FIRST REPORT ON THE BIOLOGICAL ACTIVITIES, MOLECULAR DOCKING AND STUDY OF THE TOXICITY OF TWO OLEORESINS AS WELL AS THEIR MAIN CONSTITUENTS

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ABSTRACT

The objective of this work was to evaluate the *in vitro* and *in silico* antibacterial, anti-inflammatory and antioxidant activities of two oleoresins; Myrrh and Pine resin used in the Algerian traditional pharmacopoeia. The antibacterial effect of oleoresins was evaluated by the agar diffusion test against three bacterial strains; *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923) and *P. aeuroginosa* (ATCC 27853). The antioxidant activity was assessed using DPPH method and the protein inhibition denaturation test was used to evaluate the anti-inflammatory efficacy. Resins main compounds were docked *in silico* against the bacterial tyrosyl-tRNA synthetase using the Autodock Tools 1.5.7 software. This study was carried out to determine their modes of binding with the active residues of this molecular target enzyme of antimicrobial agents. Molinspiration Cheminformatics and SwissADME online tools were used to predict physicochemical and pharmacokinetic parameters while OSIRIS Property Explorer online tools were used to predict toxicity risks. The results show that the Myrrh was effective against *E. coli* and *S. aureus* (17 mm) and that the Pine resin was similarly effective against *E. coli* (11 mm) and S. *aureus* (10 mm), but *P. aeruginosa* was completely resistant. The antioxidant test showed that both oleoresins had considerable ability to reduce the DPPH, with good IC_{50} of 0.49 ± 0.13 and 0.53 ± 0.06 mg/ml, respectively, compared to the BHT (0.89 ± 0.45 mg/ml). Both oleoresins had a remarkable anti-denaturation effects. The data of *in silico* studies revealed that all phytocompounds fit into the active pocket of the target enzyme and the binding energies ranged between -10.06 (Dehydroabietic acid) and -4.3 kcal/mol (D-glucuronic acid). The toxic and pharmacokinetic characteristics are, mostly, satisfying except for some compounds which have shown toxic effects, in particular Limonene, 4-allylanisole and Vanillin. We conclude that the extracts and their primary phytocompounds can enhance the antibacterial, antioxidant, and anti-inflammatory existing drugs without side effects.

Keywords: Biological activities, molecular docking, Myrrh, Pharmacokinetics, Pine resin, Toxicity.

INTRODUCTION

Recently, interesting effects, notably antiabacterial, antioxydant and antiinflammatory of natural substances (polyphenols, terpenes, alkaloids) have been proven by in-depth studies concluding that these compounds could be used for the prevention and treatment of different diseases and pathologies. Among the current health problems, there is antibiotic resistance, oxidative stress and free radicals as well as the side effects caused by existing anti-inflammatories in the pharmaceutical industry. On the other hand, medicinal herbs are rich treasure troves of many natural bioactive compounds by synthesizing diversity of secondary metabolites which prominently function to protect plants against predators and microbes [1]. Oleoresin is a product of the secondary metabolism of plants, it defends the plant against animals, fungi, and bacteria[2], [3]. Oleoresin is a complex mixture of turpentine (volatile fraction), and rosin (non-volatile fraction) [4], it contains mainlyditerpenes, sesquiterpenes [3], resin acids [5] and phenolic compounds that are determinant for their potential for application [6]. Myrrh is an aromatic gum resin [7], it is the exudates created by the bark of trees in the genus *Commiphora*, particularly *C. myrrha* [8] which is grown in East Africa, Saudi Arabia, and India [9]. This resin produces the characteristic odour, ranging in colour from yellowish-brown to reddish-brown and it comprises 3-8% essential oil, 30-60% water-soluble, and 25-40% alcohol-soluble components [10]. It is one of the most known natural antimicrobial agents [7], [11] mainly due to its rich composition of tannins, flavonoids, alkaloids, glycosides, steroids, saponins, and terpénoïdes [12]. Moreover, Myrrh contains many active ingredients with strong anti-inflammatory effects, among which myrrh steroid, guggulsterone [13] and antioxidant effects [8], [9]. Myrrh has been shown to be potent antiseptic, antioxidant, and anti-inflammatory natural substance because of its furanosesquiterpenes, b-sitosterol and alcohol-soluble resin constituents [14]**.** The genus *Pinus*, belonging to the conifer family [15] has its gravity center in Northern Africa, mainly in Algeria and Tunisia where it constitutes the most important massive [16] occupying 880 000 ha in Algeria [17]. The raw pine resin combines ease extraction, low cost and renewable source [18]. The advantage of this resin is that the user does not run the risk of confusing it with other products (when buying) or with other plants (during harvesting) in addition of the ease with which the resin is used as well as the sensation approved by the patients [17]. This resin is composed of 15% turpentine[15], a complex mixture of mono and sesquiterpenes, and a non-volatile rosin fraction, whose major components are diterpenes [19], it acts as a natural biocide, protecting the whole plant against xylophagous agents like insects and bacteria [18]. In traditional phytotherapy, Pine resin is used for the treatment of muscular pains, as a disinfectant of the respiratory and urinary tracts and as antifungal in North Africa [20] anti-inflammatory [21]or as a natural remedy for tiredness and used as anti-aging, anti-inflammatory, antineoplastic, antibacterial, immunomodulating, related to their effects on the activity of cyclo-oxygenase, and anticancer [16]. In Algeria, this Pine is used for external use, in the prevention and treatment of respiratory infectious diseases, fungal infections and for internal use against respiratory diseases or as suppositories against diseases of the urinary system [17]. Tyrosyl-tRNA synthetases (TyrRSs) are essential enzymes for all living organisms [22] so that they are ideal targets for the development of new antibacterial agents. They play a critical role in protein biosynthesis [23], more specifically, they are important for the correct linkage of amino acids to cognate tRNA in order to maintain the fidelity of protein synthesis [24] and thus are essential for cell viability and growth [23]. This work studies, *in vitro*, some biological properties of two resins widely used in traditional medicine in Algeria in addition to the *in silico* study of each component and its toxicity. To our knowledge, no such study has been found dealing with these bioactivities.

MATERIAL AND METHODS

Myrrh, called in the local dialect “Mor we sbor” and Pine resin, locally called “Elk snouber” are obtained from a local herbalist. Myrrh is in the form of dark brown or even black crystals while Pine resin is yellow (**Figure 1**), they are then ground into a powder.

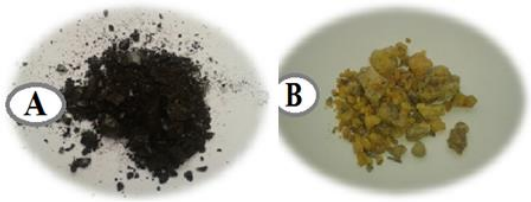

Figure 1: Photo of the drugs; A: Myrrh, B: Pine resin

In vitro antibacterial activity:

The antibacterial activity of the resins was evaluated by the Disk diffusion method on three bacterial strains from the American Type Culture Collection (ATCC): *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 25923. Six millimeters diameter sterile filter paper, impregnated with 20 µl of the oleoresin dilutions in DMSO (dimethyl sulfoxide) at a rate of 200, 150, 86 and 50mg/ml, are deposited on a pre-inoculated Mueller-Hinton (MHA) agar with an inoculum standardized to ≈10^8

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cells/ml then incubated at 37°C for 24 hours and the diameters of the zones of inhibition are measured around each disk [25]. Gentamicin (10 µg/disk) is used as positive control. To find out whether the effect of des is bactericidal or bacteriostatic, a sample from the zone of inhibition is inoculated in MHA, then incubated and examined with the naked eye. Bacterial growth indicates a bacteriostatic effect, while an absence of growth indicates a bactericidal effect.

Antioxidant activity:

It is based on reduction of the violet DPPH (2,2-diphenyl-1-picrylhydrazyl) radical by the antioxidant via a hydrogen atom transfer mechanism to cause a change in the colour to stable yellow DPPH molecules. The remaining violet DPPH radical is measured by a UV-Vis spectrophotometer nm to determine the antioxidant activity. Practically, a volume of 25µl of different concentrations of resins is mixed with 625µl of a methanolic solution of DPPH at 0.004%. Absorbance is measured at 517 nm after 30 min of incubation in the dark [26]. Butylated hydroxytoluene (BHT) is used as a positive control. The ability to scavenge the DPPH radical is calculated as follows:

$$\%\ \textbf{of DPPH scavenging effect} = \frac{(At - Ac)}{Ac}$$

Where: At represents the absorbance of the test and Ac represents the absorbance of the reference.

Antiinflammatory activity:

This test was done according to the method of [27]. A volume of 500 µl (1%) bovine serum albumin was added to 100 µl of resin solution (250, 500, and 1000 µg/ml). This mixture was kept at room temperature for 10 minutes, followed by heating at 51°C for 20 minutes. The resulting solution was cooled down to room temperature and absorbance was recorded at 660 nm. Acetyl salicylic acid was used as a positive control and percent inhibition for protein denaturation was calculated as follows:

$$\%\ Inhibition = \left(100 - \frac{A1 - A2}{A0}\right) x\ 100$$

Where A1 is the absorbance of the sample, A2 is the absorbance of the product control and A0 is the absorbance of the positive control.

GraphPad Prism 5 was used to create the graphs for the *in vitro* activities.

***In silico* study**

The selection of the phytocompounds:

The phytoconstituents that were predicted *in silico* were chosen based on extensive literature on the chemical composition of these resins (**Table a**). We proceeded to a virtual screening of the major components of the resins each separately to get an idea of the compound or compounds responsible for the activities using PubMed, Science direct, Scopus, Springer link, J-STORE and other databases were investigated.

Table a: The main compounds of the two resins according to the literature.

Resin	Phytocompound	Reference
Myrrh	4-methyl-glucuronogalactone ,furanoeudesma-1,3-diene, transβ-ocimene, D-glucuronic acid	[70]
	Furanoeudesma-1,3-dien, Curzerene	[71]
	(E)-guggulsterone, (Z)-guggulsterone, curzerenone, furanoeudesma-1,3-diene, myrrhone	[54]
Pine resin	Caffeic acid, Vanillin, M-methoxymandelic acid oumethyl ester of salicylic acid, 3,5-Bis(1,1-dimethylethyl) catechol	[46]
	α-Pinene, β-pinene α-Pinene, β-pinene, abietic acid, limonene, pimaric acid, dehydroabietic acid α-Pinene, β-pinene, limonene α-Pinene, β-pinene, abietic acid, limonene, pimaric acid, abietic acid, and neoabietic acid	[72] [4] [73] [5]
	α-Pinene, β-pinene, -terpinolene, delta 3 carene	[74]
	α-Pinene, β-pinene, limonene, 4-allylanisole, pimaric acid, dehydroabietic acid, abietic acid, and neoabietic acid	[35]

***In silico* antibacterial activity:**

The molecular interaction of the compounds was studied against the three-dimensional structure bacterial enzyme named tyrosyl-tRNA synthetase TyrRS (PDB code 1JII) which is responsible of the aminoacylation reaction. The main compounds differing in their structure and functional groups (**Figure 2**) were evaluated for their *in silico* activity in inhibition of this enzyme. In order to investigate the binding pose of those *in vitro* potent extracts, constituents were compared to gentamicin (a standard antibiotic). The two-dimensional structures (SDF format) of the tested compounds and the chemical identifier (CID) of the 3-D structure of the phytocompounds and the gentamicin were obtained from PubChem database.

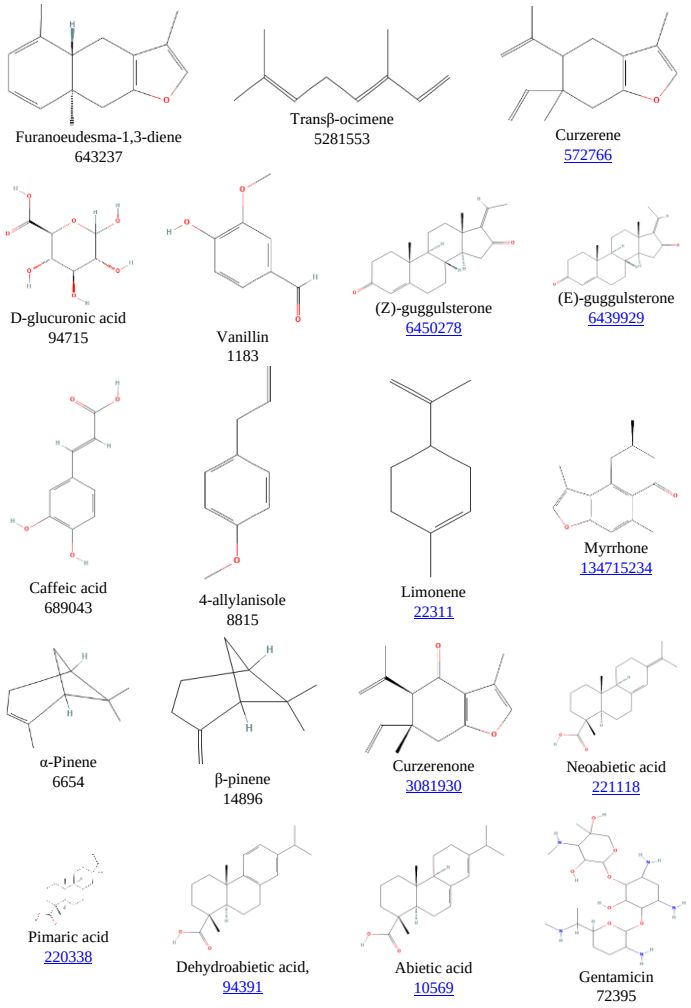


Figure 2: The 2d structures of the tested compounds.

Docking analysis and protein preparation:

To predict the affinity of each compound to the binding site of the bacterial TyrRS, AutoDock Tools 1.5.7 software was used. It is based on gradient optimization method in its local optimization process to rank the ligands based on empirical binding free energy (DG in kcal/mol) function [28]. The PDB files construction of the enzymes as target were acquired from Protein Data Bank (PDB) and used as a static structure (**Figure 3**). Water molecules were deletes, the polar hydrogen and Kollman charges were added to the protein residues and the natural ligand was removed, the natural ligand was removed and the PDBQT format files were prepared. Lamarckian Genetic was utilized as the docking algorithm with 10 runs. The SDF files of tested compounds were converted to PDB file using open Babel 2.4.1 software. Two-dimensional interactions were visualized using Discovery Studio Visualizer v.16.1.0.15350 and graphs were drawn using GraphPad Prism 5 software version 5.03. The ligands are represented in different colours, H-bonds (distance range A˚) and the interacting residues are represented in ball and line model representation.

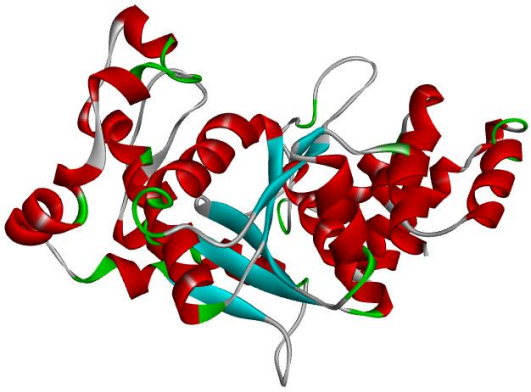


Figure 3: The crystal structure of the TyrRS (1JII).

Drug likeness, ADME and toxicity prediction:

Lipinski's rule was used to evaluate the drug-likeness property of phytocompounds, it defines four simple physicochemical parameter ranges [29]. In general, an orally active drug fulfill the following criteria: Not more than 5 hydrogen bond donors (n-OH and n-NH), not more than 10 hydrogen bond acceptors (n-ONs), molecular weight (MW) less than 500 D, not more than one violation and octanol-water partition coefficient (milogP) should be not more than 5 [30]. Molinspiration Cheminformatics and SwissADME online tools were used to predict physicochemical and pharmacokinetic parameters while OSIRIS Property Explorer online tools were used to predict toxicity risks.

RESULTS

Antibacterial activity:

The results show that *E. coli* and *S. aureus* are very susceptible to the two oleoresins notably at 200mg/ml with 17 and 11 mm by myrrh extract and 10 to 17 mm by pine resin extract respectively (**Table b, Figure 4**). On the other hand, *P. aeruginosa* is completely resistant with no inhibition zones.

Table b: The inhibition zones of the two oleoresins against the tested bacteria.

	Myrrh				Pine resin				GM
Concentration (mg/ml)	200	150	86	50	200	150	86	50	10µg
E. coli	17 ± 1	12± 1.41	12± 00	10± 00	11,3 ±1	11.5± 0,7	11.66+/-	11 +/-	40
S. aureus	17±0	13	7±0	-	10 ± 1	10	15 +/-	12,6 +/-	40
P. aeruginosa	-	-	-	-	-	-	-	-	27

n=3±SD, (-): no activity, +/-: decrease in bacterial load. GM: Gentamicin

After sub culturing from the zones of inhibition, there was development of the two strains, which shows that the activity was bacteriostatic by the two resins.

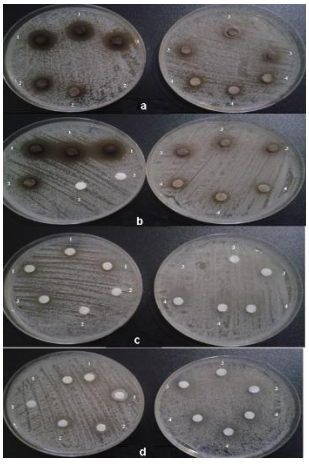


Figure 4: Zones of inhibition; a: Myrrh vs *S. aureus*, b: Myrrh vs *E. coli*, c: pine resin vs S. *aureus*, d: Pine resin vs *E. coli*. 1(50 mg/ml), 2(86 mg/ml), 3(150 mg/ml), 4 (200mg/ml).

Antioxidant activity:

The antioxidant activity of the extracts was evaluated *in vitro* by the DPPH free radical reduction method. The results obtained from the two oleoresins are presented with the reference molecule (BHT) by the curves representing the percentages of inhibition as a function of the concentrations. Pine resin and Myrrh are most active with the lowest (**Figure 5**). IC_{50} values when compared with that of BHT.

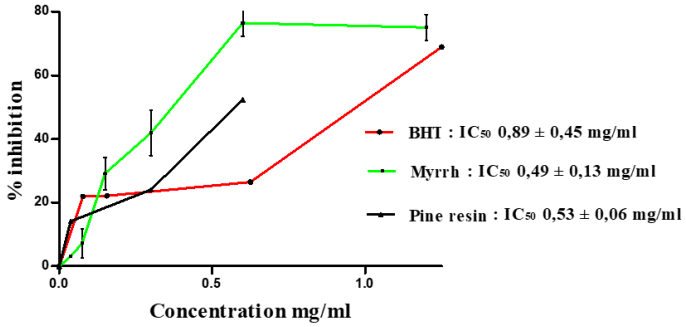


Figure 5: The antioxidant activity curve of the two oleoresins compared to that of the reference (n=3±SD)

Antiinflammatory activity:

The anti-inflammatory activity of the two oleoresins was evaluated *in vitro* by the protein denaturation inhibition method. Pine resin exhibits a remarkable dose-dependent anti-inflammatory effect, higher than that of salicylic acid, where increasing the concentration of Pine resin solution increased the percentage inhibition of protein denaturation (**Figure 6**). However, Myrrh has a lower anti-inflammatory activity inversely proportional to the concentration.

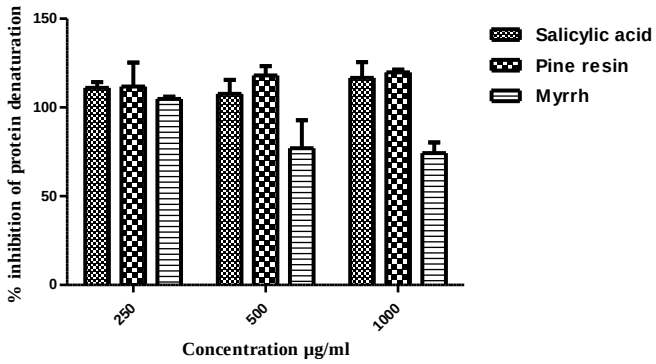


Figure 6: The antiinflammatory activity of Myrrh and that of pine resin (n=3 ±SD).

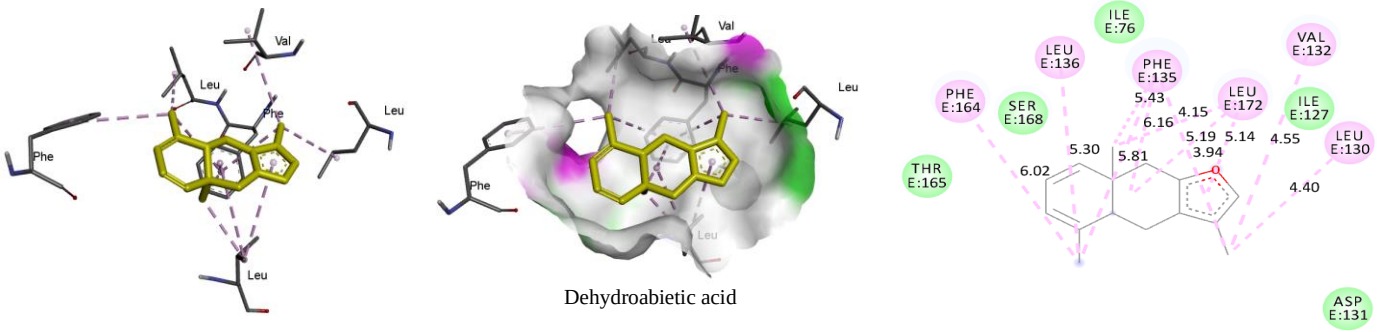
Molecular docking:

It is found that all the molecules integrate at the active site of the enzyme but with different scoring. **Table c** shows the molecular docking parameters of the phytoconstituents with the TyrRS. The Dehydroabietic acid has the best binding energy (-10.06 kcal/mol) which is much higher than that of gentamicin (-5.59 kcal/mol). The D-glucuronic acid is the most weakly integrated with -4.3 kcal/mol.

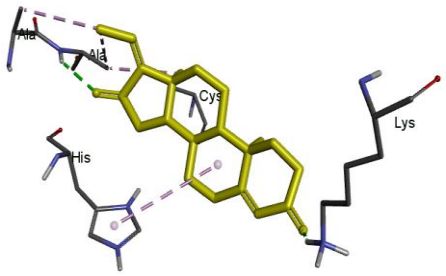
Table c: Phytocompounds scoring results

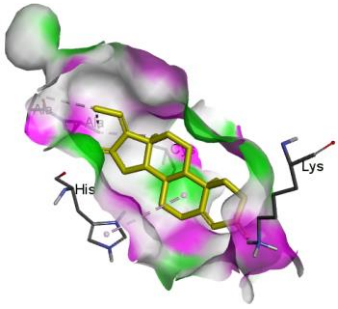
Compound	Best run	Free energy of binding (kcal/mol)	Inhibition Constant, Ki (uM)	vdW + Hbond + desolv Energy (kcal/mol)	Amino acids involved in the active site
Dehydroabietic acid	9	-10.06	42.01	-10.96	Pi-Alkyl :Phe 164, Phe 135, Leu 136, 172,130, Val 132. Vdw : Thr 165, Ser 168, Ile76,127
(Z)-guggulsterone	8	-8.17	1.02	-7.85	**H-bond**: Ala 51Lys 82 Pi alkyl: Ala 50, His 48, Cys 35. Vdw; Gly 192, 36, 47Asp 38,78, 194, Ile52, Leu 49, Phe37,Thr40, Gln 195
(E)-guggulsterone	5	-7.99	1.38	-7.94	**H-bond:** Ile 239, Pi alkyl: Phe231, His 45 Vdw: Thr238, Ile46, Gly 47, 232, 192, Val 223, Ser 193Leu222, Lys 233, 230
Abietic acid	3	-7.7	2.25	-8.06	**H-bond**: Thr200, Tyr147 Pi-Alkyl: Ala215, Ile206, Leu203, Arg207 Vdw, Arg214,Glu204, Lys151, Thr17Ala201
Neoabietic acid	3	-7.34	4.20	-6.25	**H-bond**: Ala215, Arg 214 Pi-Alkyl: Arg207, Leu203. Vdw:Asp18, Thr17,200, Trp196, Glu204
Myrrhone	2	-6.86	9.36	-6.74	Pi-Alkyl: Leu68,Cys35, Tyr169 Vdw: Thr73,Tyr34,Gln173,189,195, Asn128, Asp78, 38,176,Gly 189,191,192,Ile190,Phe37 . Pi-Sigma Gly 36
Pimaric acid	4	-6.84	9.65	-6.08	Pi-Alkyl: Phe164, 167, Met 148, 171, Leu 136 Vdw: Lys 141, Phe 135, 143, Gly 140, Ser 168
Furanoeudesma-1,3-diene	5	-6.26	25.98	-6.26	Pi-Alkyl: Phe 164, 135, Leu 136, 172, 130, Val 132. Vdw: Thr 165, Leu 136, 172,130, Val 132
Curzerene	4	-5.99	40.63	-6.46	**H-bond**: Val 223 Pi-Alkyl: Ala51, 50, Ile220 Vdw: His 48, Ser 193, Phe 231,Val 223
Curzerenone	3	-5.97	41.96	-6.59	Pi-Alkyl: Val 132, Leu 172,75,130, Ile76, Phe 135,Vdw : 131, Pi-Sigma :Ile127
Caffeic acid	9	-5.68	69.04	-5.13	**H-bond**: Ala 215, Arg 214, 207, Glu 204 Vdw: Thr 200, Ile 206 Amide: Leu 203
Gentamicin	6	-5.59	80.41	-5.24	**H-bond**: Glu 300, 301,309, Thr 297 Vdw: Lys 296, 304
α-Pinene	4	-5.26	139.33	-5.26	Pi-Alkyl: Tyr 266, Phe 254, 231, Lys 225 Vdw: Asp 262, Trp 255, Asn 257
β-pinene	9	-5.08	189.01	-5.06	Pi-Alkyl: Trp 255, Lys 225, Tyr 266 Vdw: Asp 262, 259, Thr 258,Asn 257, Phe 231, Pi-Sigma: Phe 254
Limonene	2	-5.07	193.60	-5.35	Pi-Alkyl: Leu 68, Tyr 34, Vdw: Phe 37
4-allylanisole	5	-4.5	505.54	-5.41	Pi-Alkyl: Leu 68,Vdw : Gln 173, Tyr 34, Phe 37, CH bond: Gln 189, Ile 190
Vanillin	8	-4.48	3.05	-4.20	**H-bond**: Gly 232, Lys 233, Asp 259, Asp 262 Vdw: Thr 234, Lys 230, Tyr 266,Pi-cation: Lys 225 (connecting the ring of the ligand to the positive charge NH_3^+ of LYS 225)
Transβ-ocimene	2	-4.42	572.36	-5.32	Pi-Alkyl: Cys35, Leu 68 Vdw: Tyr 34, Phe 37, Ile 190
D-glucuronic acid	4	-4.3	708.88	-4.23	**H-bond**: Ala 215, Arg 214, Glu 204 Vdw: Leu 203, Arg 207, Thr 200

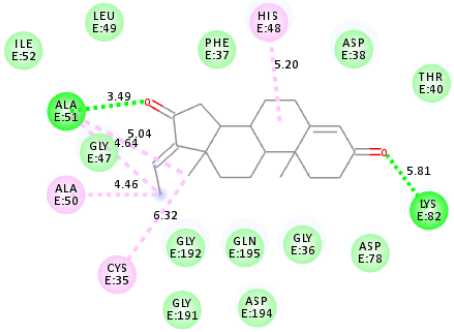
The interactions, in the binding site of TyrRS with the molecular surface around the studied ligands, show the electron donor region as a pink area and the green area represents the electron acceptor region. The 2d structures show the amino acids involved in the active site of the enzyme as well as the nature and the distances of the bonds established (**Figure 7**).



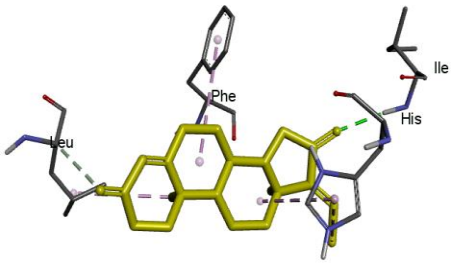
Dehydroabietic acid

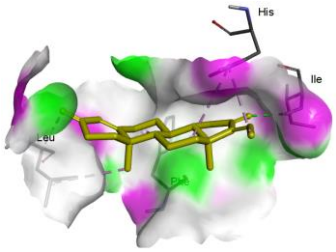


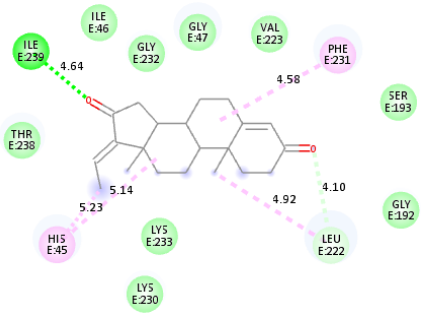




(Z)-guggulsterone







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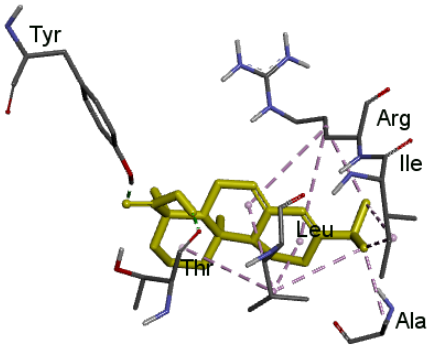
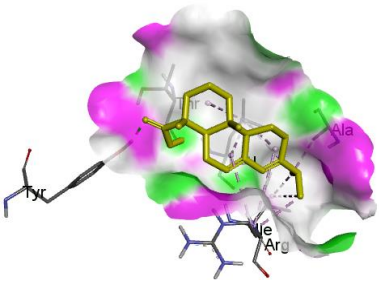
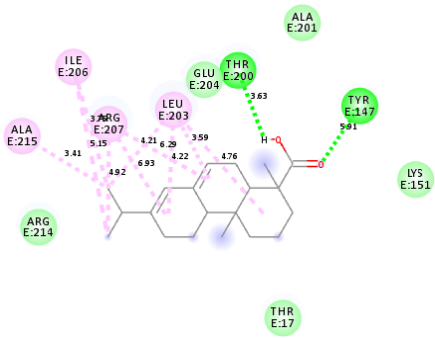
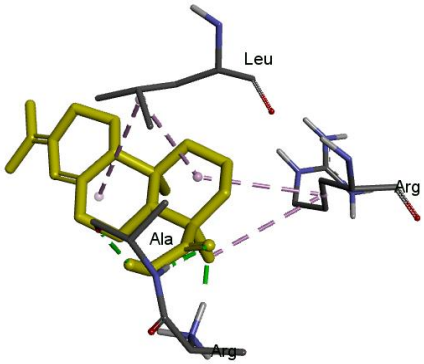
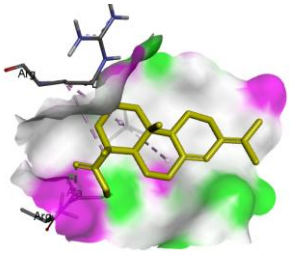


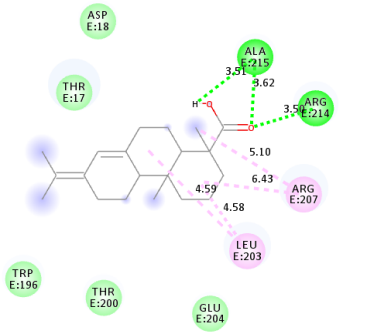




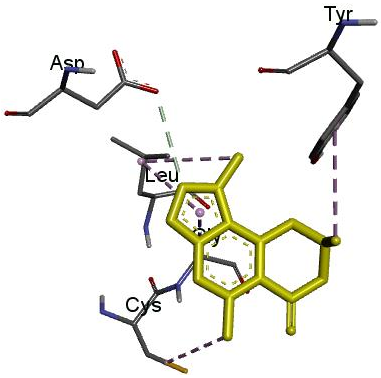
Abietic acid

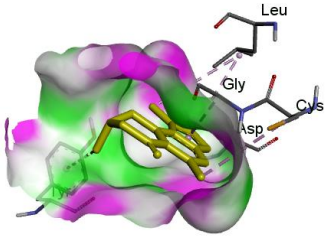


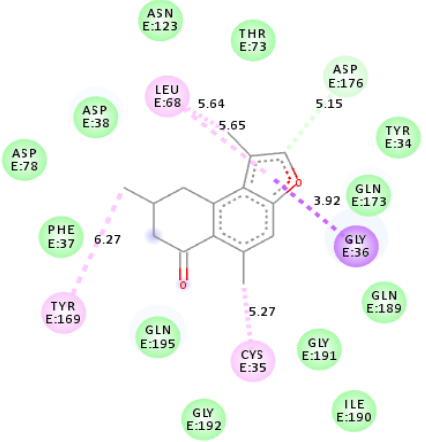




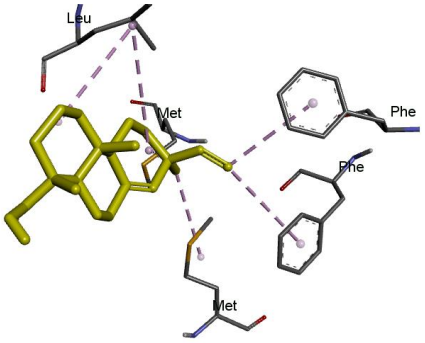
Neoabietic acid

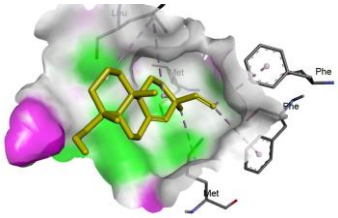




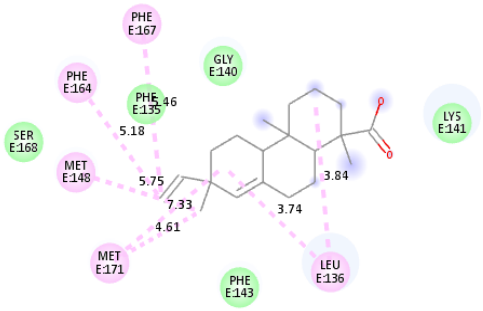


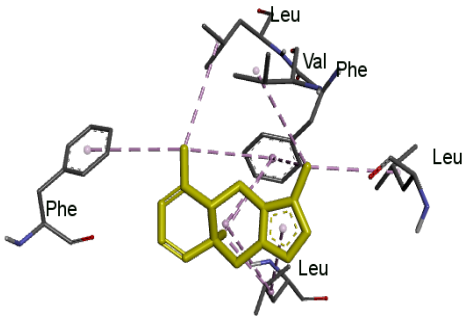
Myrrhone

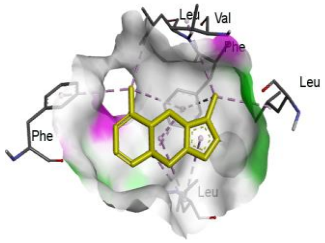




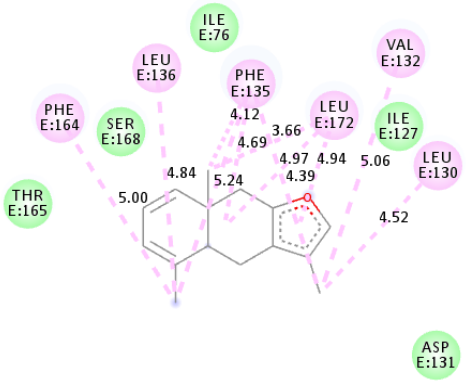
Pimaric acid

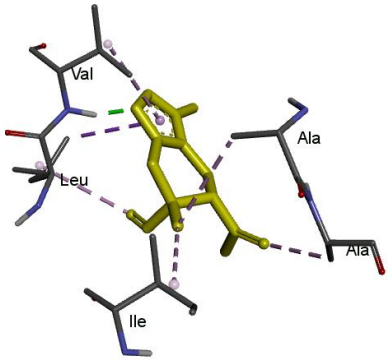


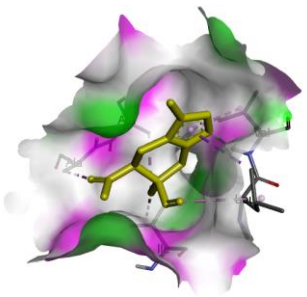




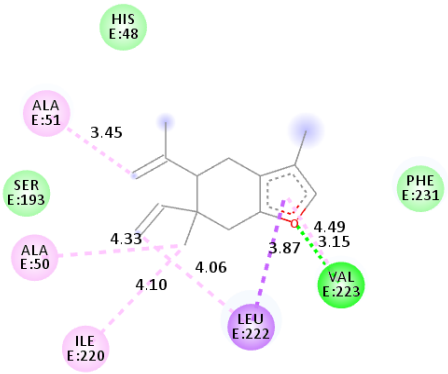
Furanoeudesma-1,3-diene

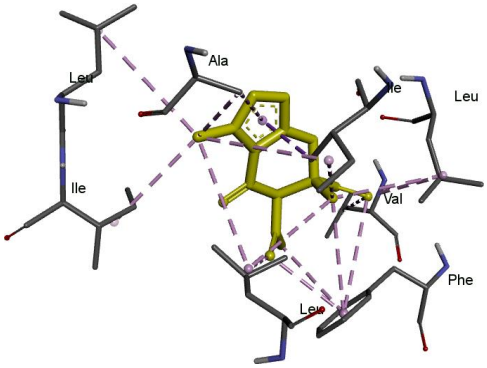


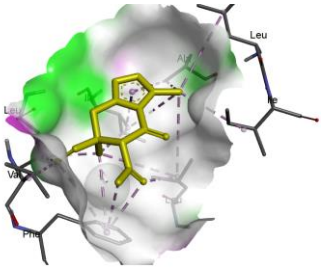




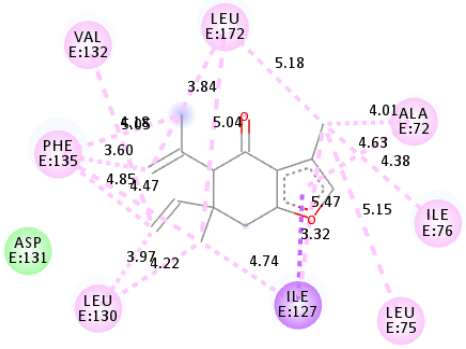
Curzerene

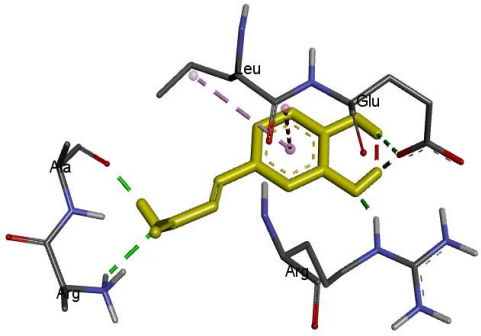


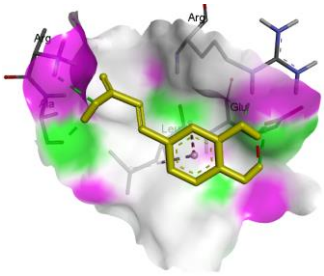




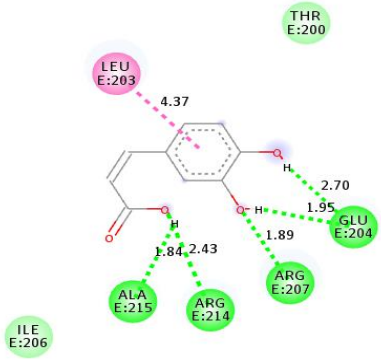
Curzerenone

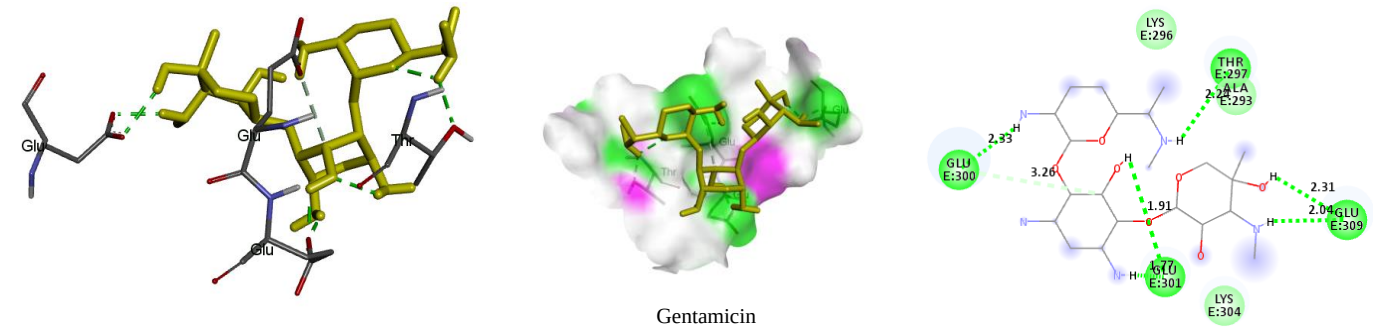




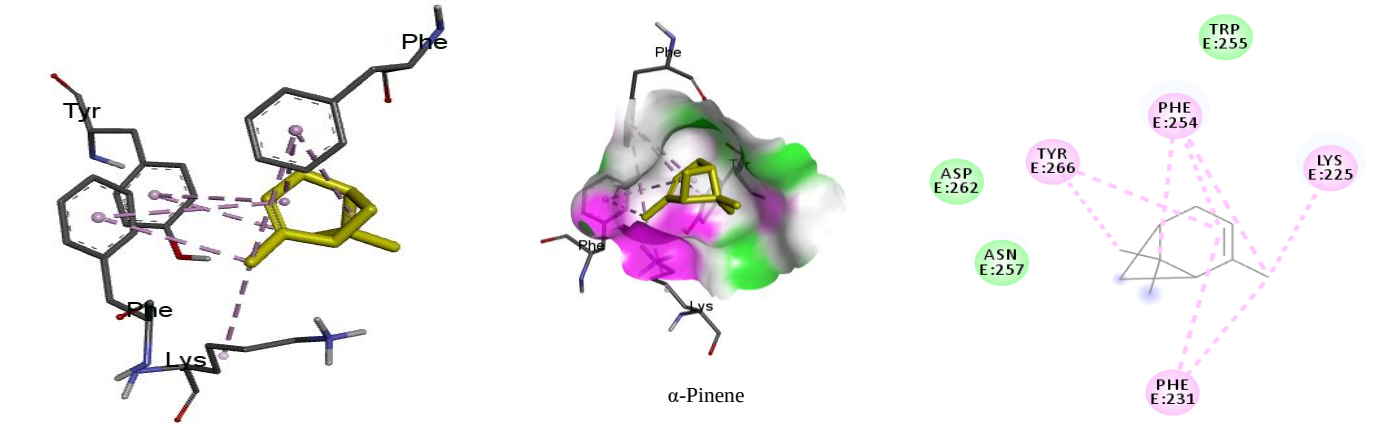


Caffeic acid

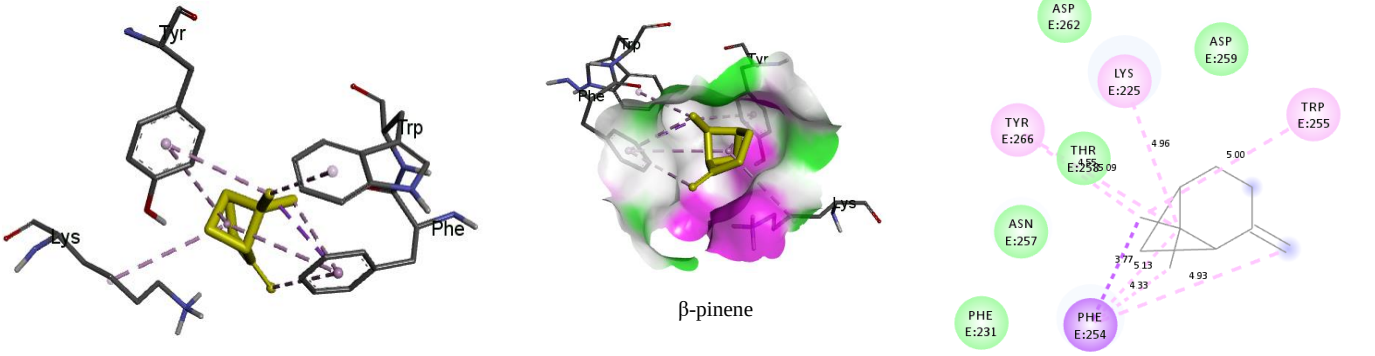




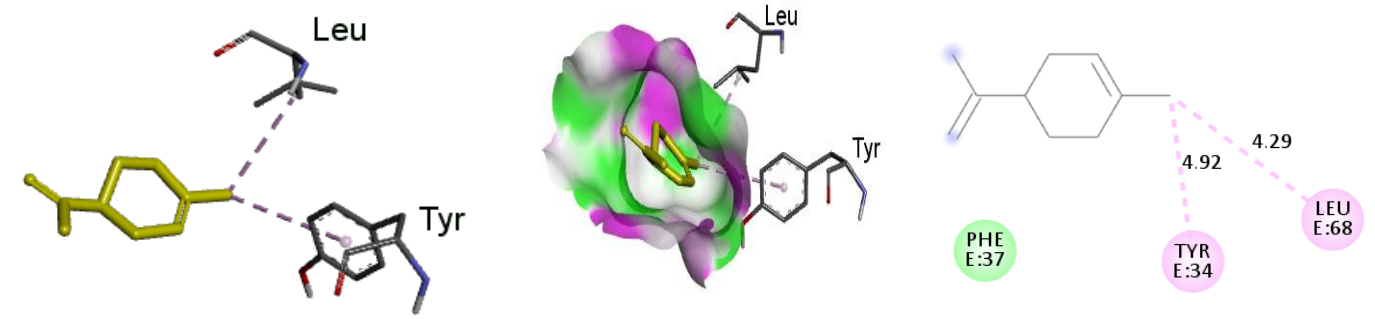
Gentamicin



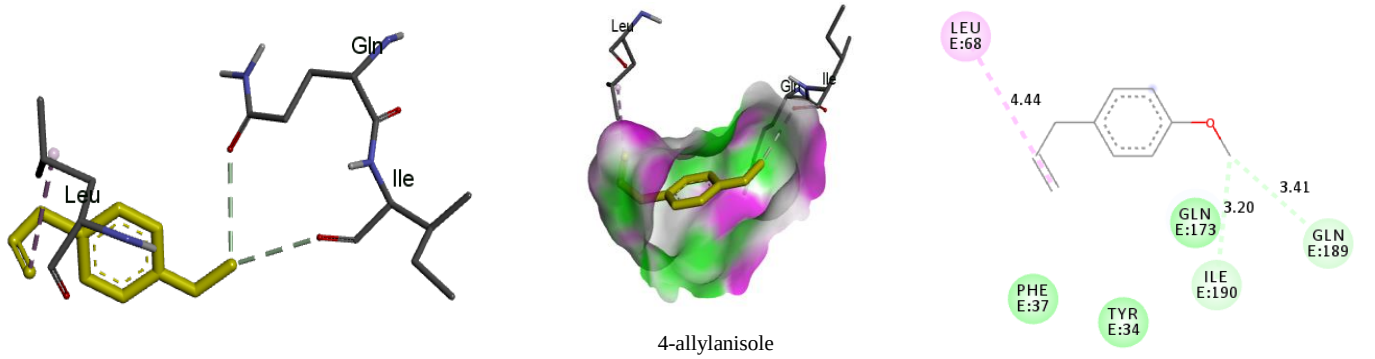
α-Pinene



β-pinene



Limonene



4-allylanisole

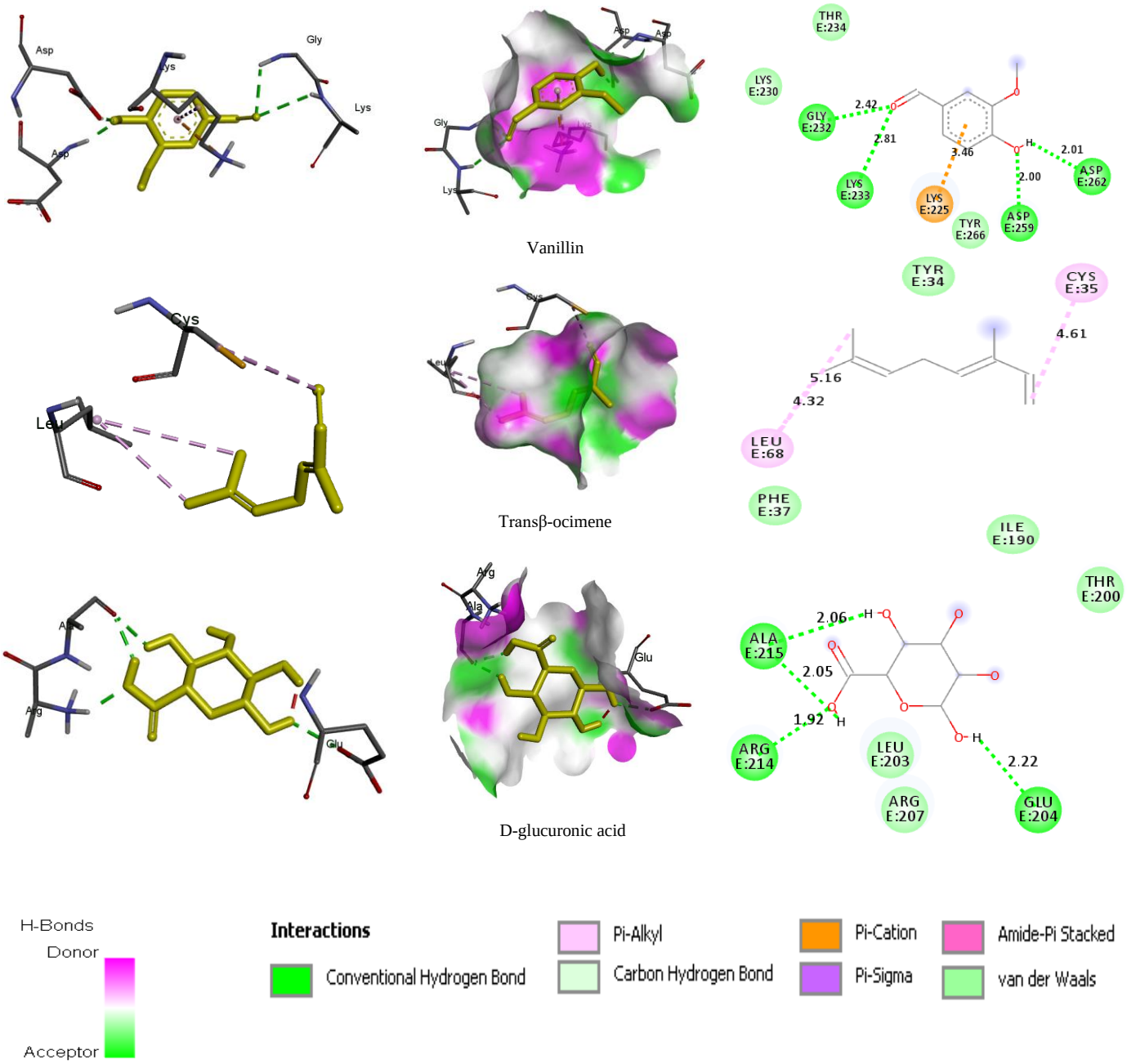


Figure 7: Interaction and bond distances of ligands inside the active site pocket as shown by molecular surface maps.

Drug likeness and toxicity prediction:

The drug likeness filters allow the early preclinical development of drugs by avoiding costly late step preclinical and clinical failure [28]. The drug-likeness properties of the phytocompounds were analysed using the SWISS ADME online tool and found that most compounds followed Lipinski's rule of five, similar to that of gentamicin. Similarly for toxicity, some compounds have toxic effects, in particular Limonene, 4-allylanisole and Vanillin (**Table d**).

TABLE D: CALCULATED PHYSICOCHEMICAL AND PHARMACOKINETIC PARAMETERS OF THE DOCKED PHYTOCOMPOUNDS

COMPOUND		ABIETIC ACID	4-ALLYLANISOLE	A-PINENE	B-PINENE	CAFFEIC ACID	CURZERENE	CURZERENONE	DEHYDRO-ABIETIC ACID	FURANOEUDESMA-1,3-DIENE	GENTAMICIN	D-GLUCURONIC ACID	(E)-GUGGULSTERONE	(Z)-GUGGULSTERONE	LIMONENE	MYRRHONE	NEOABIETIC ACID	TRANSB-OCIMENE	PIMARIC ACID	VANILLIN
PHYSICOCHEMICAL AND PHARMACOKINETIC PARAMETERS (MOLINSPIRATION CHEMINFORMATICS)																				
MILOGP < 5		5.01	2.82	3.54	3.33	0.94	4.54	3.88	5.67	4.15	-4.21	-2.77	3.62	3.62	3.62	3.50	5.31	3.97	4.96	1.07
TPSA (OA) < 500		37.30	9.23	0.00	0.00	77.75	13.14	30.21	37.30	13.14	199.74	127.44	34.14	34.14	0.00	30.21	37.30	0.00	37.30	46.53
MW < 500 (G/MOL)		302.46	148.21	136.24	136.24	180.16	216.32	230.31	300.44	214.31	477.60	194.14	312.45	312.45	136.24	228.29	302.46	136.24	302.46	152.15
MV		312.49	154.12	151.81	152.37	154.50	226.85	229.03	306.28	215.38	450.66	153.99	312.85	312.85	157.30	217.86	312.46	161.69	312.72	136.59
NON < 10		2	1	0	0	4	1	2	2	1	12	7	2	2	0	2	2	0	2	3
NOHNH < 5		1	0	0	0	3	0	0	1	0	11	5	0	0	0	0	1	0	1	1
LIPINSKI'S VIOLATION		1	0	0	0	0	0	0	1	0	2	0	0	0	0	0	1	0	0	0
SOLUBILITY AND PHARMACOKINETICS PROPERTIES (SWISSADME)																				
WATER SOLUBILITY		MS	S	MS	S	SV	MS	MS	MS	MS	SH	SH	MS	MS	MS	MS	MS	S	MS	SV
GASTROINTESTINAL ABSORPTION		H	H	L	L	H	H	H	H	H	L	L	H	H	L	H	H	L	H	H
LOG K_P : SKIN PERMEATION: CM/S		-4.75	-4.81	-3.95	-4.18	-6.58	-4.32	-4.84	-4.72	-5.00	-12.12	-9.15	-5.41	-5.41	-3.89	-5.14	-4.52	-4.11	-4.20	-6.37
CYTOCHROMES INHIBITORS	CYP1A2	NO	YES	NO	NO	NO	NO	YES	NO	NO	NO	NO	NO	NO	NO	YES	NO	NO	NO	NO
	CYP2C19	YES	NO	NO	NO	NO	YES	YES	YES	YES	NO	NO	YES	YES	NO	YES	YES	NO	YES	NO
	CYP2C9	YES	NO	YES	YES	NO	YES	YES	YES	NO	NO	NO	YES	YES	YES	NO	YES	NO	YES	NO
	CYP2D6	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	YES	NO	NO	NO	NO
	CYP3A4	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO
TOXICITY RISKS (OSIRIS PROPERTY EXPLORER)																				
MUTAGENIC		NO	YES	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	YES	YES	NO	NO	NO	YES
TUMORIGENIC		NO	YES	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	YES	NO	MR	NO	MR	NO
IRRITANT		NO	YES	YES	YES	NO	NO	NO	NO	NO	NO	NO	NO	NO	YES	NO	NO	NO	NO	YES
REPRODUCTIVE EFFECTIVE		NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	YES	YES	NO	NO	NO	NO	YES

miLogP: Logarithm of partition coefficient between n-octanol and water. TPSA: Topological polar surface area. MW: Molecular weight. MV: Molecular volume. nON: Number of hydrogen bond acceptors. nOHNH: Number of hydrogen bond donors. No: no indication found, MR: medium risk. S: Soluble. MS: Moderate to soluble. SH: Soluble to highly soluble. SV: Soluble to very soluble. H: High.

DISCUSSION

Natural resins are products widely used in traditional medicine, either boiled, macerated, prepared as an ointment, or used directly in raw resin form [12]. These natural drugs have more effective therapeutic advantages because of their multi-target and multi-channel characteristics [13]. In this study, the universal solvent DMSO was chosen because it is often used as the vehicle control-of-choice for both *in vitro* and *in vivo* studies high-throughput screening with low toxicity [31] due to its broad solubilizing capability [32] especially its potential in enhancing the solubility of weakly soluble drugs [33]. As the lack of effective treatments for various infections would cause more deaths [34], the rediscovery of old active drugs has become a priorities to tackle the antimicrobial resistance which is considered as one of the major threats for the near future. Therefore, the researchers turned over to plants with antimicrobial properties. Regarding the tested strains in this study, the diameters of inhibition values of the Myrrh oleoresin reached 17 mm for both *E. coli* and *S. aureus* at 200mg/ml which is close to the results of [35] where the antibacterial activity of the aqueous extract of myrrh had minimum zones of inhibitions against *E. coli* (12 mm) and *S. aureus* (16 mm). [36], [7] also reported that the minimum inhibitory concentrations of myrrh had good antimicrobial effects against *S. aureus* and *E. coli* strains. These inherent antibacterial properties were attributed to sesquiterpenes and furanosesquiterpenoids [14]. Pine resin was less active with diameters between 11 to 15mm of less dense areas and 10 to 11mm for *S. aureus* and *E.coli* respectively. A better result was recorded by [37] where Pine resin was characterized by pronounced bactericidal effects with 15 and 19 mm for *E. coli* and *S. aureus* respectively at only 100mg/ml. It is known that rosin acids enhance the disruption of the bacterial membrane [38], for instance; high concentration of abietic acid may damage cell walls to some degree. The activities recorded were bacteriostatic, this suggests that some resin acids does not annihilate bacteria and instead inhibits their acidogenic ability and growth [39]. *P. aeruginosa* was completely resistant. Likewise, no zones of inhibition were seen on plates seeded with two strains of *P. aeruginosa* [40] Furthermore, species belonging to the genera *Pseudomonas* were able to grow on Pine resin medium by degrading specific resin acids such as dehydroabietic or isopimaric acid [41]. But these findings contradict those of [36], [7] by Myrrh resin where this strain was susceptible to myrrh extract and Pine resin with 25mm of inhibition zone [37]. *P. aeruginosa* is included in the class of superbugs and listed by the WHO as "critical". In *P. aeruginosa*, intrinsic resistance is the result of the increasing prevalence of efflux pumps, the reduced permeability of the cell membrane, and the target site alteration [23]. The DPPH test provides useful information on the antioxidant capacity to donate hydrogen atoms, on the reaction's reducing capacity and on mechanism between the free radical and the antioxidant [42]. The Myrrh was the most active with the best IC_{50} (0.53 ± 006 mg/ml), that is probably due to the Curzerene known to possess antioxidant and free radical neutralizing properties [43] and guggulsterone having a strong antioxidant property by inhibiting intracellular ROS level [44] but Glucuronic acid exhibited almost no antioxidant activity by DPPH method [45]**.** Also, Pine resin had higher activity than BHT. [46] found the same using lyophilized aqueous extract which turned out to have considerable anti-oxidant activity with only 83.64 µg/ml. At the molecular level, Abietic acid showed a higher antioxidant activity, with an EC_{50} value of 1.65 mg/ml [47] even if less effective than the BHT (0.84 mg/ml). Within the same concept, antioxidant properties in microemulsions and emulgels containing abietic acid increase as its concentration increases [48]. On the other hand, antioxidant properties of the Caffeic acid were previously reported [49], [50], [51]. Pine resin showed an anti-inflammatory effect, higher than that of salicylic acid. However, Myrrh has a lower anti-inflammatory activity. The myrrh is one of the most frequently used gum resin as a remedy for inflammatory problems [13] even in Algeria such as rheumatic pains, amenorrhea, [52],tendonitis [36] and promoting wound healing after tooth extraction [53].

It contains many active phytosteroids with strong anti-inflammatory effects, among which myrrh steroid, guggulsterone which can improve acute pancreatitis [13], [54] and abietic acid [55]. The latter demonstrated anti-inflammatory properties *in vivo* when administered orally or topically [48]. In addition, (Z)-guggulsterone significantly inhibited oxidative stress in the study of [44]. The aminoacyl-RNA transferase are clinically validated [34] and ideal targets for the development of new antibacterial agents for several reasons; they play a critical role in protein biosynthesis as they are required for a correct attachment of amino acids to their corresponding tRNA molecule [34] and thus are essential for cell viability and growth [23]. To determine the mechanism of binding of the main compounds of resins, molecular docking of these molecules was done. The best docking solutions were selected based on the docking score and the best binding pose of the ligands. Protein-ligand binding affinity is essential in understanding molecular recognition and helps to identify a lead compound [56]. Despite that GM mode of action is related to protein synthesis inhibition [57], the binding affinities for TyrRS of (Z)-guggulsterone, (E)-guggulsterone, Myrrhone, Furanoeudesma-1,3-diene, Curzerene, Curzerenone (in Myrrh), Dehydroabietic acid, Abietic acid, Neoabietic acid, Pimaric acid, and Caffeic acid (in Pine resin) were higher in comparison with Gentamicin even so all docked compounds had interaction with TyrRS (**table 3**). These molecules could, among others, play an important role in inhibiting the biosynthesis of bacterial proteins. Guggulsterones, Abietic and neoabietic acids, Caffeic acid and Curzerene show multiple intermolecular hydrogen bonding interactions completely different from that of Gentamicin (**Figure 7**) which suggests that small molecules can integrate in different places of the active site. Generally, the bonds formed have short link distances compared to the standard; 3.94-6.16 Å in Dehydroabietic acid and a maximum of 2.33 Å in Gentamicin therefore stronger. It has been observed that the interactions also involve electrostatic bonds. It was reported earlier that Pimaric acid [58], Abietic acid, Neoabietic acid and Isopimaric acid exercised an antibacterial effects against *S.aureus* [13] and *S. epidermidis* [47] and that dehydroabietic acid was the most potent of the resin acids tested with the largest zones that were free of growth were [40]. The abietic acid may interfere with the function of the efflux pump mechanism [59] by its carboxylic functionality, which interacts with the lipid component of the bacterial cellular membrane allowing this molecule to penetrate inside the membrane and altering the membrane functions [47]. *P. aeruginosa* is responsible for severe nosocomial infections [60], it is able to resist many of the currently available antibiotics [61]. Generally, *P. aeruginosa* has multiple resistance mechanisms; this bacterium contains numerous efflux systems (more than 50) that may potentially affect the concentration of the compound inside the cell [23], a low outer membrane permeability and unlike most bacteria, it contains another synthetases aminoacyl-tRNA synthetases (TyrRZ), yet the two forms of the enzyme complete identical functions. In the study of [23], four compounds identified that inhibited the activity of *P. aeruginosa* TyrRS but only one inhibited the activity of TyrRS-Z . this can be explained by the fact that our compounds cannot join the TyrRS of *Pseudomonas* by this impermeability or that these compounds act only on the TyrRZ which does not affect the mechanism of aminoacylation. Toxicity risks and pharmacokinetic studies, such as solubility, absorption, distribution and metabolism of resin components were evaluated using OSIRIS Property Explorer, Molinspiration Cheminformatics and SwissADME online tools. Abietic, dehydroabietic and neoabietic acids have a low lipophilicity according to Lipinski rules where the physicochemical parameters are associated with acceptable aqueous solubility and intestinal permeability and comprise the first steps in oral bioavailability [29]. Globally, Myrrh is a safe, natural flavoring substance approved by the US Food and Drug Administration [62] and acute toxicity study of guggulsterone-(Z) demonstrated that it did not show any sign of toxicity or abnormal symptom in the rats of dosing suggesting its potential clinical safety [44]. Furthermore, *in vitro* analysis indicated that abietic acid exhibited no cytotoxicity to epithelial cells and mesenchymal fbroblasts [39]. The more negative the log Kp in cm/s, the less skin permeant is the molecule [63]. The skin permeability, Kp, values of all compounds ranged from −3.89 to -9.15 cm/s suggesting good skin permeability of the majority of compounds with the exception of Caffeic acid, Furanoeudesma-1,3-diene, D-glucuronic acid, guggulsterones, Myrrhone, Vanillin and Gentamicin (-6.58, -5.00, -9.15, -5.41, -5.14, -6.37 and -12.12 cm/s respectively). The knowledge about interaction of molecules with cytochromes is essential. P450 (CYP) superfamily of isoenzymes is a key player in drug elimination through metabolic biotransformation by processing small molecules synergistically to improve protection of tissues and organisms [63]. Moreover CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4 are vital for this biotransformation [64]. The consumption of phytochemicals can interfere with the drug-metabolizing activity of such (CYP) enzymes which can pose serious health consequences [12]. Except for Caffeic acid, D-glucuronic acid, Transβ-ocimenen, Vanillin and 4-allylanisole, all docked molecules acted on CYP2C19, responsible for the metabolization of various xenobiotics, including proton pump inhibitors [65] or CYP2C9 which contributes to the metabolism of many drugs, including several NSAIDs [66]. The toxicity prediction indicates a potential risk of specific toxicity. All compounds would be safe and expected no toxicity regarding mutagenicity, tumorigenicity, irritation, and effect on the reproductive system except Limonene, 4-allylanisole and Vanillin besides Pinenes which are Irritant. The toxicity of limonene has been reported to be significantly higher than that of other monoterpenes [67] and similarly, α and β pinene provoke erythema on the skin of the guinea-pigs at a concentration of 100% [68]. It should be underlined that it is not obvious to compare an activity of the compounds alone with a whole extract because these compounds generally act in synergy. Studies comparing the action of whole plant extracts to the action of purified preparation show that, in many cases, the potency of the purified preparation declines at each fractionation [69]. Despite the great discoveries in therapy, the Algerian population still has recourse to natural resources for several reasons such as the high and lack of availability of the cares, especially in rural areas, and the confidence inherited for generations in these treatments regarding the absence of adverse effects. Resins being complex mixtures of several compounds with different chemical groups and could be promising alternatives to fight numerous health problems. Oleoresins are an important renewable and sustainable resource of active compounds. The study of *in vitro* antiinflammatory and antioxidant effects of Myrrh and Pine resin were satisfying. On a molecular level, the *in silico* study demonstrated that the compounds adhere to the active site of the bacterial TyrRS which can constitute a favorable target in particular in MDR superbugs. Based on ADMET prediction analysis, isolated compounds may be safe candidates in this investigation except Limonene, Vanillin and 4-allylanisole. This study must be completed by the *in vivo* study using other evaluation techniques, in addition to the *in silico* study by docking other bacterial targets such as topoisomerase and other proteases. We conclude that the mechanism of action of one molecule may not be the same for another one and a single compound can have multiple targets. Therefore, it has become essential to exploit new bacterial targets and to multiply complementary therapies as alternatives.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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