Essential oil from Galls formed on leaves of *Pistacia atlantica* Desf.: New *in-vitro* and *in-silico* studies of anti-inflammatory activities

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Abstract

Inflammation is an important component of the immune response to pathogens and damaged cells. This study aims to assess and compare of anti-inflammatory activities in-vitro and in-silico of essential oils from galls formed on leaves of *Pistacia atlantica* Desf from two regions in Algeria (four samples from *Ain-oussera* and four samples from *Laghouat*). The anti-inflammatory activity was achieved by Nitric oxide inhibitory activity and viability of LPS-activated RAW 264.7 macrophages, and inhibition activity of 15-Lipoxygenase (LOX), quercetin was used as a positive control. The essential oils from leave gall of *Pistacia atlantica* species is able to inhibited 15-LOX enzyme with IC_{50} 0.004mg/mL and also inhibited the production of NO produced by macrophages (99.17%) without significant cytotoxic effect (78% cell viability). The molecular docking study confirm the inhibitory effect of the major compounds of the studied essential oil, even ADMET analysis indicate that the major compounds are not toxic.

Keywords: Galls of *Pistacia atlantica*, essential oil, anti-inflammatory activities, molecular docking, ADMET analysis

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List of "Abbreviations"

A	Essential oil samples from *Ain oussera* region
A°	Angstrom
ADMET	Absorption, Distribution, Metabolism, Excretion and Toxicity
ATCC	American Type Culture Collection
cLogP	calculated logP (P = octanol-water partition coefficient)
DMEM	Dulbecco's modified Eagle's medium
FCS	Foetal calf serum
iNOS	Nitric Oxide Synthase
L	Essential oil samples from *Laghouat* region
LOX	Lipoxygenase
LPS	Lipopolysaccharide
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-di-phenyl-tetrazolium bromide
NO	Nitric oxide
PBS	Phosphate buffered saline
PDB	Protein Data Bank

1. Introduction

Inflammation is an important component of the immune response to pathogens and damaged cells. It is characterized by increased temperature, redness, pain, swelling and sometimes, loss of tissue function in a chronic situation (MacNaughton, 2006). It is a complex biological response of vascular tissues to infection by pathogens, damaged cells or irritants. This usually leads to the release of a variety of inflammatory mediators that in turn induce enzyme synthesis such as; lipoxygenase (LOX) and inducible nitric oxide synthase (iNOS), leads to high concentrations of inflammatory nitric oxide (NO) and leukotrienes (Brain and Williams, 1990). LOXs are the key enzymes in the biosynthesis of leukotrienes from fatty acids producing inflammatory lipid mediators, therefore provoking inflammation-related diseases (Nathan, 1992). LOX and iNOS enzymes are involved in a wide variety of inflammatory conditions, and represent a target for anti-inflammatory therapy. *Pistacia atlantica* Desf, (*elbetoum*, *botma* or *btouma*, in local Arabic), is a beautiful tree which has been described in various scientific works. Several authors (Barra et al., 2007; Belhadj, 2001; Lahlou, 2004) describe the *Pistacia atlantica* Desf as a valuable species because of its uses in the treatment of eczema, diarrhoea, asthma, stomach-ache. In recent publications, this species has been known for its potential antimicrobial (Pourya et al., 2018; Rigane et al., 2017),antioxidant (Benamar et al., 2018; Toul et al., 2017), antidiabetic (Shahouzehi et al., 2018) and anti-inflammatory properties (Amri et al., 2018). According to Hammer et al. (2002), the galls have been shown to protect their occupants from natural enemies, such as predators and parasites, by various chemical and mechanical means.

The chemical composition of the essential oils of the galls of two regions *Ain oussera* and *Laghouat* was reported by Sifi et al. (2015b), which was identified thirty-five (35) different compounds in this essential oil. The essential oil samples are rich in monoterpenes [22.9 - 97.7%] for *Ain-oussera* and [89.16 - 94.26%] for *Laghouat*, with a dominance of hydrocarbon monoterpenes [88.48 - 95.05%]. The majority compounds identified are: *α-Pinene* [59.01% - 81.50%], *β-Pinene* [16.17% - 14.20%] and *Sabinene* [26.11% - 28.04%]. Other minor

compounds such as: *Limonene* [5.51% - 7.17%], *p-Cymene* [7.57% - 11.39%], *Terpinene*-4-ol [15.9% - 20.86%] and *α-Terpineol* [3.41% - 6.72%]. γ-Terpinene were also identified.

According to our knowledge, no study has reported on the anti-inflammatory activity of essential oil of galls part from *Pistacia atlantica* Desf species. In this research, we studied the variability of anti-inflammatory activity by the inhibition of 15-lipoxygenase (LOX) and inhibition of the production of NO in LPS-activated RAW 264.7 macrophages, of essential oils of galls of *Pistacia atlantica* Desf from two areas in Algeria (*Ain-oussera* and *Laghouat*). The molecular docking study was made to be confirmed if there's a significant anti-inflammatory activity of major compounds of the studied essential oil.

This study is a continuation of previous studies on the chemical composition (Sifi et al., 2015b) and antimycobacterial, antioxidant and cytotoxic activity (Sifi et al., 2015a) of essential oil from galls of *P. atlantica* Desf and antimicrobial activity (Sifi and Yousfi, 2020).

2. Materials and Methods

2.1. Plant samples

The galls of *Pistacia atlantica* Desf were randomly collected from the bottom of the trees branche during summer 2013 (the end of July and early August period), from two different locations in Algeria: *Aïn-Oussera* (4 samples) and *Laghouat* (4 samples); respectively located at 210 and 450 kilometres south of Algiers, the capital of Algeria. Professor Salima Benhouhou, a botanist from the National Institute of Agronomy (Algeria) confirmed the identity of the plant material, and voucher specimen (PAUG-52S/08/10), was deposited in the herbarium of Fundamental Sciences Laboratory at the University of Amar Telidji Laghouat (Algeria).

2.2. Extraction of essential oil

The essential oil was obtained by hydro-distillation using Clevenger type apparatus, for a period of three hours. Traces of water was removed from the essential oil with anhydrous sodium sulphate, filtered and stored at 4°C until analysis.

2.3. Chemicals

$Fe_2(SO_4)_3$ and linoleic acid were purchased from Merck, Darmstadt and Schuchardt (Germany) respectively. Xylenol orange was obtained from Searle Company, England. Foetal calf serum (FCS) and Dulbecco's modified Eagle's medium (DMEM) were provided by Highveld Biological, Johannesburg, South Africa. Phosphate buffered saline (PBS) and trypsin were purchased from Whitehead Scientific, South Africa. Quercetin, 3-(4,5-dimethylthiazol-2-yl)-2,5-di-phenyl-tetrazolium bromide (MTT) and was purchased from Sigma-Aldrich St. Louis, MO, USA. Tris-HCl and 15-lipoxygenase from Glycine max were provided by Sigma, Germany.

2.4. Anti-inflammatory activity

Nitric oxide inhibitory activity and viability of LPS-activated RAW 264.7 macrophages

Cell culture. The RAW 264.7 macrophages cell line was purchased from the American Type Culture Collection (ATCC TIB-71, Rockville, MD, USA), and cultured in a plastic culture flask in DMEM containing L-glutamine supplemented with 10% FCS and 1% PSF solution at 37°C with 5% CO_2. Cells were seeded (10^4 per well) in 96 well-microtitre plates and activated LPS alone (control) or with samples at different concentrations (0.004, 0.02, 0.06 and 0.2mg/mL). Quercetin (0.001, 0.002, 0.01 and 0.02mg/mL) was used as a positive control (Mu et al., 2001).

Measurement of NO produced. The amount of nitric oxide released was determined by the *Griess* reagent as reported previously (Dzoyem et al., 2015). After 24 h incubation, 100 µL of supernatant from each well of cell culture plates was transferred into 96-well microtitre plates and an equal volume of Griess reagent was added. The absorbance of the resultant solutions was determined on a BioTek Synergy microplate reader after 10 min at 550 nm. The concentrations of nitrite were derived from regression analysis using serial dilutions of sodium nitrite as a standard. Percentage inhibition was calculated based on the ability of compounds to inhibit nitric oxide formation by cells compared with the control (cells in media without compounds), which was considered as 0 % inhibition.

Cell viability. The number of viable cells was determined as previously described by Mosmann (1983) on the macrophage cells with few modifications. Briefly, the cells were topped up with

200µL DMEM after removal of media. 30µL of 15mg/mL MTT were added to each well and cells were incubated at 37°C with 5% CO_2. The medium was aspirated after 2h, and the formazan salt formed was dissolved using DMSO. The absorbance was read at 570 nm on a BioTek Synergy microplate reader. Cell viability percentage was then calculated with reference to the control (cells with LPS only considered as 100% viable).

15-Lipoxygenase inhibition assay

The assay was performed according to a previously described procedure (del Carmen Pinto et al., 2007) with slight modifications. The assay is based on measuring the formation of the complex Fe^{3+}/xylenol orange in a spectrophotometer at 560 nm. 15-Lipoxygenase from Glycine max was incubated with essential oil or standard inhibitor (the concentration ranges from 0.001mg/mL to 1mg/mL) at 25°C for 5 min. Then linoleic acid (final concentration, 140 µM) in Tris-HCl buffer (50mM, pH 7.4) was added and the mixture was incubated at 25°C for 20 min in the dark. The assay was terminated by the addition of 100 µL of FOX reagent consisting of sulphuric acid (30 mM), xylenol orange (100 µM), $Fe_2(SO_4)_3$ (100 µM) in methanol/water (9:1). For the control, only LOX solution and buffer were pipetted into the wells. Blanks (background) contained the enzyme LOX during incubation, but the substrate (linoleic acid) was added after the FOX reagent. The lipoxygenase inhibitory activity was determinated by calculating the percentage of the inhibition of hydroperoxide production from the changes in absorbance values at 560 nm after 30 min at 25°C.

$$\%\ \boldsymbol{Inhibition} = \left[\frac{(\boldsymbol{A_{control}} - \boldsymbol{A_{blank}}) - (\boldsymbol{A_{sample}} - \boldsymbol{A_{blank}})}{\boldsymbol{A_{control}} - \boldsymbol{A_{blank}}}\right] \times \mathbf{100}$$

Where, $A_{control}$ is the absorbance of control well, A_{blank} is the absorbance of blank well and A_{sample} is the absorbance of sample well. Quercetin was used as a positive control. Essential oil concentration providing 50% inhibition (IC50) was calculated from the graph plotting percentage of LOX inhibition against essential oil concentration.

2.5. Molecular docking

Through this work, the anti-inflammatory activity is tested using two different assays, the inhibition of 15-lipoxygenase (15-LOX) is studied above in vitro, in this part we choose four major compounds in the best essential oil (*Ain-oussera* sample) to dock them inside the human 15- LOX then determining their interaction with it's active site.

The ligands are alpha-pinene, camphene, myrcene, and sabinene, where their structures are presented in **Fig. 1**. They were obtained from PubChem database (Kim et al., 2016) and assembled with Discovery Studio visualizer v4.0 to PDB files. The human 15-LOX was downloaded from the protein data bank with PDB ID: 3O8Y. The protein must be prepared by removing all unnecessary water molecules, heteroatoms, any ligands and co-crystallized solvent. Polar hydrogens and partial charges were supplied to the structure using Autodock tools (ADT) (version 1.5.4). We have performed the molecular docking using the AutoDock Vina program (Trott and Olson, 2010) in an eight CPU station. The software uses rectangular boxes for the binding site, the center of the box has been set and displayed using ADT. The grid box is 22*20*20 with x=2.198, y=22.816, z=-0.235 with one Å separated grid points positioned in the middle of the active site for each protein. To regard the flexibility of side chain during this specific docking, we have assigned flexible torsions in the ligands, and we allowed the acyclic dihedral angles to rotate freely (Martins et al., 2011). We have simulated the default settings, except that the number of output conformations was set to one. The number of docking runs was set at 10 runs. All these solutions are very well studied. The "random seed" is random. The preferred conformations were those of lower binding energy within the active site. Finally, we have loaded the generated docking results into Discovery Studio visualizer, v 4.0.

2.6. ADMET analysis

To evaluate the drug-likeness prediction of the four studied monoterpenes in order to study their effects on human body, they were subjected to Lipinski filter in which an orally bio-active drug is expected not to violate more than one of the criteria for drug-likeness namely: cLogP, hydrogen donor and acceptor molecular mass, and molar refractive index (Nickel et al., 2014).

The predicted Absorption Distribution Metabolism, Excretion and Toxicity (ADMET) study were analyzed using SwissADME server (http://www.swissadme.ch/index.php) (Daina et al., 2017). We inserted the canonical SMILES of the four monoterpenes into the server online to calculate ADMET properties using default parameters.

3. Results

3.1. Anti-inflammatory activity

Nitric oxide inhibitory activity and viability of LPS -activated RAW 264.7 macrophages

The potential anti-inflammatory activity of essential oils of *Pistacia atlantica* galls from two study regions (*Ain-oussera* and *Laghouat*) was investigated using NO production in LPS-activated by RAW (264.7) macrophage cells. The essential oils had an effect on NO production and cell viability (**Fig. 2**). All essential oil samples tested inhibited NO production. At the highest concentration used (0.2mg/mL). With the *Laghouat* samples L2, L5 and L6, which had a percentage inhibition of 84.01%, 20% and 79.52% respectively all other samples led to > 90% inhibition of NO production. Although the samples (A2 and A6) have good activities against NO production at a concentration of 0.2mg/mL, the inhibitory effect appears to be due to their cytotoxicity against macrophage cells with cell viability percentages of 11.62% and 1.29%, respectively. In the same concentration, the samples A1 and A4 inhibited the production of NO (99.17 and 99.13% respectively) without significant cytotoxic effect (78.91 and 70.22%). At the lowest concentration (0.004mg/mL), all samples had a low inhibitory activity of NO production that did not exceed 30%. Quercetin at a concentration of 0.01 mg/mL, led to a percentage inhibition of 73.99% with a cell viability of 75.43%. The essential oil samples therefore had a modest anti-inflammatory activity compared to quercetin.

15-lipoxygenase inhibition assay

The results of 15-LOX inhibition assay are shown in **Table 1.** Quercetin was used as a positive control. It exhibited a strong inhibitory activity of 15-LOX; with an IC_{50} value of 0.005 mg/mL. The activity of the extracts varied between 0.0041 and 0.0376 mg/mL. Comparing with

quercetin, the most active extracts were obtained with the sample of *Ain-oussera* (A1) and (A4); with IC_{50} values of 0.0041 and 0.0056 mg/mL, respectively. The sample of Laghouat (L6) had a moderate activity. The IC_{50} value is 0.0298 mg/mL. Other samples of essential oils had a low inhibitory activity varying between 0.0319 and 0.0376 mg/mL.

3.2. Molecular docking

The in vitro study is done with the soybean LOX but in the molecular docking, we have used the human 5- LOX, according to a comparison of the binding site of human 5-LOX with that of soybean LOX-3 has revealed that the amino acids in the binding sites of human 5-LOX have much similarity with those in the soybean LOX-3 enzyme (Lončarić et al., 2020). The active site of the human 5-LOX is composed from these amino acids: Leu368, Ile406, Ala410, Leu414, Ile415, Leu619, Val604, and Leu607. We have docked the known inhibitors: zileuton as the commercial drug with the quercetin, in order to discuss our tested molecules with the best inhibitors. We have saved a ratio repeating for all the inhibitors as 100%, which confirm the stability of those complexes. The interactions within the complexes with energy values are presented in **Table 2**.

The results show that all ligands interact with the hydrophobic cavity formed by the amino acids Ala410, Leu414, His367, His372, Ile415, and Leu607 with two hydrophobic interactions types: π-alkyl and alkyl-alkyl (**Fig. 3**). The number of these interactions is high and depends on the ligand as we record for camphene (11 interactions), myrcene (9 interactions), for alpha pinene (11 interactions), for sabinene (12 interactions). These numbers improve the complexes stability. Another interactions have been saved for the standard ligands, as for zileuton, we have recorded one hydrogen bond formed with Gln363 with interatomic distance (ID) of 2.55A°. In addition, for quercetin, we have found one hydrogen bond with Ile363 with ID of 2.14A°. The presence of hydrogen bonds is due to the heteroatoms as oxygen and nitrogen in both ligands, which are completely absent in the monoterpenes. All the complexes have shown nearly the same values of energy. The in-silico study is in accordance with the in vitro obtained results, showing that this essential oil is potent inhibitor for the 5-LOX enzyme.

3.3. Pharmacokinetic properties of the four studied compounds

The result generated from the Lipinski and ADMET filtering analyses are represented in **Table 3**. The four compounds fulfilled the requirement for Lipinski analysis of the rule of-five with corresponding favourable predicted ADMET parameters. The predicted physiochemical properties for bioavailability of the lead compounds, was further represented in **Fig. 4**. The ADME/tox and pharmacokinetic properties from the filtering analyses suggested the four monoterpenes with a high probability of absorption, subcellular distribution (which is mitochondria for the four compounds), and AMES toxicity parameter. The four studied compounds were indicated to be non-carcinogenic with high level of the gastrointestinal absorption index.

4. Discussion

The differences we observed in the chemical composition of the essential oils of galls from the sampled sites could be explained by: the nature of the site's soil; the nature of the sample (male or female) and the climate. According to Chu and Kemper (2001), the presence and concentration of certain chemical compounds fluctuate according to the season and the maturation of the plant.

On the basis of our study and the work carried out by other authors (Gourine et al., 2010; Mecherara-Idjeri et al., 2008; Tzakou et al., 2007) on the chemical composition of the essential oils of the leaves and fruits of the *Pistacia atlantica* tree, we note that the different aerial parts of the tree (leaves, fruits and galls), exhibit wide variations in chemical composition, with each of the major compounds; which confirms the existence of several chemotypes within this same species.

In regards to LOX inhibition enzyme, several essential oils and their compounds have shown inhibitory efficacy of lipoxygenase (Albano et al., 2012; Baylac and Racine, 2003; Choi and Hwang, 2004; Miguel, 2010; Viljoen et al., 2006) in which , the essential oils of *Artemisia campestris* L. and *Juniperus phoenicea* L. collected from the region of Jebel Amour located in

the steppe zone of Algeria had a good anti-inflammatory activity, Bakchiche et al. (2013) attributed this activity to the presence of α-pinene and limonene in these essential oils. A more recent study by Sharopov et al. (2015) on the essential oils of several aromatic plants from Tajikistan shows that the essential oils of *Galagania fragrantissima* and *Origanum tyttanthum* had the highest activity with IC_{50} values of 0.0073 and 0.0147 mg/mL respectively. Most of the 5-lipoxygenase inhibitors appear to be phenolic compounds and fatty acid analogues such as linoleic acid, oleic acid and arachidonic acid (Baylac and Racine, 2003; Frum and Viljoen, 2006; Paubert-Braquet et al., 1997). Our essential oil samples had a high percentage of α-Pinene (81.5%) as major compound and low percentage of fatty acid as minor compounds (palmitic (0.91%) and myristic (6.03%)), probably the results of 15-LOX inhibition due to the presence of synergically activity of both compounds.

On the other hand, the study carried by Lin et al. (2008) on the anti-inflammatory activity of *Cinnamomum insularimontanum* essential oil by nitric oxide (NO) inhibitory activity assay showed a significant inhibitory activity of NO production with IC_{50} values of 0.0186 and 0.0131 mg/mL respectively for crude essential oil and citral. Another study carried out by Yoon et al. (2010) on biological effects of D-limonene on the production of pro-inflammatory cytokines and inflammatory mediators in RAW 264.7 macrophages, shown to be an effective inhibitor of NO induced by LPS in macrophage cells. These inhibitory effects of D-limonene included a dose-dependent decrease in iNOS protein expression. Therefore, our simple essential oils of *Pistacia atlantica* galls contained 7.17% Limonene. Probably, the inhibitory effect of NO production may be result from the inhibition of expression of iNOS enzyme by the presence of limonene in our samples of essential oils.

5. Conclusion

The variation of the chemical composition of essential oils of *Pistacia atlantica* species shows that the region has a direct effect on the synthesis of secondary metabolites (essential oil),

which influences the anti-inflammatory activity. In general, essential oils from leave gall of *Pistacia atlantica* species is able to inhibited 15-LOX enzyme with IC_{50} 0.0041mg/mL and also inhibited the production of NO produced by macrophages, with low cytotoxicity against healthy cells (Sifi et al., 2015a). The molecular docking study confirm the inhibitory effect of the major compounds of the studied essential oil, even ADMET analysis indicate that the major compounds are not toxic.

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Conflict of interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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Table 1. IC_{50} values (mg/mL) of 15-LOX inhibition by essential oil samples

IC_{50} (mg/mL) of 15-lipoxygenase inhibition				
Ain-oussera		***Laghouat***		***Quercetin***
A1	0.0056 ± 0.0002	**L2**	0.0346 ± 0.0014	0.005 ± 0.0001
A2	0.0290 ± 0.0012	**L3**	0.0319 ± 0.0013	
A4	0.0041 ± 0.0004	**L5**	0.0376 ± 0.0016	
A6	0.0341 ± 0.0014	**L6**	0.0298 ± 0.0012	

Table 2. The results of interactions between ligands and human 5-LOX.

Ligand	**Repeating ratio%**	**Free binding energy (kcal mol−1)**	**Closest residues**	**Hydrophobic interactions**	**Hydrogen bonds**	**Length (Å)**
Camphene	100	-6.1	Phe421, Ile415, Leu368, Phe177, His367, Leu607, His372	Alkyl-alkyl; π-alkyl; π-sigma	/	/
Myrcene	100	-5.4	His367, Leu368, His372, Phe421, Leu414, Ala410, Phe177, Leu607	Alkyl-alkyl ; π-alkyl ; π-sigma	/	/
Alpha pinene	100	-5.0	Ala410, Leu414, Ile415, Leu607, His367, His372	Alkyl-alkyl; π-alkyl	/	/
Sabinene	100	-5.9	Ala410, Leu414, His367, His372, Phe421, Phe177	Alkyl-alkyl; π-alkyl	/	/
Quercetin	100	-5.5	Leu607, Ala603, His367, Ile678, His372, Leu414, Phe421, Tyr181, Asn425	π- π T-shaped; π-alkyl	Ile673	2.14
Zileuton	100	-6.0	His367, Leu414, Leu410, Ile406, Leu368, Gln363	π- π T-shaped; π-alkyl, alkyl	Gln363	2.55

Table 3. ADMET profiling enlisting absorption, metabolism and toxicity related drug like parameters of all the four selected monoterpenes.

Models	Camphene	Alpha pinene	Myrcene	Sabinene
A. Absorption				
Blood-Brain Barrier	+	+	+	+
Human Intestinal Absorption	+	+	+	+
Caco-2	+	+	+	+
Human oral bioavailability	+	+	-	+
Skin Permeation	-4.13 cm/s	-3.95 cm/s	-4.17 cm/s	-4.94 cm/s
P-glycoprotein Inhibitor	Non Inhibitor	Non Inhibitor	Non Inhibitor	Non Inhibitor
B. Metabolism				
P-gp Substrate	Non Substrate	Non Substrate	Non Substrate	Non Substrate
CYP450 1A2 Inhibitor	Non Inhibitor	Non Inhibitor	Non Inhibitor	Non Inhibitor
CYP450 2C9 Inhibitor	Non Inhibitor	Inhibitor	Non Inhibitor	Non Inhibitor
CYP450 2D6 Inhibitor	Inhibitor	Non Inhibitor	Non Inhibitor	Non Inhibitor
CYP450 2C19 Inhibitor	Non Inhibitor	Non Inhibitor	Non Inhibitor	Non Inhibitor
CYP450 3A4 Inhibitor	Non Inhibitor	Non Inhibitor	Non Inhibitor	Non Inhibitor
C. Toxicity				
AMES mutagenesis (probability)	-(0.99)	-(1)	-(0.92)	-(0.92)
Carcinogens (probability)	- (0.72)	-(0.72)	+(0.54)	-(0.86)
Hepatotoxicity (probability)	- (0.75)	-(0.75)	-(0.82)	-(0.82)
Acute Oral toxicity (kg/mol)	2.088	1.527	1.660	2.319

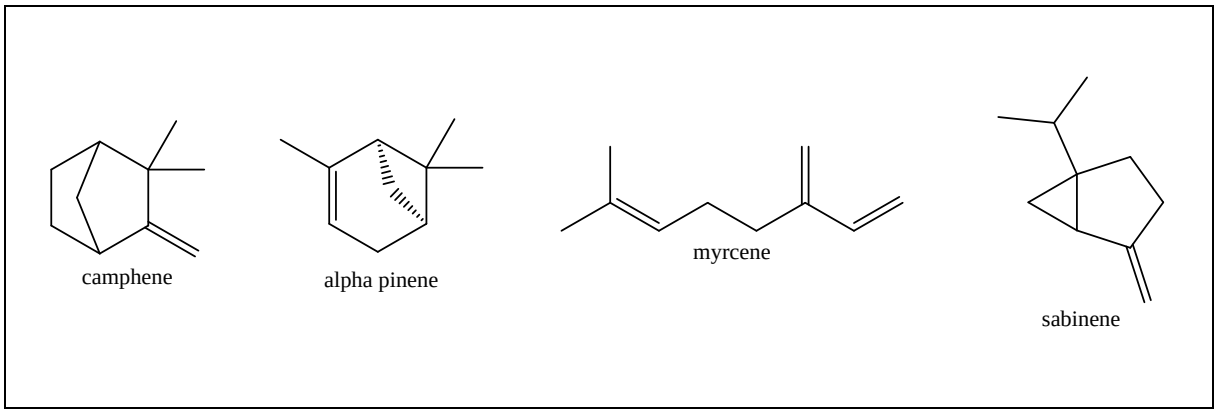


Fig. 1. Structures 2D of ligands used in the molecular docking

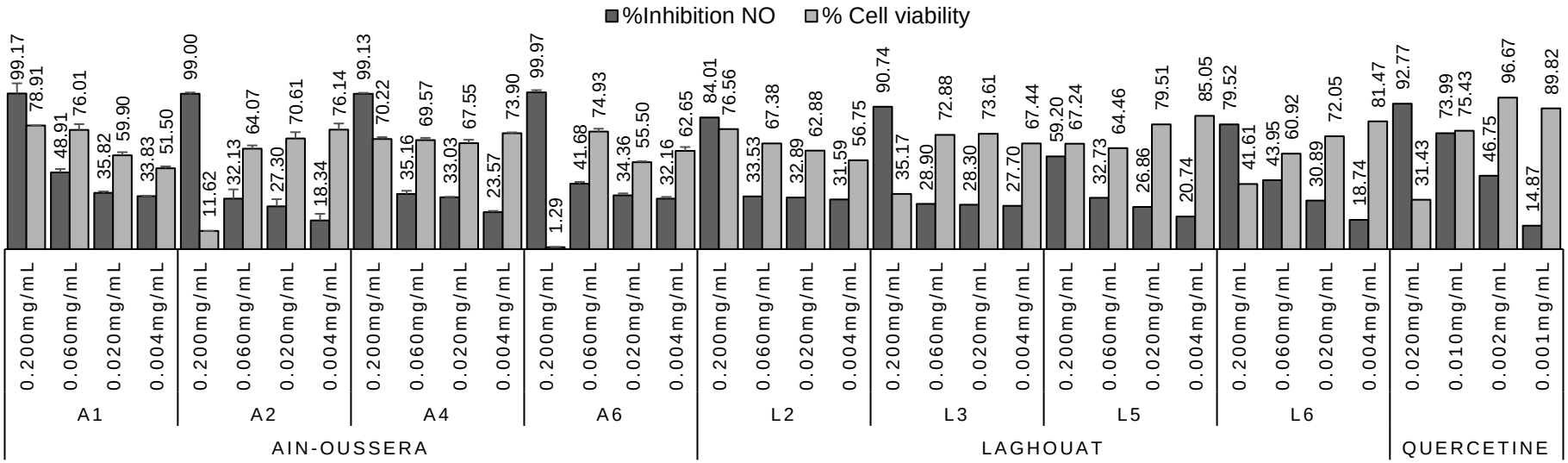


Fig. 2. Histograms showed the inhibition % of NO produced by macrophage cells type (Raw264.7) and the cell viability % using the essential oils of *Pistacia atlantica* galls from two study region (*Ain-oussera* and *Laghouat*).

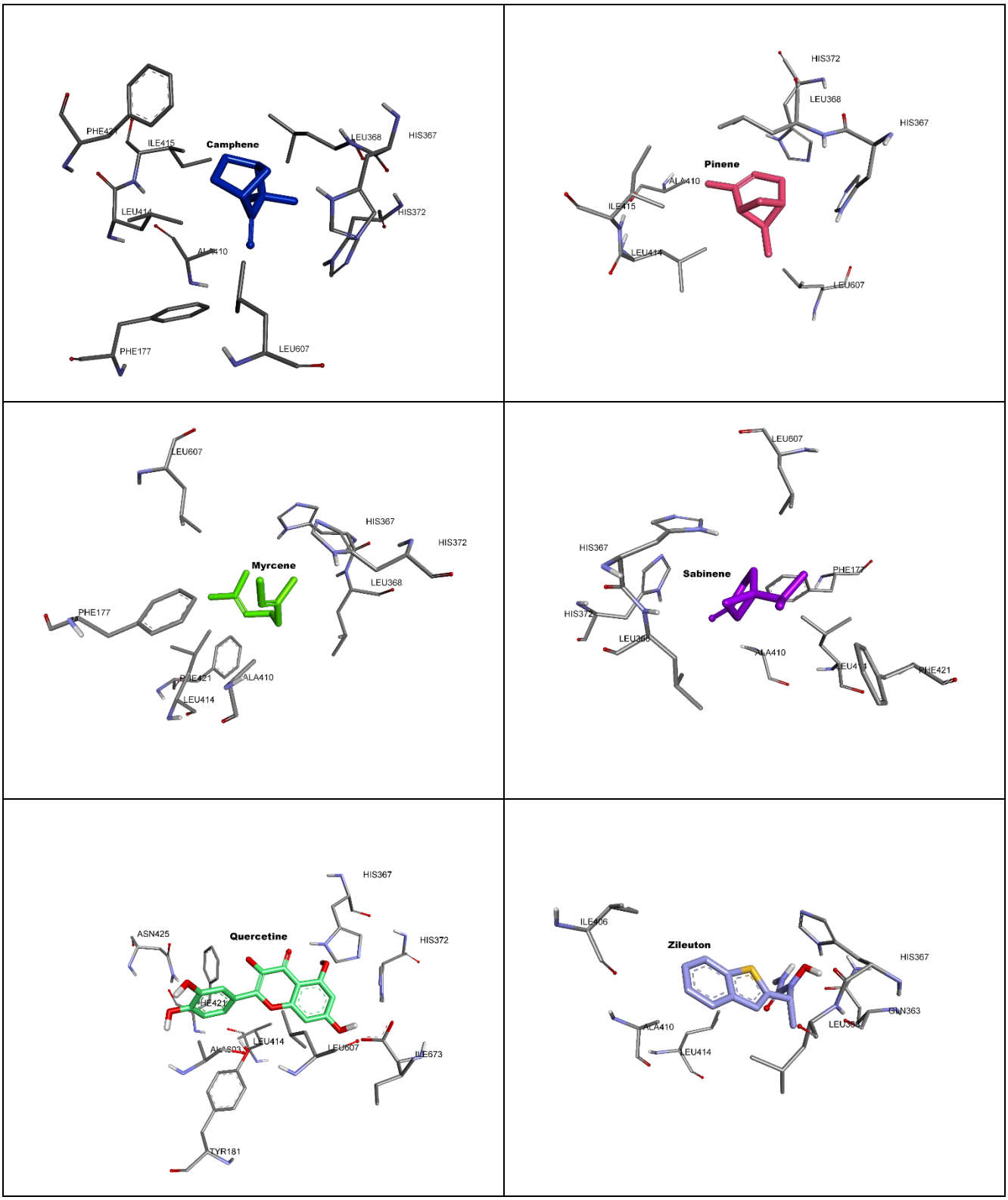
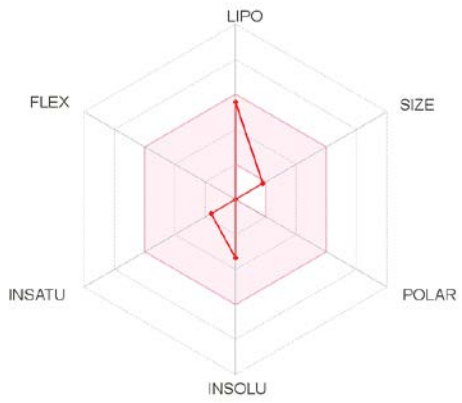
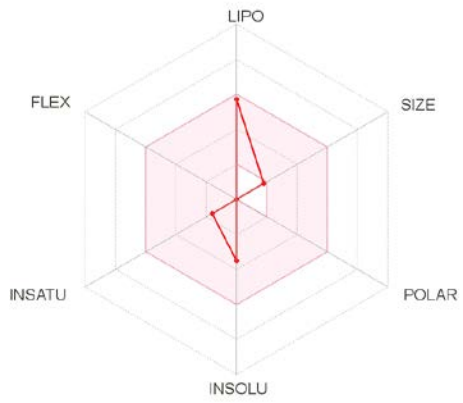


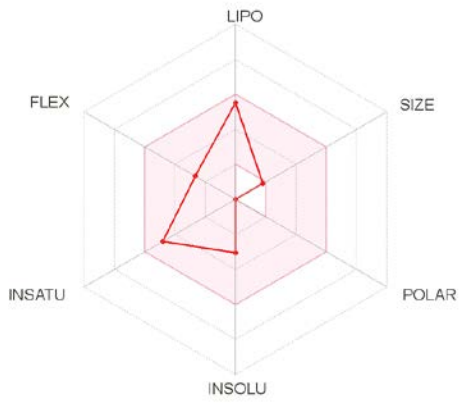
Fig. 3. Best docking poses of Binding mode of (alpha pinene, camphene, myrcene, sabinene, quercetin and zileuton) at the active site of human 15-LOX. Ligands are illustrated in different colours, labelled, and rendered as sticks.



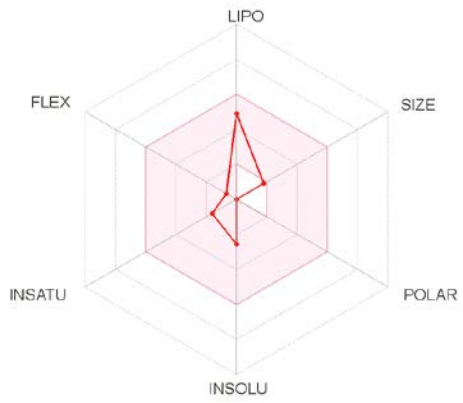
Camphene



Alpha pinene



Myrcene



Sabinene

Fig. 4. Summary of pharmacokinetic properties of the four studied monoterpenes. The colour space is the suitable physiochemical space for oral bioavailability.