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STUDY OF BIO-INSECTICIDAL POWER OF TWO GENUS *RHUS TRIPARTITUM* AND *RHUS PENTAPHYLLA* OF FAMILY ANACARDIACEA IN WESTERN ALGERIA

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Abstract

The invasion of insect pests is causing significant losses on crops and agricultural land, for this reason locust control remains one of the major concerns in the strategy of protecting crops in arid and semi-arid regions. The insecticide used are generally chemical and toxic and they have harmful effects on human health and the environment, so we will proceed with biological control. Our work consists in studying the bio-insecticidal power of the essential oils of *Rhus Tripartitum* and *Rhus Pentaphylla* of the Anacardiaceae family in the region of Tlemcen by molecular modeling methods. Our work consists in studying the inhibition of the α-amylase enzyme of the species *Dociostaurus maroccanus* which is a digestive enzyme by molecular docking. Hexadecanoic acid, represents the best inhibitor of the α-amylase enzyme to disrupt the digestive system of califer *Dociostaurus maroccanus*. The molecular dynamics simulation study showed good result for the hexadecanoic acid is a fuctional inhibitor for the activity of α-amylaseenzyme. The result obtained confirms the bio-insecticide effect of the genus Rhus family of Anacardiaceae.

Key words: Bio-insecticide; Rhus Tripartitum; Rhus Pentaphylla, α-amylase; Molecular docking

Introduction

All over the world, several locust species are likely to cause signifiant damage to agronomic heritage. Due to favourable ecological conditions, these pests can be harmful when they proliferate and cause damage to crops through their gregarisms : among these pests the Moroccan locust *Dociostaurus maroccanus - Thumberg*, 1815(Bendjelloun *et al*., 2014.). The economic and ecological impact of locusts on crops and pastures has long been recognized.

The use of chemical pesticides to control these pests is a significant humanitarian and environmental crisis; that is why biological control gains our attention.

Aromatic plants have a substantial asset owing to the scalable discovery of the applications of their essential oils in health care as well as their purposes in other areas of economic interest. Their multiples uses mean that they are experiencing an increasing demand in global markets. The long-standing popularity of essential oils and aromatic plants in general remains related to their medicinal properties, in this case the anti-inflammatory, antiseptic, antiviral, antifungal, bactericidal, antitoxic, insecticide, invigorating, stimulating and calming properties (Ouhererre *et al*., 2018).

In terms of area and bioclimatic diversity, Algeria is known for its richness in aromatic and medicinal plants (Bekkal brikci, 2019, Bahareh *et al*., 2016). The use of aromatic plants spans a broad spectrum. The technique involves extracting components from the essential oils of these aromatic plants to control these pests.

In this study, we were focused on the interactions of molecules which underlie most biological mechanisms. My work consists of studying the inhibition of the enzyme α-amylase of the species Dociostaurus maroccanus. The enzyme selected for our study is a digestive hydrolase involved in power generation in insects. These enzymes are synthesized and secreted by the epithelial cells of the insects midgut and play an essential role in their digestion and survival. This enzyme was purified from the midgut

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of Dosciostaurus maroccanus (Hassan, 2009).

This inhibitory action is done by ligands from a series of plants in Anacardiaceae family, in order to produce a bio-insecticide. The following plant species were used: Rhus pentaphylla and Rhus tripartitum.

Throughout our work, we will conduct theoretical studies to find the most consistent complex (Enzyme-Ligand) to result in the best enzyme-amylase inhibitors in order to limit the population of Dociostaurus maroccanus.

Materials and Methods

Ligands and protein preparation

The ligands used in this work are selected based on the most important yield (performance) in each part of the plant (leaf and fruit) table 1.

Table 1: Molecules present in plant essential oils.

Plant species	Essential oils %
Rhus pentaphylla	-Spathulenol (14,9) -hexadecanoic acid (31,5)
Rhus tripartitum	Hydrolysable tannin

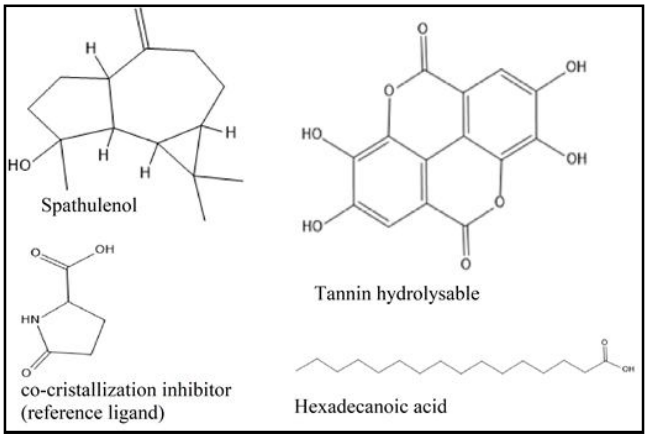

Fig. 1: Ligands Structure.

Table 2: Some properties of molecules that have been tested.

Ligands	Molecular weight (g/mol)	TPSA (Å²)
Spathulenol	220.35	20.2 (1: don, 1: acc)
Hexadecanoic acid	256.42	6.4 (1: don, 2: acc)
Hydrolysable tannin	1701.2	778(25: don, 46: acc)

The chemical structure of these ligands was achieved by the database "Pubchem" Fig. 1. We also drew the ligands with "Chemdraw" software version 12.0.2.1076, then we saved them in the format « *.Mol* », then we optimized the ligands with "Hypercham" software version 8.0.10 and saved them again in the format ". Mol" for molecular docking or molecular docking using the Molecular Operating Environment software called "MOE". In this study, the preparation of the enzyme was carried out from downloading the enzyme with the code 1CLV from "RCSB" or "PDB" (Protein Data Bank) with a resolution of 2Å table 3.

Molecular docking protocol

The working method we have adopted is based on molecular modeling. Docking essentially consists of two stages:

Molecular Docking is the selection stage of placing the ligand in the active site of the protein and sampling possible positions, conformations and orientations (poses) to have the most stable complexe (Mezhoud).

Scoring is the classification stage, which consists of

Table 3: Some enzyme properties.

Protein	Classifi-cation	Method	Resolu-tion	Chain Number	Chain Length
1CLV	HYDRO	X-Ray	2 A°	Two	503
	-LASE	diffraction		chains	

Source : www.RCSB.org

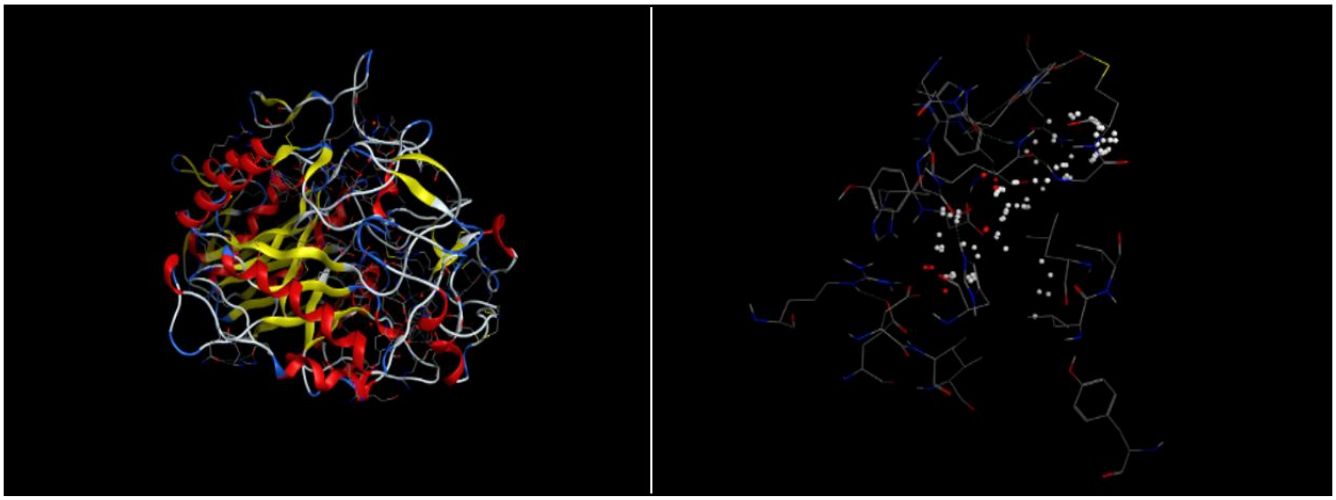

Fig. 2: The α-amylase enzyme 3-dimensional (3D) structure. The structure was taken from Protein Data Bank online server (https://www.rcsb.org/) and Molecular Operating Environment (MOE).

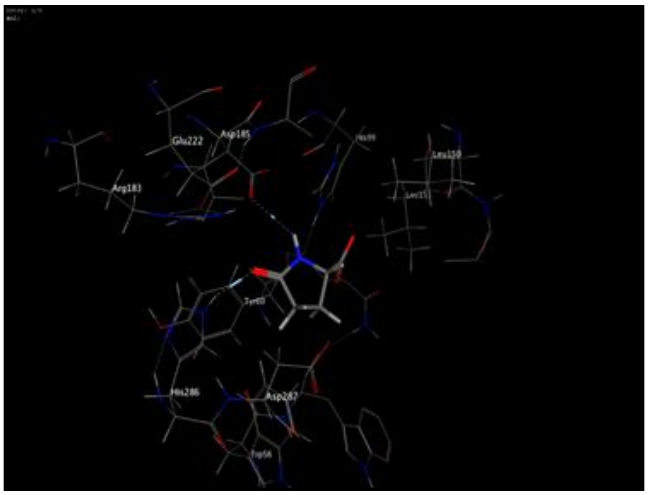

Fig. 3: 3D structure of active site enzyme.

assessing the affinity between the ligand and the protein and giving a score or binding poses achieved through the docking phase. This score helps us to keep only the best pose among all the one proposed (Nadhir *et al.,* 2010).

Molecular dynamics simulation:

The molecular dynamics simulation study was carried out for the ligand that was declared as the best among the selected molecules. From the analysis of the results, it was declared that, hexadecanoic acid was the best ligand among the selected ligands and molecules. The molecular dynamics simulations study of hexadecanoic acid and 1CLV docked complex was performed by iMODS. It is a fast, user-friendly and effective molecular dynamics simulation tool that can be used efficiently to investigate the structural dynamics of the protein complexes. It provides the values of deformability, B-factor (mobility profiles), eigenvalues, variance, co-varience map and elastic network. For a complex or protein, the deformability depends on the ability to deform at each of its amino acid residues. The eigenvalue has relation with the energy that is required to deform the given structure and the lower the eigenvalue, the easier the deformability of the complex.

Moreover, the eigenvalue also represents the motion stiffness of the protein complex. IMODS is a fast and easy server for determining and measuring the protein flexibility (Awan *et al.,* 2017; Prabhakar *et al.,* 2016; L_opez-Blanco *et al.,* 2014; Lop_ez-Blanco *et al.,* 2011).

Results and Discussion

Molecular docking simulation

In order to find the residues that form the largest active site of amino-acids on the enzyme to get an intercation with ligands, we used the "Finder site" module on the "MOE" software. It should be noted that the enzyme 1CLV contains 20 enzymatic cavities whose largest active site is made up of 21 amino acids that are:LEU 49-TRP 56-TRP 57-TYR 60-GLN 61-HIS 99-GLY 102-MET 103-LEU150-VAL151-LEU153-ARG183-ASP185-ALA186-GLU222-ILE224-HIS286 ASP287-ARG290- ASN331-ASP332.

After the enzyme and ligands preparation, the next stage is the positioning of these inhibitors in the active site of the 1CLV enzyme. This requires the docking module using the "MOE" software. Once the ligand-receptor complex is formed Fig. 4, it will adapt the most stable conformation, namely with the lowest energy level. Once the complex is formed we will analyze the results obtained.

In order to compare the stability of the ligands reviewed in the active site of the 1CLV enzyme, we conducted molecular docking between the enzyme and the ligands; results for each ligand are classified and cumulated on table 4.

Ligands are ranked in increasing order, with the lowest score with a better inhibitor.

The table above allows us to conclude that the highest score obtained after docking is that of hexadecanoic acid with a score of -5,7057 kcal/mol, followed by spathulenol with a score of -4,9324 kcal/mol and finally the hydrolysable tannins with a score of -4,5634 kcal/mol compared to the reference ligand with a score of -3,5513 kcal/mol.

Table 4: The docking results (binding energy) of all ligands and the controls along their respective number of hydrogen bonds as well as interacting amino acids.

Ligands	Score	Interaction with residues	Interaction type	Distance (A°)	Energy
hexadecanoic acid	-5,7057	/	/	/	/
Spathulenol	-4,9324	OD1-ASP185	H-donor	3,15	-1,9
hydrolysable tannin	-4,5634	NE2 – HIS286	H-acceptor	3,06	-3,3
Co-cristallisation ligand	-3,5513	OD1 – ASP185	H-donor	3,41	-0,7
		NE2 - HIS286	H-acceptor	3,04	-4,4
		ND1 – HIS99	ionic	3,87	-0,8
		NE2 – HIS99	ionic	3,23	-3,1
		NE2 - HIS99	ionic	3,83	-0,9

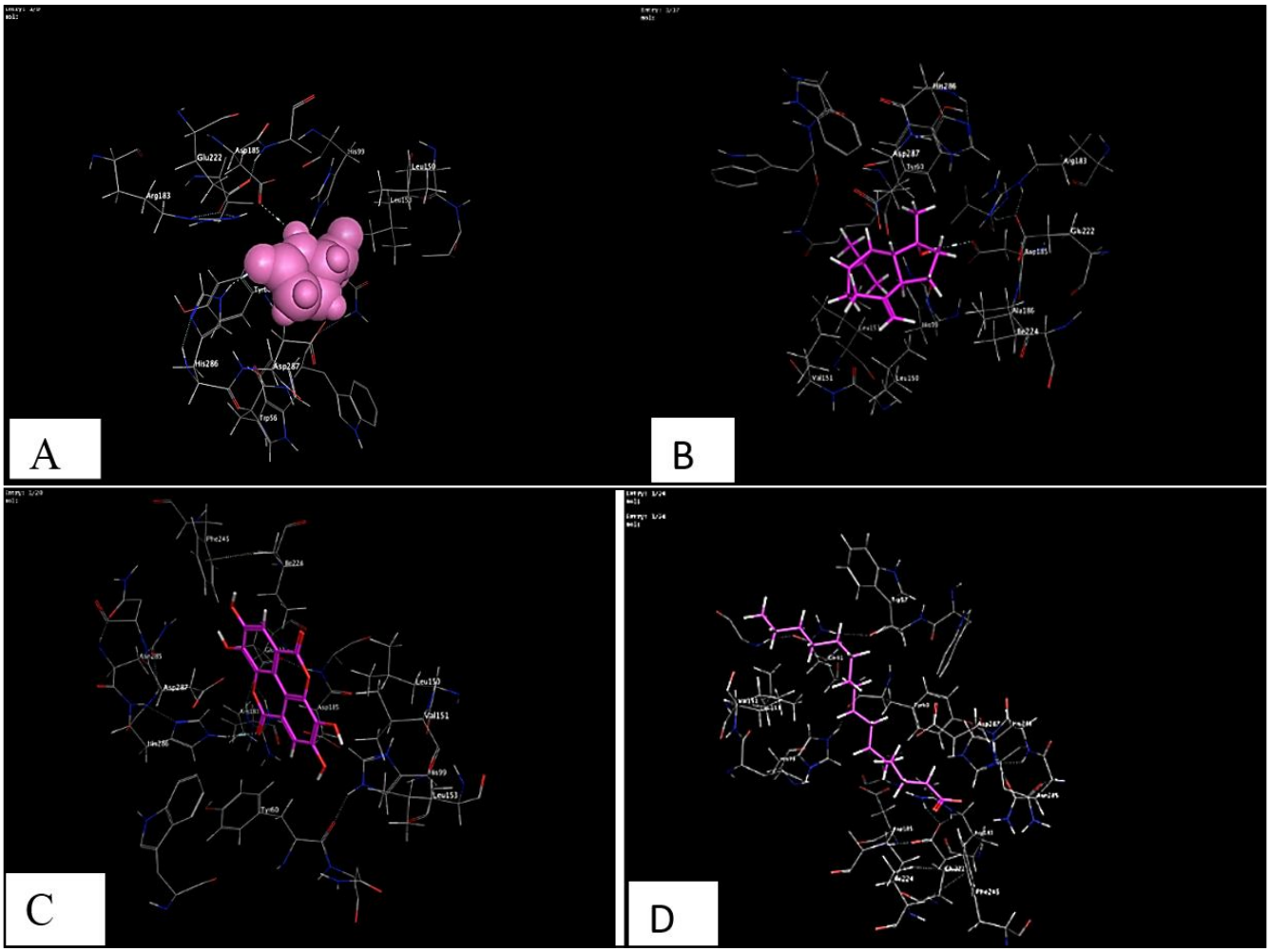

Fig. 4: 3D representations of the best pose interactions between the ligands and their receptor. A. interaction between co-cristallization ligand and 1CLV, B. interaction between Spathulenol and 1CLV, C. interaction betweentannin hydrolysable and 1CLV, D.interaction between hexadecanoic acid and 1CLV. The 3 D representations of the best pose interactions between the ligands and their respective receptors were visualized using Molecular Operating Environment (MOE).

Based on the results obtained, hexadecanoic acid is considered to be a better inhibitor for 1CLV by the post-docking score, however, other spathulenol ligands and hydrolysable tannins can be considered as good 1CLV inhibitors.

Enzyme-ligand interaction

Interactions between 2.5 and 3.1 are considered strong and interactions between 3.1 and 3.55 are assumed to be average. Interactions above 3.55 are weak or absent (Marianna *et al*., 2006). We have shown the interactions between the ligands and the enzyme Fig. 6.

This leads to the view that we have average interactions between the spathulenol and the active site of the α-amylase enzyme with a distance of 3.06A. This interaction is between OD1 and the amino acid ASP185 present in the enzymatic cavity of our enzyme with an energy of -1,9kcal/mol. On top of that, we noticed an interaction between the hydrolysable tannins and the active site of the enzyme at a distance of 3.06A and it is done between NE2 and the amino acid HIS 286.

Molecular dynamics simulation

The molecular dynamics simulation results are showed in the Fig. 5.

Fig. 5a illustrates the normal mode analysis (NMA) of Hexadecanoic acid- 1CLV complex. The deformability graph of the complex illustrates the peak in the graphs correspond to the regions in the protein with deformability Fig. 5b. The Bfactor graph of the complex gives easy visualization andunderstanding of the comparison between the NMA and the PDB field of the complex Fig. 5c. The eigenvalue of the complex is illustrated in Fig. 5d. The docked complex generated eigenvalue of 3.500969e-04. The variance graph indicates the individual variance by red colored bars and cumulative variance by green colored

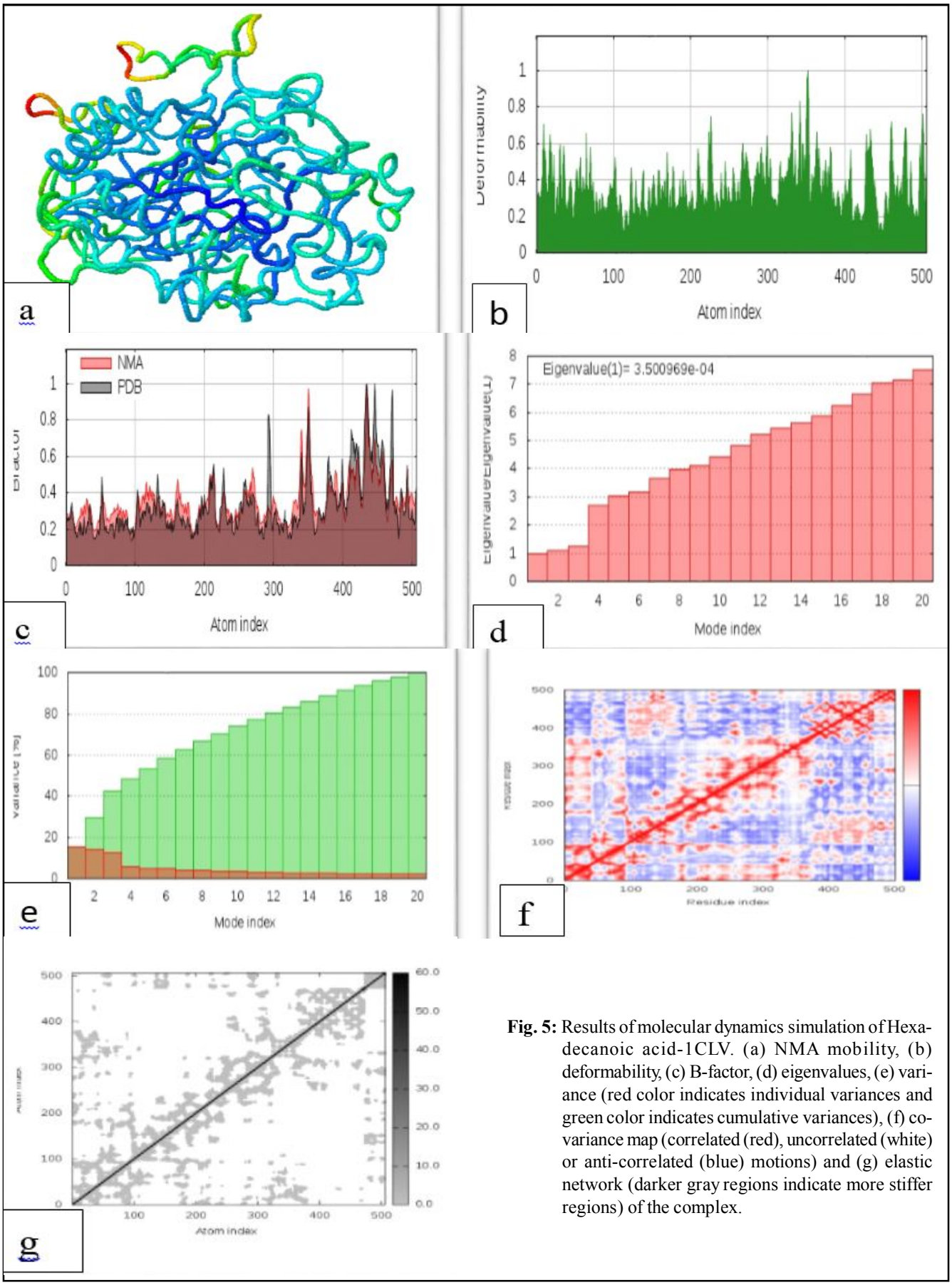

Fig. 5: Results of molecular dynamics simulation of Hexadecanoic acid-1CLV. (a) NMA mobility, (b) deformability, (c) B-factor, (d) eigenvalues, (e) variance (red color indicates individual variances and green color indicates cumulative variances), (f) covariance map (correlated (red), uncorrelated (white) or anti-correlated (blue) motions) and (g) elastic network (darker gray regions indicate more stiffer regions) of the complex.

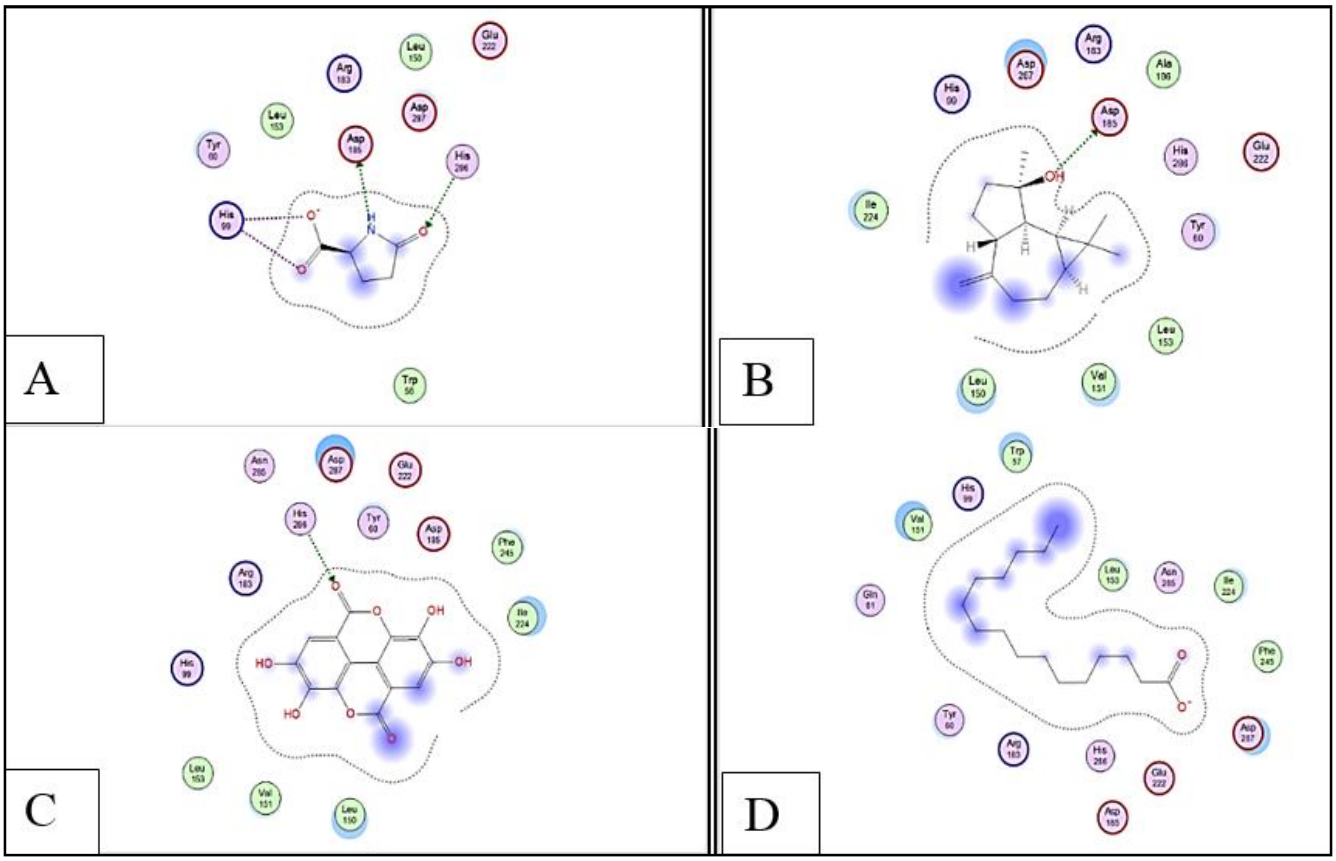

Fig. 6: 2D representations of the best pose interactions between the ligands and their receptor. A. interaction between co-cristallized ligand and 1CLV, B. interaction between Spathulenol and 1CLV, C. interaction between hydrolysable tannin and 1CLV, D. interaction between hexadecanoic acid and 1CLV. The 2 D representations of the best pose interactions between the ligands and their respective receptors were visualized using Molecular Operating Environment (MOE).

bars Fig. 5e. Fig5 fillustrates the co-variance map of the complexes where the correlated motion between a pair of residues is indicated by red color, uncorrelated motion is indicated by white color and anti-correlated motion is indicated by blue color. The elastic map of the complex shows the connection between the atoms and darker gray regions indicate stiffer regions Fig. 5g.

From the molecular dynamics study of hexadecanoic acid- 1CLV docked complex, it is clear that the complex had a very good amount of deformability Fig. 5b as well as it had low eigenvalue of 3.500969e-04, for this reason, this lower eigenvalue, represent the easier the deformability of the complex Fig. 5d and also represents the motion stiffness of the protein complex. However, the variance map showed high degree of cumulative variances than individual variances Fig. 5e. The covariance and elastic network map also produced satisfactory results Fig. 5f and 5g.

The damage caused by locusts poses a threat to the stability of agricultural areas. The damage and devastation attributed to these insects is as much a result of larvae activity as that of adults (Acheuk, 2012). Locust control remains a major concern in the strategy to protect crops in arid and semi-arid regions already subject to climatic variations. Among these, the chemical controlhas been the most exploited in decades: the insecticides used are usually neurotoxic. This means of control has pernicious effects on human health and the environment. Biological control remains the only way to eliminate them. Essential oils are, by definition, secondary metabolites produced by plants as a defence mechanism against Phytophagous pests (Cseke *et al.,* 1999).

More than 2000 plants species with insecticide properties have been identified (Regnault-Roger *et al.,* 1997). Therefore, the use of plant-based extracts as natural insecticides is of purely ecological interest and least harmful for the environment (Hamid Oudjana, 2017).

Several studies have highlighted the effectiveness of many essential oils applied as bio-insecticides. Among this work, we can mention : the power of essential oils of medicinal plants of *Pistacia lentiscus* and *Marrubium vulgaris* against agressive bio-agents of the olive tree, that *Pistacia lentiscus* plays a major role in biological control (Senouci, 2016). The toxicity study of extracts

from three acridifuge plants on fifth-stage larvae and adult *Schistocerca gregaria* (Ould el Hadj *et al.,* 2003).The study of the leaves *of Schinus molle*, which is a species belonging to the anacardiaceae family, causes nearly 73% mortality rate on adults of *Drosophila melanogaster*. This further suggests that anacardiaceae family has a bio-insecticide potency against insects.

Conclusion

In the present essay, we focused on the molecular interactions between the α-amylase enzyme of the digestive system of *Dosciostaurus marrocanus*. We theoretically tested the use of molecular modeling more precisely the molecular docking of the "MOE" software and Molecular Dynamics simulation; the inhibitory potency of ligands resulting from the extraction of essential oils from the "leaves and fruit" part of the following plants: *Rhus pentaphylla* and *Rhus tripartitum* that belong to the anacardiaceae family. As well, after having an overview of the disadvantages of chemical insecticides on human health and the environment ; based on previously achieved results, it can be concluded that hexadecanoic acid of the species *Rhus pentaphylla* has an inhibitory potency of the α-amylase enzyme to control *Dosciostaurus marrocanus* as a bio-insecticide and limit species outbreaks.

References

ACHEUK Fatma (2012). Evaluation des effets du Teflubenzuron et de l'extrait methanolique de la plante haplophyllum tuberculatum (Rutacee) sur le developpement et la reproduction du criquet migrateur :Locusta migratoria (Linne,1758) (Orthoptera, oeudipodinae). These doctorat en science agronomique. Ecole national superieure agronomique El harrach Alger.

Awan, F., A. Obaid, A. Ikram and H. Janjua (2017). Mutation-structure function relationship based integrated strategy reveals the potential impact of deleterious missense mutations in autophagy related proteins on hepatocellular carcinoma (HCC): A comprehensive informatics approach. *International Journal of Molecular Sciences,* **18(1):** 139. doi: 10.3390/ijms18010139.

Bahareh Rafiei, Mohammad Ghadamyari, Sohrab Imani, Vahid Hosseininaveh and Ali Ahadiyat (2016). Purification and characterization of α-amylase in Moroccan locust, Dociostaurus maroccanus Thunberg (Orthoptera: Acrididae) and its inhibition by inhibitors from *Phaseolus vulgaris* L., *Toxin Reviews,* **35:** 3-4, pp :90 97.

Bekkal brikci Sohayb (2019). Contrôle biologique du nematode des Citrus Tylenchulus*emipenetrans (Tylenchylidae) par des substances naturelles*. Memoire master en ecologie. Universite de Tlemcen.

Bendjelloun M., L. El ghadraoui, D. Essakhi J. Alfiguigui and ET N. Errabhi (2014). Contribution à l'etude de la diversite des orthopteres acridiens dans le Moyen Atlas (Maroc). Laboratoire EFE, FST, Universite Sidi Mohamed Ben Abdellah, Fes-Maroc. AFPP-Dixieme conference internationale sur les ravageurs en agriculture Montpellier -22 et 23 octobre 2014.

Cseke, L.J. and P.B. Kaufman (1999). *How and why these compounds are synthesized by plants*. Pages 37-90 in P.B. Kaufman, L.J. Cseke, S. Warber, J.A. Duke et H.L. Brielmann (eds.), Natural Products from Plants. CRC Press, Boca Raton, FL.

Hamid Oudjana Aicha (2017). *Cholinesterases et toxicite d'extraits de quelques plantes acridicides ou acridifuges chez Schistocerca gregaria (Forskål, 1775)*. These doctorat en science biologique. Universite KASDI Merbah Ourgla.

Hassan Amhamdi, Fatima Aouintil, Jean Paul Wathelet and Ali Elbachiri (2009). Chemical Composition of the Essential Oil of *Pistacia lentiscus* L. from Eastern Morocco. *Rec. Nat. Prod.,* **3:** 2. Pp:90- 95. karim Mezhoud.Docking Principes et methodes.

Lopez-Blanco, J.R., J.I. Garz_on and P. Chac_on (2011). iMod: Multipurpose normal mode analysis in internal coordinates. *Bioinformatics,* **27(20):** 2843–2850. doi:10.1093/bioinformatics/btr497.

Lopez-Blanco, J.R., J.I. Aliaga, E.S. Quintana-Ort_ý and P. Chac_on (2014). iMODS: Internal coordinates normal mode analysis server. *Nucleic Acids Research,* **42(W1):** W271–276. doi:10.1093/nar/gku339.

Marianna Usai, Giorgio Pintore, Bruno Tirillini and Mario Chessa (2006). Essential Oil Composition of Different Aerial Parts of *Pistacia terebinthus* L. Growing Wild in Sardinia. *Article in Journal of Essential Oil Research,* **18:** July/ August :383-385.

Nadhir Gourine, Isabelle Bombarda.

Mohamed Yousfi and Emile M. Gaydou (2010). Chemotypes of Pistacia atlantica Leaf Essential Oils from Algeria. *Natural product communication,* **5(1):** pp : 115-120.

Ouhererre Asmaa and Abidat Rabiaa (2018). *Caracterisation et l'effet de l'epoque de recolte sur la composition des huiles essentielles de Schinus molle L*. memoire de master en microbiologie applique.Universite DJILLALI BOUNNAMA Khmiss meliana.

Ould el Hadj M., A. Tankari Dan-Badjo, F. Halouane and S. Doumandji (2003). *Etude comparative de la toxicite des extraits de trois plantes acridifuges sur les larves du cinquieme stade et sur les adultes de Schistocerca gregaria Forskal, 1775 (Orthoptera-Cyrtacantha-cridinae). Courrier du Savoir,* **3:** pp :81-86. Annales de l'INRAT, n° 76, Tunisie:pp : 73-92.

Prabhakar, P.K., A. Srivastava, K.K. Rao and P.V. Balaji (2016). Monomerization alters the dynamics of the lid region inCampylobacter jejuni CstII: An MD simulation study. *Journal of Biomolecular Structure and Dynamics,* **34(4):**

778–791. doi:10.1080/ 07391102.2015.1054430.

Regnault-Roger, C. and A. Hhamraoui (1997). Control of phytophagous insects by aromatic plants and their allelochemical molecules, Ed Acta bot. Gallica, pp 401-412.

Senouci Hanane (2016). Antiparasitic and antifungal activities of essential oils and hydrolates of two medicinal plants Pistacia lentiscus and Marrubium vulgaris against the pests of the olive tree, Tlemcen, Abou Bekr Belkaid University.