 2005, Hocine Laouer et al. 2009
Menth-2-en-1-ol (cis-p-)	Youcef Hadef et al. 2007
Pinocarveol-trans	Youcef Hadef et al. 2007
Verbeneol-trans	Youcef Hadef et al. 2007

Dodecene-1	Youcef Hadef et al. 2007
Terpineol-4	Youcef Hadef et al. 2007
Benzoic acid-4-methyl, methyl ester	Youcef Hadef et al. 2007
Thymoquinone	Youcef Hadef et al. 2007
Thymol	Youcef Hadef et al. 2007, Kabouche a et al. 2005, Hocine Laouer et al. 2009
Carvacrol	Youcef Hadef et al. 2007, Kabouche a et al. 2005, Hocine Laouer et al. 2009
α-Copaene	Youcef Hadef et al. 2007, Kabouche a et al. 2005, Hocine Laouer et al. 2009
Tetradecene-1	Youcef Hadef et al. 2007
β-Bourbonene	Youcef Hadef et al. 2007, Kabouche a et al. 2005, Hocine Laouer et al. 2009
β-Caryophyllene	Youcef Hadef et al. 2007, Hocine Laouer et al. 2009
β-Humelene	Youcef Hadef et al. 2007
β-Elemene	Youcef Hadef et al. 2007

allo-aromadendrene	Youcef Hadef et al. 2007
γ-Muurolene	Youcef Hadef et al. 2007, Kabouche a et al. 2005, Hocine Laouer et al. 2009
β-Silenene	Youcef Hadef et al. 2007, Kabouche a et al. 2005
β-Bisabolene	Youcef Hadef et al. 2007
δ-Cadinene	Youcef Hadef et al. 2007, Kabouche a et al. 2005, Hocine Laouer et al. 2009
Sesqui phellandrene	Youcef Hadef et al. 2007
N-tridecanol	Youcef Hadef et al. 2007
Decanoic acid,ethyl ester	Youcef Hadef et al. 2007
Caryophyllene oxide	Youcef Hadef et al. 2007, Kabouche a et al. 2005, Hocine Laouer et al. 2009
Myrcene	Kabouche a et al. 2005, Hocine Laouer et al. 2009
3-Octanol	Kabouche a et al. 2005
Limonene	Kabouche a et al. 2005, Hocine Laouer et al. 2009

cis-Sabinene hydrate	Kabouche a et al. 2005, Hocine Laouer et al. 2009
Camphor	Kabouche a et al. 2005
Borneol	Kabouche a et al. 2005, Hocine Laouer et al. 2009
Isoborneol	Kabouche a et al. 2005
Dihydrocarveol	Kabouche a et al. 2005
cis-Carveol	Kabouche a et al. 2005
Carvone	Kabouche a et al. 2005, Hocine Laouer et al. 2009
Eugenol	Kabouche a et al. 2005
α-Cubebene	Kabouche a et al. 2005
α-Humulene	Kabouche a et al. 2005, Hocine Laouer et al. 2009
Germacrene D	Kabouche a et al. 2005, Hocine Laouer et al. 2009
α-Muurolene	Kabouche a et al. 2005, Hocine Laouer et al. 2009

α-Farnesene	Kabouche a et al. 2005
Spathulenol	Kabouche a et al. 2005
Cubenol	Kabouche a et al. 2005
α-Cadinol	Kabouche a et al. 2005
α-Bisabolol	Kabouche a et al. 2005
Myrcenyl acetate	Kabouche a et al. 2005
Octadecane	Kabouche a et al. 2005
Nonadecane	Kabouche a et al. 2005
(E)-2-hexenal	Hocine Laouer et al. 2009
(Z)-3-hexenol	Hocine Laouer et al. 2009
3-octanone	Hocine Laouer et al. 2009
δ -3-carene	Hocine Laouer et al. 2009

b-phellandrene	Hocine Laouer et al. 2009
(Z)-b-ocimene	Hocine Laouer et al. 2009
(E)-b-ocimene	Hocine Laouer et al. 2009
dehydro-p-cymene	Hocine Laouer et al. 2009
terpinolene	Hocine Laouer et al. 2009
terpinen-4-ol	Hocine Laouer et al. 2009
a-terpineol	Hocine Laouer et al. 2009
dihydrocarvone	Hocine Laouer et al. 2009
methyl thymol methyl ether	Hocine Laouer et al. 2009
β -cubebene	Hocine Laouer et al. 2009
(Z)- α -bisabolene	Hocine Laouer et al. 2009
δ -cadinene	Hocine Laouer et al. 2009

(E)- α -bisabolene	Hocine Laouer et al. 2009

Annexe 2: Chemical composition of essential oils of *Thymus algeriensis*.

Tricyclene	Abdenour Ait-Ouazzou et al. 2011, Milos Nikolic et al. 2013, Dob T et al. 2006, Nacim Zouari et al. 2012
***α*-Thujene**	Abdenour Ait-Ouazzou et al. 2011, Milos Nikolic et al. 2013, Dob T et al. 2006, Nacim Zouari et al. 2012
***α*-Pinene**	Abdenour Ait-Ouazzou et al. 2011, Milos Nikolic et al. 2013, Dob T et al. 2006, Nacim Zouari et al. 2012
Camphene	Abdenour Ait-Ouazzou et al. 2011, Milos Nikolic et al. 2013, Nacim Zouari et al. 2012
Thuja-2,4(10)diene	Abdenour Ait-Ouazzou et al. 2011,
Sabinene	Abdenour Ait-Ouazzou et al. 2011, Milos Nikolic et al. 2013, Dob T et al. 2006, Nacim Zouari et al. 2012
***β*-Pinene**	Abdenour Ait-Ouazzou et al. 2011, Milos Nikolic et al. 2013, Nacim Zouari et al. 2012
Myrcene	Abdenour Ait-Ouazzou et al. 2011, Dob T et al. 2006
3-Octanol	Abdenour Ait-Ouazzou et al. 2011

***α*-Phellandrene**	Abdenour Ait-Ouazzou et al. 2011, Milos Nikolic et al. 2013, Dob T et al. 2006, Nacim Zouari et al. 2012
***α*-Terpinene**	Abdenour Ait-Ouazzou et al. 2011, Milos Nikolic et al. 2013, Dob T et al. 2006, Nacim Zouari et al. 2012
***p*-Cymene**	Abdenour Ait-Ouazzou et al. 2011, Milos Nikolic et al. 2013, Dob T et al. 2006, Nacim Zouari et al. 2012
Limonene	Abdenour Ait-Ouazzou et al. 2011, Milos Nikolic et al. 2013, Dob T et al. 2006, Nacim Zouari et al. 2012
***β*-Phellandrene**	Abdenour Ait-Ouazzou et al. 2011
1,8-Cineole	Abdenour Ait-Ouazzou et al. 2011, Milos Nikolic et al. 2013, Dob T et al. 2006, Nacim Zouari et al. 2012
(*E*)-*β*-Ocimene	Abdenour Ait-Ouazzou et al. 2011
***γ* -Terpinene**	Abdenour Ait-Ouazzou et al. 2011, Milos Nikolic et al. 2013, Nacim Zouari et al. 2012
Terpinolene	Abdenour Ait-Ouazzou et al. 2011, Dob T et al. 2006, Nacim Zouari et al. 2012
***p*-cymenene**	Abdenour Ait-Ouazzou et al. 2011
Linalool	Abdenour Ait-Ouazzou et al. 2011, Milos Nikolic et al. 2013, Dob T et al. 2006, Nacim Zouari et al. 2012
6-Camphenol	Abdenour Ait-Ouazzou et al. 2011, Nacim Zouari et al. 2012

***trans*-Pinocarveol**	Abdenour Ait-Ouazzou et al. 2011
***cis*-Verbenol**	Abdenour Ait-Ouazzou et al. 2011
Camphor	Abdenour Ait-Ouazzou et al. 2011, Milos Nikolic et al. 2013, Dob T et al. 2006, Nacim Zouari et al. 2012
***δ*-Terpineol**	Abdenour Ait-Ouazzou et al. 2011
Borneol	Abdenour Ait-Ouazzou et al. 2011, Milos Nikolic et al. 2013, Dob T et al. 2006, Nacim Zouari et al. 2012
Terpinen-4-ol	Abdenour Ait-Ouazzou et al. 2011, Milos Nikolic et al. 2013, Dob T et al. 2006
***p*-Cymen-8-ol**	Abdenour Ait-Ouazzou et al. 2011, Nacim Zouari et al. 2012
***α*-Terpineol**	Abdenour Ait-Ouazzou et al. 2011, Milos Nikolic et al. 2013, Dob T et al. 2006, Nacim Zouari et al. 2012
***trans*-dihydrocarvone**	Abdenour Ait-Ouazzou et al. 2011
Isobornyl formate	Abdenour Ait-Ouazzou et al. 2011
Carvacrol, methyl ether	Abdenour Ait-Ouazzou et al. 2011, Dob T et al. 2006
Carvenone	Abdenour Ait-Ouazzou et al. 2011, Dob T et al. 2006, Nacim Zouari et al. 2012

Isobornyl acetate	Abdenour Ait-Ouazzou et al. 2011, Milos Nikolic et al. 2013
Thymol	Abdenour Ait-Ouazzou et al. 2011, Milos Nikolic et al. 2013, Dob T et al. 2006, Nacim Zouari et al. 2012
Carvacrol	Abdenour Ait-Ouazzou et al. 2011, Milos Nikolic et al. 2013, Dob T et al. 2006, Nacim Zouari et al. 2012
***α*-Cubebene**	Abdenour Ait-Ouazzou et al. 2011
***α*-Ylangene**	Abdenour Ait-Ouazzou et al. 2011
***α*-Copaene**	Abdenour Ait-Ouazzou et al. 2011, Dob T et al. 2006, Nacim Zouari et al. 2012
***β*-Bourbonene**	Abdenour Ait-Ouazzou et al. 2011, Nacim Zouari et al. 2012
Caryophyllene <(*Z*)	Abdenour Ait-Ouazzou et al. 2011
***α*-Gurjunene**	Abdenour Ait-Ouazzou et al. 2011, Nacim Zouari et al. 2012
***β*-Caryophyllene**	Abdenour Ait-Ouazzou et al. 2011, Milos Nikolic et al. 2013, Dob T et al. 2006
***β*-Copaene**	Abdenour Ait-Ouazzou et al. 2011
Aromadendrene	Abdenour Ait-Ouazzou et al. 2011, Milos Nikolic et al. 2013, Dob T et al. 2006, Nacim Zouari et al. 2012

α-Himachalene	Abdenour Ait-Ouazzou et al. 2011
α-Humulene	Abdenour Ait-Ouazzou et al. 2011, Milos Nikolic et al. 2013, Nacim Zouari et al. 2012
Alloaromadendrene	Abdenour Ait-Ouazzou et al. 2011, Milos Nikolic et al. 2013, Nacim Zouari et al. 2012
***trans*-Cadina-1(6),4-diene**	Abdenour Ait-Ouazzou et al. 2011
γ -Muurolene	Abdenour Ait-Ouazzou et al. 2011
Germacrene D	Abdenour Ait-Ouazzou et al. 2011, Milos Nikolic et al. 2013, Dob T et al. 2006, Nacim Zouari et al. 2012
cis-β-guaiene	Abdenour Ait-Ouazzou et al. 2011
Valencene	Abdenour Ait-Ouazzou et al. 2011
Bicyclogermecrene	Abdenour Ait-Ouazzou et al. 2011, Milos Nikolic et al. 2013, Nacim Zouari et al. 2012
α-muurolene	Abdenour Ait-Ouazzou et al. 2011, Dob T et al. 2006
β-Himachalene	Abdenour Ait-Ouazzou et al. 2011, Dob T et al. 2006
β-Bisabolene	Abdenour Ait-Ouazzou et al. 2011, Milos Nikolic et al. 2013

γ -Cadinene	Abdenour Ait-Ouazzou et al. 2011, Milos Nikolic et al. 2013, Dob T et al. 2006, Nacim Zouari et al. 2012
δ-Cadinene	Abdenour Ait-Ouazzou et al. 2011, Milos Nikolic et al. 2013, Dob T et al. 2006, Nacim Zouari et al. 2012
***trans*-Calamenene**	Abdenour Ait-Ouazzou et al. 2011
***trans*-Cadina-1(2),4-diene**	Abdenour Ait-Ouazzou et al. 2011
α-Cadinene	Abdenour Ait-Ouazzou et al. 2011
Spathulenol	Abdenour Ait-Ouazzou et al. 2011, Milos Nikolic et al. 2013, Nacim Zouari et al. 2012
Caryophyllene oxide	Abdenour Ait-Ouazzou et al. 2011, Milos Nikolic et al. 2013, Nacim Zouari et al. 2012
Tau-cadinol	Abdenour Ait-Ouazzou et al. 2011
1-Octen-3-ol	Milos Nikolic et al. 2013, Dob T et al. 2006
β-Myrcene	Milos Nikolic et al. 2013, Nacim Zouari et al. 2012
δ3 -Carene	Milos Nikolic et al. 2013
trans- β -Ocimene	Milos Nikolic et al. 2013, Nacim Zouari et al. 2012

cis-Sabinene hydrate	Milos Nikolic et al. 2013, Dob T et al. 2006, Nacim Zouari et al. 2012
***α*- Terpinolene**	Milos Nikolic et al. 2013
cis-Chrysanthenol	Milos Nikolic et al. 2013
cis-Dihydrocarvone	Milos Nikolic et al. 2013, Dob T et al. 2006
Thymol methyl ether	Milos Nikolic et al. 2013, Nacim Zouari et al. 2012
Bornyl acetate	Milos Nikolic et al. 2013, Dob T et al. 2006, Nacim Zouari et al. 2012
Thymol acetate	Milos Nikolic et al. 2013
Carvacrol acetate	Milos Nikolic et al. 2013
trans-*γ*-Bisabolene	Milos Nikolic et al. 2013, Dob T et al. 2006
***α* -Guaiol**	Milos Nikolic et al. 2013
epi- *α* -Cadinol (t-cadinol)	Milos Nikolic et al. 2013
***α* -Eudesmol**	Milos Nikolic et al. 2013

Eudesm-3-en-6-ol	Milos Nikolic et al. 2013
(Z)-b-Ocimene	Dob T et al. 2006
(E)-b-Ocimene	Dob T et al. 2006
trans-Sabinene hydrate	Dob T et al. 2006, Nacim Zouari et al. 2012
Isomenthone	Dob T et al. 2006
Linalool acetate	Dob T et al. 2006
Eugenol	Dob T et al. 2006
Neryl acetate	Dob T et al. 2006
β **-Elemene**	Dob T et al. 2006, Nacim Zouari et al. 2012
β **-Cedrene**	Dob T et al. 2006
α **-Santalene**	Dob T et al. 2006
β **-Gurjunene**	Dob T et al. 2006

dehydro Aromadendrene	Dob T et al. 2006
***γ* -Gurjunene**	Dob T et al. 2006
epi-Cubebol	Dob T et al. 2006
Elemol	Dob T et al. 2006, Nacim Zouari et al. 2012
Ledol	Dob T et al. 2006, Nacim Zouari et al. 2012
Longiborneol	Dob T et al. 2006
Cedrol	Dob T et al. 2006
***α* -Bisabolol oxide B**	Dob T et al. 2006
cis-*α*-Atlantone	Dob T et al. 2006
trans-*α*-Atlantone	Dob T et al. 2006
Verbenene	Nacim Zouari et al. 2012
cis-Linalool oxide	Nacim Zouari et al. 2012

p-Menth-2-en-1-ol	Nacim Zouari et al. 2012
Campholenal	Nacim Zouari et al. 2012
Nopinone	Nacim Zouari et al. 2012
Pinocarveol	Nacim Zouari et al. 2012
p-Menth-4(8)-ene	Nacim Zouari et al. 2012
Pinocarvone	Nacim Zouari et al. 2012
4-Terpineol	Nacim Zouari et al. 2012
Myrtenal	Nacim Zouari et al. 2012
trans-Piperitol	Nacim Zouari et al. 2012
Verbenone	Nacim Zouari et al. 2012
trans-Carveol	Nacim Zouari et al. 2012
Cuminal	Nacim Zouari et al. 2012

Linalyl acetate	Nacim Zouari et al. 2012
Cuminol	Nacim Zouari et al. 2012
p-Mentha-1,4-dien-7-ol	Nacim Zouari et al. 2012
Terpenyl acetate	Nacim Zouari et al. 2012
Carvacryl acetate	Nacim Zouari et al. 2012
trans-Caryophyllene	Nacim Zouari et al. 2012
Eremophilene	Nacim Zouari et al. 2012
Palustrol	Nacim Zouari et al. 2012
1,6-Germacradien-5-ol	Nacim Zouari et al. 2012
Viridiflorol	Nacim Zouari et al. 2012
Ledol	Nacim Zouari et al. 2012

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