In silico investigations of some Cyperus rotundus compounds as potential anti-inflammatory inhibitors of 5-LO and LTA4H enzymes

Fares Fenanira, Abderrahmane Semmeq, Yacine Benguerba, Michael Badawib,d, Marie-Antoinette Dziurlad, Smain Amirae and Hocine Laouer

Laboratoire de Valorisation de Naturel et biological Resources, University Ferhat Abbas, Sétif, Algeria; Laboratoire de Physique et Chimie Théoriques (UMR 7019), CNRS-Université de Lorraine, Saint-Avold, France; Laboratoire des Matériaux Polymères Multiphasiques, LMPMP, Université Ferhat Abbas, Sétif, Algeria; IUT de Moselle-Est, Université de Lorraine, Saint-Avold, France; Laboratory of Phytotherapy Applied to Chroniques Diseases, University Ferhat Abbas, Sétif, Algeria

Communicated by Ramaswamy H. Sarma

ABSTRACT

The present study aimed to experimentally identify the essential oil of Algerian Cyperus rotundus L. and to model the interaction of some known anti-inflammatory molecules with two key enzymes involved in inflammation, 5-Lipoxygenase (5-LO) and leukotriene A4 hydrolase (LTA4H). Gas chromatography/mass spectrometry (GC/GC-MS) revealed that 92.7% of the essential oil contains 35 compounds, including oxygenated sesquioperpenes (44.2%), oxygenated monoterpenes (30.2%), monoterpenic hydrocarbons (11.8%) and sesquiterpenic hydrocarbons (6.5%). The major identified oxygenated terpenes are humulene oxide II, caryophyllene oxide, khusinol, agaroisoipl, spathulolin and trans-pinocarveol. Myrtenol and α-terpinol are known to exhibit anti-inflammatory activities. Several complexes obtained after docking the natural terpenes with 5-LO and LTA4H have shown strong hydrogen bonding interactions. The best docking energy were found with α-terpinol, Myrtenol and khusinol. The interaction between the natural products and amino-acid residues HIS367, ILE673 and GLN363 appears to be critical for 5-LO inhibition, while the interaction with residues GLU271, HIS295, TYR378, GLU296 and ASP375 is critical for LTA4H inhibition. Molecular dynamics (MD) trajectories of the selected docked complexes showed stable backbone root mean square deviation (RMSD), supporting the stability of the natural product–enzyme interaction.

1. Introduction

Asthma is a widespread chronic inflammatory disease involving many inflammatory mediators such as small molecules, lipids, peptides, chemokines, cytokines, growth factors and enzymes (proteases) (Barnes et al., 1998; Barnes & Adcock, 2003; Busse & Lemanske, 2001). Among these enzymes, 5-lipoxygenase (5-LO) and leukotriene A4 hydrolase (LTA4H) play a key role in inflammation because they catalyse several steps in the biosynthesis of leukotriene B4 (LTB4). The enzyme 5-LO, which is also implicated in arthritis, psoriasis and heart diseases (Poeckel & Funk, 2010; Tsuji et al., 2010), contributes to the biosynthesis of LTB4 through the conversion of free arachidonic acid to an unstable epoxide intermediary, leukotriene A4 (LTA4). The enzyme LTA4H exhibits two different activities, epoxide hydrolase and aminopeptidase. The hydrolase allows LTB4 biosynthesis through LTA4 hydrolysis (Haeggström, 2001), and the aminopeptidase activity contributes to LTA4H anti-inflammatory effects by reducing the consequences of LTB4 production (Snellgrove et al., 2010). Many efforts have been performed in the development of drugs targeting LTB4 biosynthesis. Amongst these drugs, some 5-LO inhibitors were shown to be efficient (Bhatt et al., 2017) towards asthma models such as allergen-induced bronchoconstriction (Buccellati et al., 2002). Zileuton, one 5-LO inhibitor, showed satisfying effects on asthmatic patients but negative hepatotoxic effects (Atkins et al., 2007). The usefulness of synthetic compounds is limited by their high cost, poor efficiency and remarkable side effects such as myelotoxicity, gastrointestinal disturbances, cardiotoxicity and hepatotoxicity (Badria et al., 2014). To date, the most advanced inhibitors are large spectrum inhibitors targeting LTA4H aminopeptidase activity. While current efforts are deployed towards finding selective inhibitors of the hydrolase activity of LTA4H (Stsiapanava et al., 2014). However, potent LTA4H inhibitors entering clinical trials have all failed (Low et al., 2017).

Plant essential oils, which are secondary metabolites, comprise various natural bioactive compounds. They are eco-friendly, user-friendly and also exhibit multi-targeting activities (Sarma et al., 2019; Verma et al., 2019). There is a growing interest in plant biological activities contributing to ethnomedicine for the treatment of inflammatory diseases. These physical properties are often due to monoterpenes and...
sesquiterpene molecules present in plant essential oils used as herbal medications of traditional medicine (Salud et al., 2011). Adapted to warm environments (Wills, 1998), *Cyperus rotundus* L. is a *Cyperacea* spice growing in the Oued-Souf region of Sahara (Algeria). *C. rotundus* shows large intra-species morphological and genetic variations, observed all around the world (Molin et al., 2009; Okoli et al., 1997; Tayyar et al., 2003; Wills, 1998). This spice has been used to reduce inflammation and pain in traditional medicine and treat stomach troubles and diverse inflammatory disease (Ilham et al., 2018). Moreover, its tuber extract is widely used to treat bowel disorders, spasm and menstrual irregularities (Jiangsu New Medical College, 1977; Joshi & Joshi, 2000; Puratchikody et al., 2006). Several studies reported that the root of *C. rotundus* exhibits antioxidant, antimicrobial, anti-diabetic, antimutagenic, analgesic activities and has a significant anti-inflammatory activity (Dhillon et al., 1993; Kilani et al., 2007; Raut & Gaikwad, 2006; Uddin et al., 2003; Zhu et al., 1997). Chemical screening of rhizome’s extracts identified a predominance of oxygenated sesquiterpenes and monoterpenes (Sonwa & König, 2001). However, the chemical composition and pharmacological activities of the essential oil extracted from *C. rotundus* L. growing in the Oued-Souf region of Sahara (Algeria) have still to be investigated. Especially, the key oxygenated terpenes and the effect of the plant’s origin remain unclear (Pirzada et al., 2015). Moreover, only a few studies investigated the anti-inflammatory activity of *C. rotundus* plant parts and their oxygenated phytoconstituents concerning molecular targets such as 5-LO and LTA4H.

Computational virtual screening of natural products against specific enzymatic activities and *in silico* explorations have been performed to identify new plant-derived inhibitors with anti-5-LO and anti-LTA4H activities. Presently, several of them are anti-inflammatory terpenoids (El-Naggar et al., 2017). Terpenes are a large class of natural compounds with many biochemical properties. Being used as safe adjuvants with relatively low toxicity, they play roles such as scents, flavours and pharmaceuticals (Aql et al., 2007; Davies, 2009; Sapra et al., 2008). Terpenes with oxygen-containing functional groups in their hydrocarbon skeleton are the major constituents of *C. rotundus* and are responsible for its biological, pharmacological activities (Pirzada et al., 2015) and mainly for their best-described antioxidant and anti-inflammatory activities (Dang et al., 2011). Among these terpenes, Humulene oxide II (Jin et al., 2011), caryophyllene oxide (Chavan et al., 2010), khusinol (Niu et al., 2017) and agarosperol (Yadav et al., 2013) were reported to inhibit 5-lypoxigenase-catalyzed leukotriene production, to exert anti-inflammatory activity in carrageenan-induced mice paw edema, to reduce inflammation by inhibiting NO production and to reduce oxidative stress and the production of pro-inflammatory cytokines in TPA-induced mouse ear inflammation model, respectively. The cyclic terpenoids alcohol spathulenol (do Nascimento et al., 2018), trans-pinocarveol (Elizabeth, 2004; Nature Technology Inc, 2008; Symrise & Co, 2011), myrtenol (Gomes et al., 2017; Silva et al., 2014) and α-terpineol (Oliveira et al., 2012) showed significant inhibition in the Cg-induced mice paw edema, reduction of the blood concentration of an inflammatory cytokine, modulation of acute inflammation through inhibition of cytokine release and significant inhibition of the neutrophil influx in the pleurisy model, respectively.

Overall, these compounds, which combine cyclic or bicyclic structures and numerous hydrophilic groups, are exciting molecules for performing structure–activity relationship studies (Kopecná et al., 2019). Cyclic structures play a significant role in protein–ligand and protein–protein interactions (Espinoza-Fonseca & García, 2008; Lanzarotti et al., 2011). Additionally, hydroxyl (OH) functional groups (hydrophilic nature) enable water displacement, which plays a key role in ligand’s affinity (Cardoso-Teixeira et al., 2018) and probably drives the interaction between the hydrophilic groups and both the N- and C-terminal domains of catalytic enzymes (Madura, 2009). Computer modelling of protein–ligand interactions allows the understanding of interaction mechanisms, with identifying intermediate states and structural modifications induced by the ligand on enzymes (and vice versa) (Solanke et al., 2019).

Recently, a 5 ns molecular dynamics simulation was conducted on four similar inhibitors towards LTA4H to provide valuable information on their biological activities (Thangapandian et al., 2012). The most active compound was used in drug design to identify novel structures as potential LTA4H inhibitors (Thangapandian et al., 2012). In another study, a 25 ns molecular dynamics simulations were performed on the 5-LOX enzyme in complex with velutin, galangin and chrysin. Final evaluation based on MD analysis has identified novel compounds to be used as potents in the 5-LOX inhibitor design (Singh et al., 2017).

The present study was designed to screen the main active compounds of *C. rotundus* essential oil *in silico* evaluations of their inhibitory activity towards 5-LO and LTA4H. Classical molecular dynamics were performed to determine the stability of the protein–ligand complexes.

2. Materials and methods

2.1. Plant materials

*C. rotundus* was collected at the flowering stage in July from Oued-souf region located in the south of Sahara (Algeria). By collaboration with botanists at the Setif’s university, rhizomes of *C. rotundus* were identified by Pr. Paul ozenda (1920–2019), a French botanist, specialist in the plant population of the Sahara, and the Alpine massif (Paul, 1983) who was a professor at the faculty of sciences of Algiers and the French botanist Gaston Bonnier (1853–1922) (Bonnier & Cypéracées, 1990).

2.2. Plant processing

The plant roots were air-dried, grounded and submitted to hydro-distillation using a Clevenger-type apparatus (100 g, 3 h). The oil percentage content was calculated based on the
2.3. Oil composition analysis

The chemical structure of the essential oil was established by GC and GC-MS analyzes. A Varian CP-3800 gas chromatograph with a DB-5 capillary column (30 m × 0.25 mm; film thickness 0.25 μm) and a Varian Saturn 2000 ion trap mass detector was used. These analyzes were carried out under the following analytical conditions: temperatures of the injection and transfer line of 220 and 240°C; oven temperatures from 60 to 240°C at 3°C/min; helium carrier gas at 1 ml/min; injection of 0.2 μL (10% hexane solution); split ratio 1:30. The component identification method is based on the comparison of retention times with those of authentic samples. Further analysis was performed by comparing their linear retention indices related to n-hydrocarbon series and data processors to commercial (NIST, 98 and Adams) and library mass spectra based on pure substances and ingredients of recognized oils and MS literature data (Adams, 2007; Lafferty, 1998). GC/CIMS, with MeOH as the IC ionizing gas was used to analyze the molecular weights of all components.

2.4. Molecular properties of the lead compounds

The physicochemical and pharmacological properties such as partition coefficient (LogP(octanol/water)) value, molecular weight, number of hydrogen bond acceptors (HBAs), number of hydrogen bond donors (HBDs) and number of rotatable bonds for each abundant compound were analysed. Lipinski’s rule of five (Lipinski et al., 1997) was used to evaluate the potential use of the terpenoids as drugs.

2.5. In silico docking and interaction analysis

Successful structure-based modelling projects demand not only accurate software, but accurate starting structures as well. As a crucial step to meet minimum requirements for further computational calculations, all the studied target proteins and ligands were carefully prepared. And based on the empirical scoring function best conformations corresponding to the lowest energy for each component, were chosen following the docking procedure (de Lima et al., 2016; Kuca et al., 2018).

Schrödinger’s Protein Preparation Wizard was used for the protein preparations, as an initial step, high-resolution protein crystal structures of to the crystal structures of 5-lipoxygenase (5-LO, PDB code: 3O8Y) and leukotriene (LTA4H, PDB code: 3CHR) obtained from the Protein Data Bank (PDB, www.rcsb.org). The missing side chains were constructed to prepare the proteins, while removing the water molecules and ligands and adding hydrogen atoms. The protein geometry was further optimized using the OPLS-AA force field (Jorgensen et al., 1996). The chemical structures of natural products were downloaded from PubChem (Bolton et al., 2008) in the SDF-files format. The molecular structures were subjected to ligand preparation using LigPrep. Ionization/tautomer states were generated; the ligands were set at target pH = 7.4. During the process, the ligands were converted to 3D with the OPLS2005 force field.

To validate our results for the potent inhibition of LTA4H, we examined published X-ray data of LTA4H bound to SC57461A (Askonas et al., 2002; Calişkan & Banoglu, 2013; Kachur et al., 2002) and DG-051 (Davies et al., 2009; Enache et al., 2009; Sandanayaka et al., 2010). Both are potent clinical inhibitors of LTA4H with those of extracted phytochemical compounds studied herein, focusing on the active site of the enzyme one can see that both SC57461A and DG-051 encroach into the same site of the docked complexes Humulene oxide II, Spathulenol, Myrtenol and terpeneol by hydrogen bonding involved in the interaction with TYR383 and GLN136 (Supplementary Figure 1). Previous reports have cited the potential of ARM1 as a selective LTA4H inhibitor (Stsiapanava et al., 2014). In the published X-ray crystallography structure of Pro–Gly–Pro analogue (4M56.PDB) within the active site of LTA4H, the compound was oriented in the same target protein residues with actives compounds Myrtenol and Terpinol and formed common residues involved in the interaction as GLU318, TYR383 and GLY269, GLU271, respectively (Supplementary Figure 1).

The terpenoids were docked to the crystal structures using CLC Drug Discovery Workbench software (Version 2.5, CLC Bio, Boston, MA, USA). We used CLC drug discovery, which was recently introduced and gained attention among medicinal chemists (Kaushik et al., 2014). Bench mark results of CLC drug discovery software provide very accurate predictions of ligand-binding modes (83.0%) (Hartshorn et al., 2007) compared with other docking software such as Glide SP (81.8%) (Green et al., 2012), AutoDock (78%) (Forli & Olson, 2012), FlexX-HYDE (75%) (Green et al., 2012), FRED (70%) (Green et al., 2012). The docking score used the PLANTS/plp algorithm (protein–ligand ANT system) (Korb et al., 2009). This score has a good balance between accuracy and evaluation time. Before setting up the docking target, potential binding pockets were determined by applying the ‘Find Binding Pocket’ function. The predicted binding pocket that includes the receptor’s active site was selected as the center of the binding site, which had a radius of 13Å (Figure 1).

The number of docking poses for each ligand was set to 100. The conformation of the ligand was set as flexible, while the protein was kept as a rigid structure. After docking, only the best PLANTSplp scoring binding mode was returned for each ligand. The amino-acid residues interacting with the terpenoid and the binding affinities were predicted based on the docking score, hydrogen bonding and hydrophobic interactions.

2.6. Molecular dynamic simulations of the selected docked complexes

To validate the selected docked complexes and ensure that the topologies are consistent, molecular dynamics simulations were carried out with Molecular Operating
Environment (MOE) (Chemical Computing Group Inc., 1010 Sherbooke St. West, Suite 910, Montreal, QC, Canada H3A 2R7). The system was set up for simulation using a predefined water model as an explicit solvent, where the complexes were neutralized by adding Na\(^+\) and Cl\(^-\) counter ions to balance the net charges of the system. All complexes were described using the Amber99 force field (Showalter & Bruschweiler, 2007). A 100 ps equilibration and thermalization run was conducted within the isothermal–isobaric ensemble produced by the Nosé–Poincaré–Anderson (NPA) algorithm under NPT ensemble at 300 K and 1 atm. The final production run was propagated for 20 ns with an integration step of 2 fs, and an interval of 250 ps was employed for trajectory sampling. The RMSD trajectories of the simulation were analysed using the VMD software (Humphrey et al., 1996). The Ramachandran plot was used to analyze the backbone conformation of complexes structures and verify that the main chain torsion angles (\(\psi\), \(\psi\)) are stereochemically feasible (Ramachandran et al., 1963). These diagrams were displayed using rampage online server (Lovell et al., 2003)

\[
\text{RMSD}(t_1, t_2) = \sqrt{\frac{1}{N} \sum_{i=1}^{N} \sum_{j=1}^{N} m_i [r_i(t_1) - r_i(t_2)]^2}
\]

3. Results and discussion

3.1. Analysis of the composition of C. rotundus essential oil

Essential oil extraction from air-dried aerial parts provided an oil yield of 2.7%. Volatile constituents, analysed using GC and GC–MS, revealed the presence of 35 compounds accounting for up to 92.7% of the total oil composition (Table 1).

The oil contained eight monoterpenes (11.8%), eight sesquiterpenes (6.5%), 12 oxygenated monoterpenes (30.2%) and seven oxygenated sesquiterpenes (44.2%) (Table 1). Humulene oxide II (21.3%), caryophyllene oxide (13.3%), trans-pinocarveol (9.6%), myrtenol (6.5%), \(\alpha\)-pinene (4.6%), \(\beta\)-pinene (4.6%), verbenone (2.8%), khusinol (2.7%), agarospirole (2.6%), spathulhol (2.3%), \(\alpha\)-terpineol (1.9%), pinocarvone (1.4%), cyperene (1.4%), \(\alpha\)-humulene (1.3%) and \(\alpha\)-bulsone (1.1%) have been found to be the major components present in the oil. Oladipupo and Adebola (2009) examined the essential oil composition obtained from C. rotundus L. grown in South Africa. The oil contained

![Figure 1. 3D structure and binding sites with 'find binding pockets' settings of proteins 5-LO (PDB ID: 3O8Y) (a), and LTA4H (PDB ID: 3CHR) (b). The binding site volume is indicated as transparent green spheres.](image-url)

Table 1. Oil composition (%) of root Cyperus rotundus.

| No. | Constituents                      | RI     | Proportion (%) |
|-----|----------------------------------|--------|----------------|
| 1   | \(\alpha\)-Pinene                 | 941    | 4.6            |
| 2   | Thuja-2,4(10)-diene              | 959    | 0.5            |
| 3   | \(\beta\)-Pinene                  | 981    | 4.6            |
| 4   | p-Cymene                         | 1028   | 0.4            |
| 5   | Limonene                         | 1032   | 1.1            |
| 6   | Terpinolene                      | 1090   | 0.6            |
| 7   | Dehydrosabinaketone              | 1119   | 0.4            |
| 8   | \(\alpha\)-Campholenal            | 1127   | 0.5            |
| 9   | 1,8-Cineole                      | 1034   | 0.6            |
| 10  | Trans-pinocarveol                | 1140   | 9.6            |
| 11  | Cis-verbenol                     | 1142   | 0.9            |
| 12  | Pinocarvone                      | 1164   | 1.4            |
| 13  | p-Mentha-1,5-dien-8-ol           | 1168   | 1.4            |
| 14  | 4-Terpine                        | 1178   | 1.1            |
| 15  | p-Cymen-8-ol                     | 1184   | 0.9            |
| 16  | \(\alpha\)-Terpineol             | 1192   | 1.9            |
| 17  | Myrtenol                         | 1195   | 6.5            |
| 18  | Verbenone                        | 1206   | 2.8            |
| 19  | Trans-carveol                    | 1218   | 1.1            |
| 20  | Carvone                          | 1244   | 1.1            |
| 21  | \(\alpha\)-Copaene                | 1377   | 0.7            |
| 22  | \(\beta\)-Elemene                 | 1392   | 0.2            |
| 23  | Cyperene                         | 1398   | 1.4            |
| 24  | \(\beta\)-Caryophyllene           | 1419   | 0.4            |
| 25  | \(\alpha\)-Guaiene                | 1440   | 0.7            |
| 26  | \(\alpha\)-Humulene               | 1455   | 1.3            |
| 27  | \(\gamma\)-Murolene               | 1478   | 0.5            |
| 28  | \(\alpha\)-Bulsene                | 1506   | 1.0            |
| 29  | Spathulenol                      | 1577   | 2.3            |
| 30  | Caryophyllene oxide              | 1582   | 13.3           |
| 31  | Humulene oxide II                | 1608   | 21.3           |
| 32  | Caryophylla-4(14),8(15)-dien-5-ol| 1637   | 1.3            |
| 33  | T-murolol                        | 1642   | 0.7            |
| 34  | Agarospirole                     | 1647   | 2.6            |
| 35  | Khusinol                         | 1673   | 2.7            |

Total identified %: 92.7.

*Retention index relative to n-alkanes on DB-5 capillary column.
monoterpene (30.4%), including β-pinene (11.3%), α-pinene (10.8%), α-cyperone (7.9%) and Myrtenol (7.1%) as the main constituents of the total oil. In 2018, Ilham et al. (2018) evaluated the chemical composition of Cyperus rotundus EO from Turkey. Cyperene (30.5%), α-copaene (10.6%) and α-ylangene (7.7%) were found to be the main constituents. This variability may be related to genetic factors, determining the chemical polymorphism among the species, and physiological and environmental factors, namely climatic factors, soil composition, plant organs, plant age, seasonality and circadian cycle, which qualitatively and quantitatively affect the essential oil composition (De Souza et al., 2018).

3.2. Molecular properties of selected compounds

Chemical structures of the compounds selected for computational studies are shown in Figure 2.

Assessment of the drug-like properties indicated that all compounds comply with the Lipinski’s Rule of Five stating that: no more than five hydrogen bond donors, no more than 10 hydrogen bond acceptors, no more than 15 rotatable bonds, a molecular weight under 500 g/mol, and Log P (with P being the partition coefficient) less than 5. These compounds are indeed small and rather hydrophobic. For the macrocyclic compounds like humulene oxide II and caryophyllene oxide, the macrocycle was considered rigid after energy minimization to simplify the docking process. The results are given in Table 2.

3.3. Docking analysis

3.3.1. Docking of selected natural compounds

Docking studies were performed to define binding pockets, inhibitor interactions with the two enzymes, and binding energies. Zileuton and Bestatin, which are clinically well-characterized inhibitors of 5-LO and LTA4 hydroxylase, respectively (Ichinose et al., 2003; Rao et al., 2007), were used as reference inhibitors for the present in silico studies. Results obtained for the two enzymes involved in the inflammatory pathway and the eight ligands are summarized in (Table 3).

The best-selected compounds and binding residues were examined using a CLC drug discovery based on the PLANTS_pSP scoring functions and hydrogen interactions. In our analysis, α-terpineol, trans-pinocarveol, spathulenol and

Figure 2. Chemical structures of selected compounds of essential oils in root Cyperus rotundus.

Table 2. Molecular properties of the principal constituents isolated from essential oils of Cyperus rotundus.

| No. | Compounds                  | Molecular mass (g/mol) | Hydrogen bond donors | Hydrogen bond acceptors | XLogP | Rotatable bonds |
|-----|----------------------------|------------------------|----------------------|-------------------------|-------|----------------|
| 1   | Humulene oxide II          | 220.35                 | 0                    | 1                       | 3.85  | 0              |
| 2   | Caryophyllene oxide        | 220.35                 | 0                    | 1                       | 3.56  | 0              |
| 3   | Khusinol                   | 220.35                 | 1                    | 1                       | 2.92  | 1              |
| 4   | Agarospiroli               | 222.37                 | 1                    | 1                       | 3.65  | 1              |
| 5   | Spathulenol                | 220.35                 | 1                    | 1                       | 3.11  | 0              |
| 6   | Trans-pinocarveol          | 152.23                 | 1                    | 1                       | 1.79  | 0              |
| 7   | Myrtenol                   | 152.23                 | 1                    | 1                       | 1.6   | 1              |
| 8   | α-Terpineol                | 154.25                 | 1                    | 1                       | 2.60  | 1              |

Table 3. Selected bioactive components of essential oils and their docking score along with the score of H-bond formation with 5-LO and LTA4H.

| Compounds       | Docking score | H-bonds score | H-bonds interaction | H-bonds interaction |
|-----------------|---------------|---------------|---------------------|---------------------|
| Humulene oxide II | 25.88         | -2.00         | TYR383              | Zn²⁺/HIS295/GLU296  |
| Caryophyllene oxide | 10.17         | 1.27          | HIS367/GLN363       | TYR378             |
| Khusinol        | 39.24         | 3.16          | HIS367/GLN363       | Zn²⁺/HIS295/GLU296 |
| Spathulenol     | 37.52         | 3.11          | HIS367/GLN363       | TYR383/TYR378      |
| Agarospiroli    | 20.92         | 4.00          | HIS367/GLN363       | Zn²⁺/HIS295/GLU296 |
| Trans-pinocarveol | 36.85         | 4.70          | HIS367/GLN363       | ASP375/TYR267      |
| Myrtenol        | 35.31         | 2             | ILE673              | GLU271/GLN363      |
| α-Terpineol     | 42.20         | 8.43          | HIS367/GLN363/GLN557| GLU271/GLN363/GLY269|
| Drug            | 45.20         | 8.43          | HIS367/GLN363/GLN557| TYR383/TYR378/ARG563|
khusinol fitted well into the active pockets of the enzymes shown in Figure 1, indicating minimum docking energy values, and formed H-bond count by comparison to other compounds (Table 3). Interactions between these compounds and the amino acids of the catalytic sites of 5-LO and LTA4H are shown in Figures 3 and 4, respectively. In 5-LO, docking results showed that except Humulene oxide II and caryophyllene oxide, all other sesquiterpenes have similar interactions with the key amino-acid residues HIS 367 and GLN363. Alpha-terpineol exhibited an interaction mediated by H-bonds in the active sites of 5-LO and formed two H-bonds with HIS367 and GLN363 with the highest interaction energy ($\Delta E_{0} = 42.20$ kcal/mol). Khusinol and trans-pinocarveol also showed a high interaction energy ($\Delta E_{0} = 39.24$ kcal/mol and 36.85 kcal/mol, respectively), formed two of the H-bonds compared to Myrtenol (see Table 3). Spathulolin formed two H-bonds with GLN363 and one H-bond with HIS367 and exhibited a good docking score of $-37.52$ kcal/mol. Only the natural molecule Myrtenol interacted with the catalytic residue ILE673 with an interaction energy value $-35.31$ kcal/mol. Similarly, in LTA4H, $\alpha$-terpineol fits into the macromolecule's active site with the highest energy ($-43.67$ kcal/mol) and formed three H-bonds with GLU271, GLN136 and GLY269 of the active site. Myrtenol that forms the best complex with high interaction energy ($-37.40$ kcal/mol) is bound to Zn$^{2+}$ and has three H-bonds with HIS295, GLU318 and TYR383. Spathulenol shows a good interaction with coordination to Zn$^{2+}$ and forms two H-bonds with HIS295 and TYR383.

Khusinol is found to be a potential ligand, particularly in LTA4H with an interaction energy value of $-35.75$ kcal/mol.
It has coordination with Zn$^{2+}$ and two H-bonds with HIS295 and GLU296. The two other inhibitors, namely trans-pinocarveol and Agarospirol display two H-bonds with ASP375 and TYR367 and a docking score of $-44.45$ kcal/mol for the first one with only one H-bond with HIS295, and a high docking score of $-46.50$ kcal/mol for the second molecule.

### 3.3.2. Active interaction of phytochemical compounds with target proteins and binding sites

Docking results showed that the best interaction scores were obtained for α-terpineol and khusinol in 5-LO, and for Myrtenol and α-terpineol in LTA4H. Moreover, their binding sites are similar to those of Zileuton and Bestatin (Figure 5(a and b)).
For 5-LO, α-terpineol showed a hydrogen bonding with the active site residue at the carbonyl group of GLN363, at the nitrogen group of HIS367 and hydrophobic interactions with other residues. Interactions observed between α-terpineol and 5-LO involved 16 of the 20 residues of 5-LO interacting with Zileuton (Figure 6(b) and Table 4). Khusinol was oriented in the active site through hydrogen bonding of its hydroxyl branch with the nitrogen group of HIS367 and with the oxygen from the carbonyl group of GLN363 (H-bond score value of $-3.16 \text{ kcal/mol}$, Table 3), and through hydrophobic interactions with 18 other residues (Figure 6(a) and Table 4). Interactions observed between Khusinol and 5-LO involved 16 of the 20 residues of 5-LO interacting with Zileuton (Figure 6(b) and Table 4).
involved 16 of the 20 residues of 5-LO interacting with Zileuton.

In LTA4H, the best interaction set for the hydroxyl group of Myrtenol showed three hydrogen bonds as follows: the first one to the carbonyl oxygen of GLU318, the second one to the hydroxyl of TYR383 and the third one to the nitrogen group of HIS295, with an H-bond score of $-4.67 \text{ kcal/mol}$ (Table 3). Moreover, Myrtenol performs hydrophobic interactions with 14 other residues. Interactions observed between Myrtenol and LTA4H involved 13 of the 21 residues of LTA4H interacting with Bestatin (Figure 6(c) and Table 4). The hydroxyl branch of $\alpha$-terpineol suitably adjusted in the target protein LTA4H through three hydrogen bonds on the carbonyl oxygen of GLU271, GLN136 and GLY296, accompanied with an H-bond score of $-3.85 \text{ kcal/mol}$ (Table 3), and through hydrophobic interactions with 15 other residues. Interactions observed between $\alpha$-terpineol and LTA4H involved 14 of the 21 residues of LTA4H interacting with Bestatin (Table 4).
Tables and figures

3.4. Molecular dynamic simulations of the docked complexes

MD simulations were performed to determine the structural stability of α-terpineol–5LO and Myrtenol–LTA4H complexes. These complexes were selected from the structures corresponding to a minimum binding energy and then subjected to 20 ns MD simulations. The RMSD of the backbone protein for the α-terpineol–3O8Y complex presented the lowest average value of 0.020–0.044 Å range Figure 7, followed by the spathulenol and pinocarveol fitted in 5-lipoxygenase with an average RMSD = 0.022 and 0.026 Å, respectively. The complex of Khusinol–3O8Y showed the highest RMSD range and reached a 0.052–0.126 Å (Table 5). Where in LTA4H, the RMSD indicated that the Khusinol complex shows the lowest deflection and remained within 0.028–0.063 Å range. Likewise, α-terpineol and spathulenol complexes at 20 ns reached stability with an average RMSD of 0.057 and 0.067 Å, respectively. The highest deviation occurred in the myrtenol complex and reached 0.068–0.112 Å range.

3.5. Discussion

In the interaction between the natural oxygenated terpenes of the essential oil of Cyperus rotundus and the two enzymes 5-LO and LTA4H, the catalytic site’s amino-acid residues interacting with the terpenes were compared to those involved in the interaction between these enzymes and commonly used inhibitors, namely Zileuton and Bestatin. The molecules α-terpineol, khusinol, trans-pinocarveol and spathulenol fitted well in the active pocket of 5-LO, making hydrogen bonds with HIS367 and GLN363. In addition, α-terpineol and khusinol, selected for their best interaction scores, showed the same binding site as Zileuton. On the other hand, Myrtenol interacted with another catalytic site residue, ILE673. It has been demonstrated that HIS367, GLN363 and ILE673 are potential key residues for 5-LO activity. The α-amino group of HIS367 is coordinated to Fe²⁺, making a hydrogen bond with the hydroxyl groups of α-terpineol, khusinol, trans-pinocarveol and spathulenol. The acetate group of ILE673 is bound to Fe²⁺ and to the carbonyl group of Myrtenol. HIS367 and ILE673 are iron-coordinating residues (Rådmark et al., 2015), and their most likely effect appears to be on the orientation of ligands in the active site of 5-LO, it was suggested that HIS367 and ILE673 act as replaceable iron ligands (Gilbert et al., 2011). The oxygenated terpenes α-terpineol, khusinol, trans-pinocarveol and spathulenol occupied the same enzyme pocket as Zileuton with the same hydrogen bonding, which makes them good inhibitors of epoxide hydrolase.

Moreover, selected natural products α-terpineol and khusinol have hydrophobic interaction, particularly with PHE177 and TYR181, acting as cork for the active site (Rådmark et al., 2015). Ligands should enclose a ‘cage’ around the iron atom with the coordinating residues. This cork may hinder access to the catalytic site for any other molecule.

In the case of LTA4H, Zn²⁺, this latter is coordinated to GLU318 and HIS295 which bind the hydroxyl oxygen atom of khusinol, spathulenol, agarospirol and Myrtenol. This suitable position suggests that these terpenes are strong LTA4H inhibitors. Accordingly, the crystal structure of LTA4H was found to contain three proposed binding-zinc amino-acid residues, HIS295, HIS299 and GLU318 (Medina et al., 1991). Close to the prosthetic zinc, the catalytic residues TYR383 and GLU296 are bound to Myrtenol, spathulenol, humulene oxide II and khusinol. In previous studies, it was found that the catalytic residues TYR383 and GLU296 serve as a proton donor and possibly play an important role in the aminopeptidase activities of LTA4H (Blomster et al., 1995; Wetterholm et al., 1992). A glutamic acid residue GLU271 forms in the catalytic zinc vicinity a hydrogen bond with α-terpineol which contributes to both catalytic activities (epoxide and peptidase) of LTA4H (Rudberg et al., 2002). In addition, the structural determinant TYR378, which is responsible for suicide inactivation of LTA4H (Mueller et al., 1996), forms a hydrogen bond with the oxygen atom of humulene oxide II and caryophyllene oxide. ASP375 is essential only for the reaction of epoxide hydrolase (Rudberg et al., 2002). Its N-terminal group is linked to the hydroxyl oxygen atom of trans-pinocarveol. It is concluded that the inhibitor trans-pinocarveol is specific for both hydrolase and aminopeptidase activities.

Many experimental studies, along with computational ones, have been carried out on 5-LO and LTA4H. Recently, various inhibitors of 5-LO and LTA4H were reported in the literature. It was found that a series of 1,4-benzoquinones interacted with His367, Gln557, Tyr181 and Asn425 (Schaible et al., 2014). Using the same approach, miconidin acetate (MA), a natural compound from the flower bud extract of Eugenia hiemalis which has an anti-inflammatory activity by inhibition of 5-lipoxygenase (IC₅₀ = 0.3 ± 0.17 µM) (Zatelli et al., 2016), employing molecular docking, MA has been found to occupy part of the active site. It formed a hydrogen bond with the iron-coordinating residue ILE676 and hydrophobic contacts in the tunnel leading to the catalytic center (Zatelli et al., 2016). Boudreau et al. investigated structural analogues of bioactive components caffeic acid phenethyl ester, a naturally-occurring 5-lipoxygenase inhibitor. It was observed directly bound to 5-LO, forming hydrogen bonds interaction with His367 and ASN407 (Boudreau et al., 2017). UI-Haq et al. reported that using molecular structures of thiazolone analogues of 5-LO inhibitors showed that strong hydrogen bonding was formed between the carbonyl oxygen of the thiazolone ring and GLN363 (UI-Haq et al., 2016). The structures of [6]-gingerol and its derivatives were obtained from Zingiber officinale by extraction. In the computational study

| Complexes    | RMSD (Å) | Ramachandran plot region (%) |
|--------------|---------|-----------------------------|
|              | rmsdav  | rmsdmax | ramsdav | ramsdmax |
| Khusinol–3O8Y | 0.052   | 0.126   | 84.6    | 15.4    | 0.0 |
| Spathulinol–3O8Y | 0.022   | 0.049   | 83.3    | 16.7    | 0.0 |
| Pinocarveol–3O8Y | 0.026   | 0.061   | 83.3    | 16.7    | 0.0 |
| α-Terpineol–3O8Y | 0.020   | 0.044   | 84.6    | 15.4    | 0.0 |
| Khusinol–3CHR | 0.028   | 0.063   | 100     | 0.0     | 0.0 |
| Spathulinol–3CHR | 0.057   | 0.106   | 95      | 5       | 0.0 |
| Myrtenol–3CHR | 0.068   | 0.112   | 88.2    | 11.8    | 0.0 |
| α-Terpineol–3CHR | 0.046   | 0.078   | 90.9    | 9.1     | 0.0 |
of the [6]-gingerol and other gingerol derivatives inhibition of LTA4H, it was found that the phenolic OH group in [6]-gingerol is essential for inhibiting both activities of the LTA4H due to its coordination with zinc metal, its hydrogen bond with ASP375 and the hydrogen bonding between its carbonyl oxygen with GLN134. In an in silico prediction using a reverse-docking approach, [6]-gingerol gave similar inhibition results to Bestatin. It was found that, with its functional groups, seems to be able to participate in the coordination of the zinc ion with its hydroxyl group to form a hydrogen bond with GLU271 and revealed that LTA4H might be a potential target of [6]-gingerol (Jeong et al., 2009). Another computational virtual screening of a series of resveratrol (natural compounds) against LTA4H revealed that the phenolic hydroxyl formed a hydrogen bond with ASP375 (Low et al., 2017).

In this study, the selected phytochemicals gave multiple interactions with enzymes similar to Zileuton and Bestatin. The confirmation obtained after docking showed the best score for some of the components such as α-terpineneol, myrenol and khusinol and a strong interaction to inhibit the targeted pathway compared to agarospirol and spathulenol.

These properties of the terpenoid action could be due to their isoprene units. The chain’s extension increases the number of cyclizations according to their oxygenated functional groups, which allows a wide chemical reaction. Based on the RMSD measurement and protein–ligand contacts during MD simulation, the slightly higher deviation showed that the mean backbone RMSD varied between 0.052 and 0.126 Å for the 3O8Y–kusinol complex and between 0.057 and 0.106 Å for the 3CHR–pathulolinol complex. The fluctuation of RMSD results from the two bicyclic compounds’ structural geometry due to the rotation of its functional groups, which try to integrate well in the restricted binding pocket to adopt a favourable conformation. In this state, inhibitors must exhibit more flexibility than other inhibitors to achieve stability by targeting the catalytic pocket over the period of molecular dynamics, which is represented in RMSD analysis by low RMSD values. The 5-LO and LTA4H did not undergo any remarkable change during the simulation time, indicating that the skeletons are stabilized in the active sites, which is owed to hydrogen bonds between the hydroxylated hydrogen atom of the selected terpenes’ molecular structures and the amino acids located near the metal cations. The Van der Waals interactions, which form a hydrophobic pocket provided in the light of conformational stability and the unusual change of interatomic contacts, can be limited to the rigid protein docking and lead to better protein–ligand interactions.

One key ingredient of molecular dynamic approach analysis is to extract pertinent information from conformational ensembles, typically generated by relatively short (tens of ns) MD simulations (Karami et al., 2018). By using this formalism, one could rationalize long-range conformational changes effects and ensures the applicability of the method at large scale, in a computationally tractable way (Karami et al., 2018). There are several examples in the literature where MD simulations, even of only a few tens of nanoseconds. Mohd Amin et al. (2020) performed a 25 ns MD simulation of 5-LO complexed with chalcone and flavone derivatives using Desmond tool, RMSD plot for the backbone atoms revealed that the average RMSD values of 5-LO corresponding to selected compounds was 2 Å and more stable throughout the simulation. Singh et al. (2017) conducted also a 25 ns MD analysis for the 5-LO with each of the selected flavonoids. The event trajectory remained stable and exhibiting least RMSD value (0.285 ± 0.007 nm). Another report showed that the stability of LTA4H–pyrrolidine complex during MD simulation was monitored RMSD calculation. The root mean square deviation has converged at around 2.4 Å within production time 5 ns (Thangapandian et al., 2012). Torres et al. showed that the LTA4H–alpha lipoic acid complex at studied time 20 ns, the system remained stable and RMSD value displayed at around 2 Å (Torres et al., 2017).

Finally, a wide variety of computational methods allow to select a promising target-ligand from docking simulation and provide a starting point for MD simulation and have also focused more attention on structural conformations to ensure stability in allosteric binding sites (de Lima et al., 2016). Many of these for examples, scoring function methods. A typical scoring function would be able to predict the binding free energy of the protein–ligand complex and simultaneously should be fast enough to allow its application to the virtual screening studies. Also, scoring functions based on protein-ligand interactions have emerged as promising surrogates of the classical scoring functions (Ballester & Mitchell, 2010; Khamis et al., 2015; Wójcikowski et al., 2017). Furthermore, the binding affinities of top-ranked docking poses can be more accurately predicted via end-point free energy calculations such as molecular mechanics Poisson–Boltzmann or generalized Born surface area (MM/PBSA and MM/GBSA), coupled with molecular dynamic simulations (Genheden & Ryde, 2015; Homeyer & Gohlke, 2012; Hou et al., 2011). Quantum mechanics/molecular mechanics, QM/MM, are frequently used to study reactions that involve changes to covalent bonds (Senn & Thiel, 2009), in which a larger part of the system was modelled using the electronic degrees of quantum chemical approach with MM calculations, increasing performance and decreasing computational demand (Heyden et al., 2007). In recent study was developed a novel approach semi-empirical quantum mechanics scoring function methods SQM that successfully reproduces protein–ligand geometries also in cases where a halogen bond has improved the protein–ligand geometries as well as halogen-bond features, which makes it a promising tool for future computer-aided drug development (Kolár & Hobza, 2012; Kolár et al., 2013). It has to be noted that due to the simplicity of the model used, the significant improvement of the protein–ligand geometries was achieved without significant additional computational cost. Other researchers have been using SQM methods for drug-design purposes as well as Gilson et al. combined the PM6-DH + method with the COSMO implicit solvation model to rescoring 29 docked complexes in a cucurbit [7]uril host (Muddana & Gilson, 2012). The method combined with the mining minima (MM2) algorithm was able to reproduce successfully the experimental
binding energies. Ryde et al. used the SQM methods (AM1, RM1 and PM6) coupled with the MD/MM-GB/PBSA setup for protein–ligand-binding affinity predictions. They rescored events trajectories from MD simulations of 25 protein–ligand complexes. Overall, these methods showed a very good performance (Mikulski et al., 2012). Geometric complementarity, the geometric matching plays a key role in determining the structure of a complex (Connolly, 1986; Kuntz et al., 1982; Norel et al., 1994; Zielenkiewicz & Rabczenko, 1984). The three dimensional structures of most protein–ligand complexes reveal a close geometric match between interfaces of a receptor and a ligand (Fischer et al., 1995; Tsai et al., 1998). The scoring functions used practically exclusively geometric complementarity criteria (Connolly, 1986; Norel et al., 1994; Shoichet et al., 1992). In the presumption, a geometric complementarity between the complementarity interfaces of the macromolecular target and ligand is indicative of areas proving the best geometric fit (Sobolev et al., 1996). Additionally, with the advancement in computational approaches for the accurate predictions of interactions formed between drug and macromolecular target spots, it has become state of the art to be a fundamental job during the process the drug finding, of among these, computational chemogenomic approaches and proteochemometric modelling (PCM) include the two computational approaches for target-ligand-based drug–target interaction prediction, which integrate both the structural information of the compounds as well as the genomic space of target proteins in a single machine learning model. In chemogenomics, an emerging discipline area focused on the systematic examination of the biological impact of a broad series of minute molecular-weighting ligands with high quality chemical probes on a broad range of targets families. Therefore, the modulation of the target by the active compound induces a specific phenotype. Recently, Rodrigues et al. generated drug–protein complexes workflow with random forest model and it was successfully used to characterize b-lapachone as an allosertic 5-lipoxygenase inhibitor (Rodrigues et al., 2018). Proteochemometric modelling enables both interpolation and extrapolation to novel compounds and novel proteins and can fulfill the requirement in hit identification of orphan targets (Ain et al., 2014; Cortés-Ciriano et al., 2015). PCM can supply advantages in identification for novel allosteric inhibitors. Considering the induced-fit interaction between compounds and target, PCM allows discrimination between different protein conformations and binding modes (Tresadern et al., 2017). Burggraaff et al., (2019) recently predicted inhibitors for sodium-dependent glucose co-transporter 1, by fulfillment of ligand- and protein-based information into random forest models using PCM approach. The authors used a database of natural products and synthetic compounds. Thirty (30) out of 77 identified potential compounds were validated in vitro, showing low micromolar activities. Hence, docking can be used to model drug–protein complexes, involving use of the enhanced proteochemometric and chemogenomic methodologies, lead to explain the intricate mode of action for chemical compounds as well as repositioning them for new therapeutic applications (Gribble, 2012).

4. Conclusions

This study provided accurate experimental information regarding the composition of the roots of the Saharian medicinal plant C. rotundus. The natural products were identified by GC/GC–MS and accounted for 92.7% of the oil extract. Moreover, computational studies indicated that some essential oil molecules such as α-terpinel, trans-pinocarveol, Myrtenol and khusinol showed interesting potential inhibitory activity against 5-LO and LTA4H. We have demonstrated the effective binding of several essential oil components to the target enzymes, especially oxygenated sesquiterpenes and monoterpenes compounds. This study indicated that the interactions with the amino-acid residues HIS367, ILE673 and GLN363 are critical for the inhibition of 5-LO. Interactions with HIS295, GLU318, GLU296, GLU271, TYR383, TYR378 and ASP375 are essential for inhibiting LTA4H. The selected molecules’ inhibitory activities need to be further confirmed in vitro and in vivo to consider these molecules as lead compounds for drug development.

Disclosure statement

No potential conflict of interest was reported by the authors.

ORCID

Yacine Benguerba http://orcid.org/0000-0002-8251-9724

References

Adams, R. P. (2007). Identification of essential oil components by gas chromatography/mass spectrometry (4th ed.). Allured Publishing Corporation.

Ain, Q. U., Méndez-Lucio, O., Ciriano, I. C., Malliavin, T., van Westen, G. J., & Bender, A. (2014). Modelling ligand selectivity of serine proteases using integrative proteochemometric approaches improves model performance and allows the multi-target dependent interpretation of features. Integrative Biology: Quantitative Biosciences from Nano to Macro, 6(11), 1023–1033. https://doi.org/10.1039/c4ib00175c

Agil, M., Ahad, A., Sultana, Y., & Ali, A. (2007). Status of terpenes as skin penetration enhancers. Drug Discovery Today, 12(23–24), 1061–1067. https://doi.org/10.1016/j.drudis.2007.09.001

Askonas, L. J., Kachur, J. F., Villani-Price, D., Liang, C. D. D., Russell, M. A., & Smith, W. G. (2002). Pharmacological characterization of SC-57461A (3-[methyl(3-{4-(phenylmethyl)phenoxy}propyl)amino]propanoic acid HCl), a potent and selective inhibitor of leukotriene A(4) hydrolase I: In vitro studies. The Journal of Pharmacology and Experimental Therapeutics, 300(2), 577–582. https://doi.org/10.1124/jpet.300.2.577

Atkins, P. B., Dube, L. M., Walton-Bowen, K., Cameron, C. M., & Kasten, L. E. (2007). Clinical pattern of zileuton-associated liver injury: Results of a 12-month study in patients with chronic asthma. Drug Safety, 30(9), 805–815. https://doi.org/10.2165/00002018-200730090-00006

Badria, F. A., El-Naggar, M. H., Abdel Bar, F. M., & Amer, M. M. (2014). New gingerol derivative and other related compounds from Zingiber officinale. Journal of Drug Discovery and Therapeutics, 2, 53–59.

Ballester, P. J., & Mitchell, J. B. (2010). A machine learning approach to predicting protein–ligand binding affinity with applications to molecular docking. Bioinformatics (Oxford, England), 26(9), 1169–1175. https://doi.org/10.1093/bioinformatics/btq112

Barnes, P. J., & Adcock, I. M. (2003). How do corticosteroids work in asthma? Annals of Internal Medicine, 139(5 Pt 1), 359–370. https://doi.org/10.7326/0003-4819-139-5_part_1-200309020-00012
formation: Hevein-32 domain. *Scientific Reports*, 9, 1–7. https://doi.org/10.1038/s41598-019-33815-w

Sonwa, M. M., & K. (1998). Protein folding via binding and /C19 Tresadern, G., Trabanco, A. A., P. (2017). Abrogation of the peptidase activity by mutation of glutamic acid-Vallee, B. L., & Samuelsson, B. (1992). Leukotriene A4 hydrolase: Identification of allostERIC modulators of metabotropic glutamate 7 receptor using proteochemometric modeling. *Journal of Chemical Information and Modeling*, 57(12), 2976–2985. https://doi.org/10.1021/acs.jcim.7b00338

Torres, M. J., Fierro, A., Pessoa-Mahana, C. D., Romero-Parra, J., Cabrera, G., & Faundez, M. (2017). Effect of alpha lipoic acid on leukotriene A4 hydrolase. *European Journal of Pharmacology*, 799, 41–47. https://doi.org/10.1016/j.ejphar.2017.01.038

Tresadern, G., Trabanco, A. A., Pérez-Benito, L., Overington, J. P., van Vlijmen, H. W. T., & van Westen, G. J. P. (2017). Identification of allostERIC modulators of metabotropic glutamate 7 receptor using proteochemometric modeling. *Journal of Chemical Information and Modeling*, 57(12), 2976–2985. https://doi.org/10.1021/acs.jcim.7b00338

Tsai, C. J., Xu, D., & Nussinov, R. (1998). Protein folding via binding and vice versa. *Folding & Design*, 3(4), R71–R80. https://doi.org/10.1016/S1359-0278(98)00032-7

Tsuji, F., Aono, H., Tsuboi, T., Murakami, T., Enomoto, H., Mizutani, K., & Inagaki, N. (2010). Role of leukotriene B4 in 5-lipoxygenase metabolite- and allergy-induced itch-associated responses in mice. *Biological & Pharmaceutical Bulletin*, 33(6), 1050–1053. https://doi.org/10.1248/bpb.33.1050

Uddin, S. J., Mondal, K., Shilpi, J. A., & Rahman, M. T. (2006). Antidiarrhoeal activity of *Cyperus rotundus*. *Fitoterapia*, 77(2), 134–136. https://doi.org/10.1016/j.fitote.2004.11.011

Ul-Haq, Z., Khan, N., Zafar, S. K., & Moin, S. T. (2016). Active site characterization and structure based 3D-QSAR studies on non-redox type 5-lipoxygenase inhibitors. *European Journal of Pharmaceutical Sciences: Official Journal of the European Federation for Pharmaceutical Sciences*, 88, 26–36. https://doi.org/10.1016/j.ejps.2016.03.014

Verma, S. S., Rai, V., Awasthee, N., Dhasmana, A., Rajalaksmi, D. S., Nair, M. S., & Gupta, S. C. (2019). Isoehelephantopin, a sesquiterpene lactone induces ROS generation, suppresses NF-kB activation, modulates LncRNA expression and exhibit activities against breast cancer. *Scientific Reports*, 9, 1–16. https://doi.org/10.1038/s41598-019-52971-3

Wetterholm, A., Medina, J. F., Rådmark, O., Shapiro, R., Haeggström, J. Z., Vallee, B. L., & Samuelsson, B. (1992). Leukotriene A4 hydrolase: Abrogation of the peptidase activity by mutation of glutamic acid-296. *Proceedings of the National Academy of Sciences of the United States of America*, 89(19), 9141–9145. https://doi.org/10.1073/pnas.89.19.9141

Wills, G. D. (1998). Comparison of purple nutsedge (Cyperus rotundus) from around the world. *Weed Technology*, 12(3), 491–503. https://doi.org/10.1017/S0890037X00044201

Wójcikowski, M., Ballester, P. J., & Siedlecki, P. (2017). Performance of machine-learning scoring functions in structure-based virtual screening. *Scientific Reports*, 7, 46710–46710. https://doi.org/10.1038/srep46710

Yadav, D. K., Mudgal, V., Agrawal, J., Maurya, A. K., Bawankule, D. U., Chanotiya, C. S., Khan, F., & Thul, S. T. (2013). Molecular docking and ADME studies of natural compounds of Agarwood oil for topical anti-inflammatory activity. *Current Computer-Aided Drug Design*, 9(3), 360–370. https://doi.org/10.2174/1573409911309030012

Zatelli, G. A., Temml, V., Kutil, Z., Landa, P., Vanek, T., Schuster, D., & Falkenberg, M. (2016). Miconidin acetate and primin as potent 5-lipoxygenase inhibitors from Brazilian *Eugenia hiemalis* (Myrtaceae). *Planta Medica Letters*, 3, 17–19.

Zhu, M., Luk, H. H., Fung, H. S., & Luk, C. T. (1997). Cytoprotective effects of *Cyperus rotundus* against ethanol induced gastric ulceration in rats. *Phytotherapy Research*, 11(5), 392–394. https://doi.org/10.1002/(SICI)1099-1573(199708)11:5<392::AID-PTR113>3.0.CO;2-1

Zielenkiewicz, P., & Rabczenko, A. (1984). Protein–protein recognition: Method of finding complementary surfaces of interacting proteins. *Journal of Theoretical Biology*, 111(1), 17–30. https://doi.org/10.1016/S0022-5193(84)80193-9