Research Article

Hacettepe University Journal of the Faculty of Pharmacy
Volume 44 / Number 2 / June 2024 / pp. 131-142

Evaluation of Anti-inflammatory Activity and Identification of a Monoterpenoidhydroxylactone (-)-loliolide from *Tribulus terrestris* L.: *In vivo* and *In silico* Approaches

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Received date : 23.09.2022
Accepted date : 08.08.2023

DOI: 10.52794/hujpharm.1179460

ABSTRACT

The management and reduction of inflammatory incidents often require medications that can cause adverse effects. Therefore, it is crucial to discover natural anti-inflammatory substances that can offer improved therapeutic outcomes while decreasing the likelihood of adverse reactions. The main objective of this study was to evaluate the anti-inflammatory properties of *Tribulus terrestris* L (aerial parts) and identify the anti-inflammatory compounds present in the active extracts and fractions. The mechanism of action and ADMET properties of these compounds were predicted through *in silico* analysis. The efficacy of various extracts in reducing inflammation was assessed in rats with carrageenan-induced edema. The phytoconstituents of the active fraction were identified using Thermo Scientific DFS high-resolution GC-MS. GC-MS analysis revealed 13 compounds, with (−)-loliolide being the most abundant by peak area. Autodock 4.0 was employed to assess the binding affinity of the compound to three crucial enzymes implicated in the inflammatory response, namely cyclooxygenase (COX 1 and 2) and 5-lipooxygenase (5-LOX). It showed good binding energies which are lower than standard compounds. The favorable binding energy, drug-like qualities, and favorable pharmacokinetic parameters of (−)-loliolide indicate that it could be an effective inhibitor, but additional research is necessary to confirm its potential.

Keywords: (-)-loliolide, *Tribulus terrestris* L., anti-inflammatory, GC-MS analysis, COX.

1. Introduction

Inflammation is a pathophysiological response in which several mediators are activated, resulting in the accumulation of extravasated fluid rich in proteins and leukocytes, leading to edema [1]. It could be acute inflammation, which is self-limiting, or chronic inflammation, if the tissue fails to completely resolve the problem at the inflammatory site [2]. Therefore, the treatment strategy for inflammation is based on the prevention of inflammatory mediators [1].

Steroids and non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used anti-inflammatory agents [3]. Glucocorticoids, a type of steroids, are effective in reducing inflammation by binding to cortisol receptors or inhibiting phospholipase enzymes. However, their use is often restricted owing to the potential side effects associated with their use. These side effects include increased risk of infection, weight gain, and high blood sugar levels. Despite these limitations, corticosteroids remain a widely used medication for various conditions that require anti-inflammatory treatments. [3-4]. NSAIDs inhibit cyclooxygenase (COX) enzymes, which catalyze the formation of prostaglandins that are involved in inflammation. Unfortunately, the gastrointestinal effects of these drugs lead to their discontinuation in patients [5].

Secondary metabolites from plants have been an important source of various drugs since ancient times and approximately half of these drugs are derived from natural sources. Many of these compounds are widely prescribed for the treatment of inflammatory conditions [6]. Sudan has a vast variety of cultivated and natural vegetation z, owing to its unusually variable climate. These found their way into methods have been frequently and successfully utilized in folk medicine, particularly by locals in rural regions, to treat many human and animal ailments. Numerous medicinal plants have been extracted and utilized efficiently for the treatment of various inflammatory diseases, including asthma, arthritis, rheumatism, fever, edema, infections, and related ailments. Despite their widespread use, the efficacy of these plants has not been thoroughly evaluated or subjected to comprehensive scientific analysis [3].

Tribulus terrestris. L *(T. terrestris*) from Zygophyllaceae is a flowering plant that grows in tropical and temperate regions of southern Europe, South Asia, Africa, Australia, and Sudan. Plants are commonly used in traditional medicine to treat inflammatory conditions in Sudan [7]. This study aimed to evaluate the anti-inflammatory activity of the compound and to identify anti-inflammatory agents in the active extracts, as well as to predict their mechanism of action, pharmacokinetics, and toxicity profile using *in silico* methods.

2. Material and Methods

2.1. Plant Collection, Identification, Extraction and Fractionation

Plants were collected from Khartoum, Sudan. Dr. Haydar Abdul-gader, a taxonomist affiliated with the Medicinal and Aromatic Plant Research Institute in Sudan, verified the botanical identity of the plant in question. To ensure the accuracy of identification, a voucher specimen (KHPHG-F/S/8/107) was deposited at the Department of Pharmacognosy, University of Khartoum. The dried aerial parts were powdered and then subjected to two successive extractions with an adequate volume of dichloromethane and 80% methanol at room temperature for 48 h. The resulting powdered plant material weighed 200 g. The filtration of the extracts was carried out using Whatman filter paper, and the resulting filtrates were concentrated under vacuum and preserved at room temperature. The most effective extract was subjected to gradient elution on a silica gel column and the elution medium was continuously altered. To enhance the polarity, the eutrophic series was utilized as the solvent in the following order: petroleum ether, chloroform, ethyl acetate, and methanol.

2.2. Anti-inflammatory Activity Study

2.2.1. Samples preparation

The plant extracts and their fractions were mixed with water, with 0.25% sodium carboxymethyl cellulose serving as a suspending agent, and then homogenized.

2.2.2. Animals and ethical standards

Male Wistar rats weighing 200-220 grams were used to examine the anti-inflammatory properties. The animals were sourced from the Experimental Animal Care Center at the College of Pharmacy, King

Saud University, Riyadh. The rats were housed at a constant temperature of 22 ± 2 °C and a light/dark cycle of 12 h on and 12 h off. All experimental procedures, including euthanasia, blood collection, and final sacrifice, were performed in accordance with the guidelines established by the National Institutes of Health for Laboratory Animal Care and Use (NIH 1996). Ethics approval was granted by the Faculty of Pharmacy University of Khartoum Research Committee (November 14, 2016; FP/DO/RC/3-2016).

2.2.3. Carrageenan induced rat paw oedema

The effects of carrageenan-induced rat paw edema on inflammation were assessed based on these adjustments.[8]. Each animal was marked with an indelible blue ink on the right ankle in a circular manner. Subsequently, the volume of each paw was assessed using the Ugo-Basil plythesmometer, and a 0.45% sodium chloride solution was used as the displacement fluid. The immersed paws were then recorded on a digital screen. For each group, three animals were administered an intraperitoneal injection of various extracts at a dose of 2 g/kg. After 60 min, each rat was then given an intraperitoneal injection of 0.2% aqueous carrageenan using a 1-ml fine hypodermic syringe. The control group received intraperitoneal injection of the suspending agent solution at a volume equal to the injected test volume. After 60 min, carrageenan was injected intraperitoneally as described above.

After administering the carrageenan injection, the volume of the paw was determined at the designated points every 1, 2, and 3 h, and the volume of edema was calculated using the following formula:

$$\text{Percentage inhibition} = \frac{(Ct - Co)\ \text{control} - (Ct - Co)\ \text{treated}}{(Ct - Co)\ \text{control}} \times 100$$

The volume of edema in the control group is represented by Co, while the volume of edema in the test group is represented by Ct. The net edema volumes formed two hours post-carrageenan injection were used to calculate the effect of the extracts on the induced edema. Data are expressed as mean ± SEM. One-way ANOVA was used to analyze any significant differences between the control and treated groups, which were considered statistically significant at $P < 0.05$.

2.3. GC-MS (Gas Chromatography-Mass Spectroscopy) Analysis of Active Fraction

Gas Chromatography-Mass Spectrometry (GC-MS) analysis was performed using a Shimadzu GCMS-QP1020 spectrometer, which operates in the frequency range of 45–500 MHz. One milliliter of the test compound was dissolved in methanol and filtered through a syringe filter. Subsequently, 1 µL of the resulting solution was injected into the GC-MS system using a Hamilton microliter syringe. The analysis was performed under the following conditions.

Using an ionization method called Electron Impact (EI), the carrier gas was helium and the total flow rate was set at 50 ml/minute. The column flow rate was 1.6 ml/minute, and the column used was a Capillary Column-DB5 (30m×0.25 mm). The injection volume was 1µl, and the injection temperature was 240 °C.

2.4. In Silico Studies

2.4.1. ADME properties

The Swiss-ADME web tool (http://www.swissadme.ch/) was employed to predict the (-)-loliolide's Adsorption, Distribution, Metabolism, and Excretion (ADME) properties. The Swiss-ADME web tool (http://www.swissadme.ch/) was used. It is a free web tool for computing the pharmacokinetics, ADME properties, drug-likeliness, and medicinal chemistry friendliness of small molecules [9]. The SMILES format of the compounds was retrieved from the PubChem database prior to use. When the results were loaded, they were saved as CSV files for further analyses.

2.4.2. Assessment of toxicity risks and drug likeliness

The toxicity risks of (-)-loliolide were evaluated by retrieving its chemical scaffolds from the PubChem database and illustrating them using the OSIRIS Property Explorer open-source program (http://www.organic-chemistry.org/prog/peo/). This program computes the toxicity risks and drug-relevant properties of compounds and provides color-coded features.

2.4.3. Create coordinate files

The X-ray crystal structures of COX-1 (PDB ID: 6Y3C), COX-2 (PDB ID: 1PXX), and 5-LOX (PDB ID: 6N2W) were obtained from Protein Data Bank (http://www.rscb.org/pdb). Protein structures were

ISSN: 2458 - 8806

prepared by removing all water molecules and heteroatoms from the target files. The Swiss PDB viewer was employed to optimize the target structures and minimize their energy levels in V.4.1.0. [10].

2.4.4. Preparation of ligands

The PubChem database was the source of the 3D SDF structures of (-)-loliolide and two standard drugs, Ibuprofen and Meclofenamate sodium, which were downloaded (https://pubchem.ncbi.nlm.nih.gov/) and converted to the PDB format using Open Babel. Energy was minimized and converted to the PDBQT format using the graphical user interface version of PyRx virtual screening tool Python 0.8.

2.4.5. Molecular docking

The primary bioactive component of *T. terrestris*, as detected by gas chromatography-mass spectrometry (GC-MS), was chosen for molecular docking studies to gain a deeper understanding of its potential molecular interactions based on its affinity to various target enzymes. The research studies were executed using the Auto-Dock software (version 4.0) on Windows 10 (x86) operating system-based PCs. The software suite comprises MGL tools 1.5.6, which rely on Python 2.7. The interaction energy grid maps for the different atom types were calculated using Auto Grid. For every docking, a grid box was generated with a 60×60×60 grid map and grid spacing of 0.375 Å. The grid maps were centered on the predicted ligand-binding sites within the protein structures, which were determined using online tools available on the RPBS web portal (https://bioserv.rpbs.univ-paris diderot.fr/services/fpocket/) [11]. The Lamarckian Genetic Algorithm (LGA) was implemented using default settings to conduct docking simulations. These simulations were executed using Cygwin to generate grid parameter files (. gpf) and docking parameter files (.dpf, respectively) for each ligand. The resulting docked conformations for each ligand were categorized based on their binding energies and the conformation with the highest score was selected for further investigation. The pose with the lowest binding energy was chosen as the most appropriate and subsequently analyzed for interactions between the ligand and receptor.

2.4.6. Analysis and visualization

The image visualization of the molecules with the lowest binding energy within the active site was accomplished using Discovery Studio Visualizer Client, which is a 64-bit Windows application with a file size of 267 MB.

3. Results

3.1. Anti-inflammatory Activity

The methanol extract exhibited mean net edema of 0.45%±0.09 after 2 h, reflecting well anti-edematous effect (72.0% ±3.9 inhibition), unlike the dichloromethane extract, which exhibited only 0.05% ± 0.2 inhibition. Furthermore, the chloroform fraction showed the highest anti-edematous activity, with 76.1% ±1.276% inhibition compared to the other fractions (Table1)

3.2. GC-MS Analysis of T. terrestris chloroform fraction

The analysis of the active fraction using gas chromatography-mass spectrometry (GC-MS) identified 13 compounds, as illustrated in Table 2. Nevertheless, the total ionic chromatogram (TIC), as depicted in Figure 1, projected the most dominant compound by peak area to be (-)-loliolide (100 %).

Table 1. Net edema of fractions at dose of 2g/kg body weight (i.p)

Type of fraction	Mean net oedema after 2 hours (ml)*	% inhibition
Chloroform	0.400 ± 0.03	76.1±1.2
Water	0.75 ±0.04	55.2±0.5
Ethyl acetate	0.62 ±0.07	0.02±0.1
n-butanol	0.57 ± 0.03	-

*The volume of edema formed in control rats two hours after carrageenan administration was 1.675 ± 0.01 ml. Values represent the mean±S.E.M. of three rats.

3.3. *In Silico* Studies

3.3.1. Toxicity risks and drug- likeliness

It is essential to investigate the physicochemical properties and toxicity risks of lead compounds that influence their pharmacokinetic properties and, in turn, determine the ultimate biological effect. The physicochemical properties of loliolide were assessed using Lipinski's rule of five, which predicted that it had no risk of mutagenic, tumorigenic, irritant, or reproductive effects, and had a drug score greater than 0.4, as indicated in Table 3.

3.3.2. Physicochemical parameters

(-)-Loliolide fulfilled Lipinski's criteria, which are widely used to determine drug-like properties. It has a molecular weight of less than 500, with no more than 10 hydrogen bond acceptors, 5 hydrogen bond donors, and a lipophilicity (expressed as Log Po/w) of less than 5. Additionally, (-)-Loliolide has a molar refractivity between 40 and 130, and adheres to the rule of five, with no more than 4 violations of Lipinski's rule of five. (Table 4)

Molecular weight (MW), partition coefficient (Log $P_{o/w}$), number of hydrogen bond donors (NHBDs) and acceptors (NHBAs), number of rotatable bonds (NRBs), and topological surface area (TPSA)

3.3.3. ADME analysis

(-)-Loliolide exhibits high gastrointestinal absorption and can cross the BBB. It did not inhibit cytochrome enzymes and showed a skin permeability coefficient equal to -6.79 cm/s. (Table 5).

HIA, human gastrointestinal absorption; BBB, blood-brain barrier; P-gp substrate, permeability glycoprotein substrate; K*p*, skin permeability coefficient.

3.3.4. Molecular docking study

The results of molecular docking analysis are shown in Table 6, Figures 3-5. In the present study, three major enzymes were used to predict the anti-inflammatory activity of (−) - loliolide. Lead docking to the cyclooxygenases (COX-1 and 2) resulted in binding energy of-6.98 and-6.64 kcal/mol, respectively which are nearly similar to that of standard drug **Ibuprofen** (-6.83 and -6.88 kcal/mol, respectively).

Table 2. List of identified compounds by GC-MS

Number	R_t (min)	PA (%)	Name of Compound	Molecular Formula
1	5.34	1.1	Benzene, 1,4-dimethy	$C_6H_4(CH_3)_2$
2	11.8	1.6	2-(Hydroxymethyl) Benzoic acid	$C_8H_8O_3$
3	18.8	1.6	Ascaridole	$C_{10}H_{16}O_2$
4	19.3	100.0	(-)-loliolide	$C_{11}H_{16}O_3$
5	19.40	9.9	Dehydrovomifoliol	$C_{13}H_{18}O_3$
6	20.3	1.7	8-pentadecene	C15 H30
7	21.1	2.8	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$
8	22.3	2.2	E-15-Heptadecenal	$C_{17}H_{32}O$
9	22.4	0.6	2-propyldecan-1-ol	$C_{13}H_{28}O$
10	24.2	0.8	Cyclohexane	C_6H_{12}
11	26.9	1.2	1-heptadecene	$C_{17}H_{34}$
12	30.9	0.6	Eicosyl acetate	$C_{22}H_{44}O_2$
13	33.2	7.9	1,2-benzenedicarboxylic acid	$C_8H_6O_4$

R_t: Retention time; PA: Peak area

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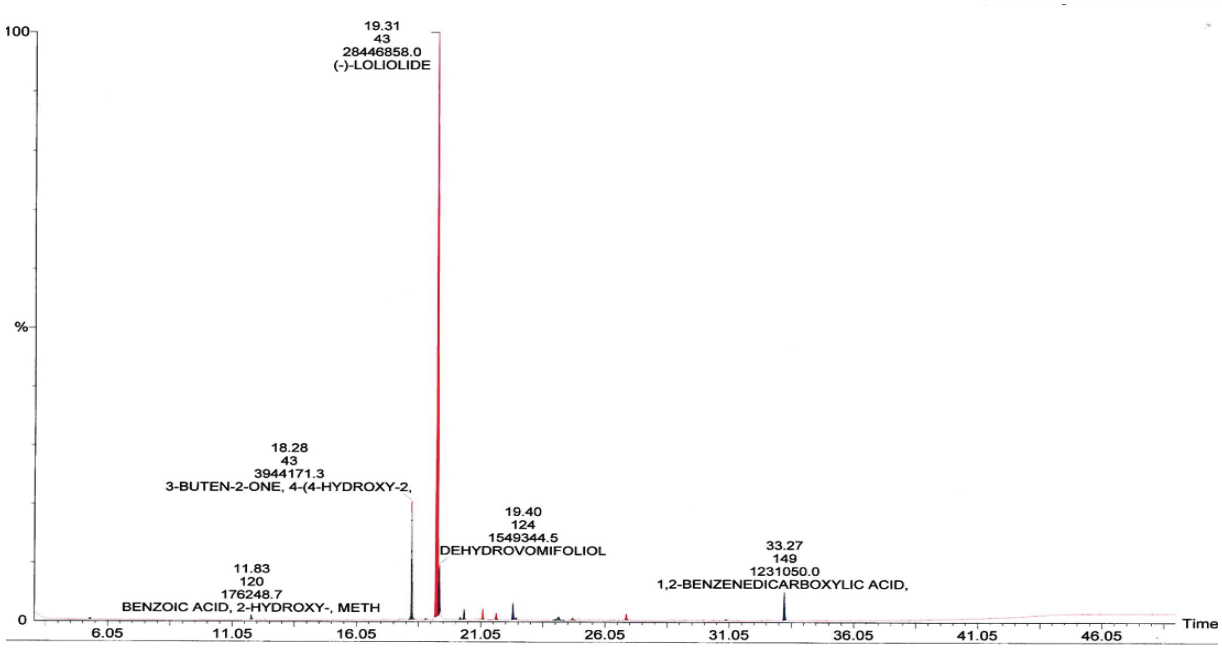


Figure 1. GC-MS Chromatogram of *T. terrestris* active fraction

Table 3. Toxicity risks and drug- likeliness predicted by OSIRIS Property Explorer

Compound name	ME	TE	IE	RE	DL	DS
(-)-loliolide	-	-	-	-	-0.422	0.49

ME: mutagenic effect, TE: tumorigenic effect, IE: irritant effect, RE: reproductive effect, DL: drug likeliness, DS: drug score, (−): no risk.

Table 4. Physicochemical properties of (-)-loliolide

MW g/ mol	NHBAs	NHBDs	Molar Refractivity	Consensus Log Po/w	[6]Rule of Five	TPSA (A°2)	NRBs	Water solubility class
196.24	3	1	52.51	1.53	0	46.53	0	Very soluble

Table 5. Predicted ADME properties of (-)-loliolide

HIA	BBB permeant	Pgp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	log Kp (cm/s)
High	Yes	No	No	No	No	No	No	-6.79

On the other hand, It exhibited binding energy (-5.25 kcal/mol) lower than standard drug **Meclofenamate sodium** (-6.89 kcal/mol).

4. Discussion

Inflammation is a disease state that encompasses numerous disorders including rheumatism, autoimmune diseases, diabetes, and cardiovascular incidents. Medications known to have adverse effects

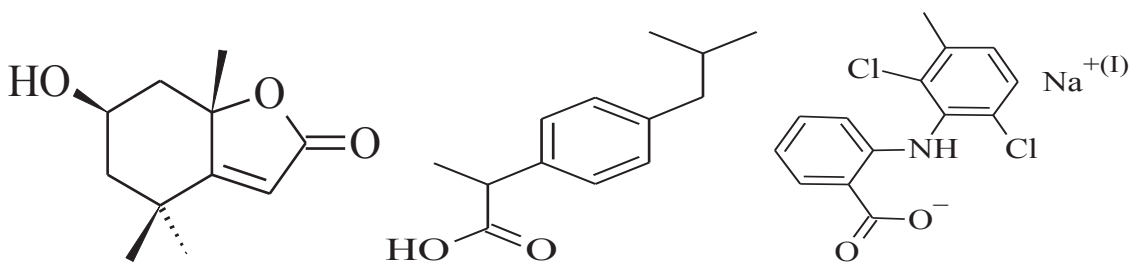
are commonly used to regulate and suppress inflammatory episodes. Therefore, it is crucial to identify natural anti-inflammatory agents that can provide enhanced pharmacological responses while minimizing adverse side effects [12]. The carrageenan-induced paw edema test is an effective method for assessing the anti-inflammatory properties of topical and systemic agents as it creates a suitable model for skin inflammation [13]. Infusion of the aerial parts of *T. terrestris* is widely used in Sudanese folk medicine as a demulcent [14]. In this study, this fraction was extracted using various solvents. The methanol extract showed significant inhibitory activity (72%) at dose of 2g/kg body weight (P value is < 0.05). This indicates that the hydroalcoholic extract of *T. terrestris* inhibits mediators related to inflammation, which has beneficial effects in various inflammatory conditions [13, 15-16]. In contrast, dichloromethane extract showed no activity at the same dose. Therefore, the methanol extract was fractionated to obtain the active agent. Chloroform and water fractions showed significant inhibitory activity (76.1 and 55.2%, respectively) at dose of 2 g/kg body weight, ethyl acetate fraction was not active the n-butanol fraction was led to an increase in edema at same dose (Table1). Reports have shown that *T. terrestris* exhibits its effects, particularly by its saponin-containing steroidal compounds that are structurally found in glycoside form and are soluble in polar residue [17].

Gas chromatography-mass spectrometry (GC-MS) is a widely utilized hyphenated analytical method in the medical and pharmaceutical sectors to identify numerous metabolites [18]. GC/MS analysis is highly valuable for identifying markers of primary metabolites because of its reproducibility and ability to utilize a constructed database [19]. Using this technique, (-)-loliolide was found to be the most abundant compound in terms of the peak area (Fig.1). It is a monoterpenoid hydroxylactone that is commonly found in plants, animals, and terrestrial and marine environments. The straightforward structure of this compound displays a diverse range of biological activities including anticancer, antibacterial, antifungal, and antioxidant properties, making it a compelling substance [20].

Following the identification of lead compounds, different *in silico* tools have been used to predict their safety and bioactivity. OSIRIS Property Explorer uses a fragmentation method to predict the probabil-

Table 6. Molecular docking results against the three target enzymes

Compound Name	Min. Binding energy on COX-2 (1pxx; kcal/mol)	Min. Binding energy on COX-1 (6y3c; kcal/mol)	Min. Binding energy on 5-LOX (6n2w; kcal/mol)
(-)-loliolide	-6.64	-6.98	-5.25
Ibuprofen	-6.88	-6.83	-
Meclofenamate sodium	-	-	-6.89



(-)-loliolide **Ibuprofen** **Meclofenamate sodium**

Figure 2. Chemical structures of ligands for molecular docking

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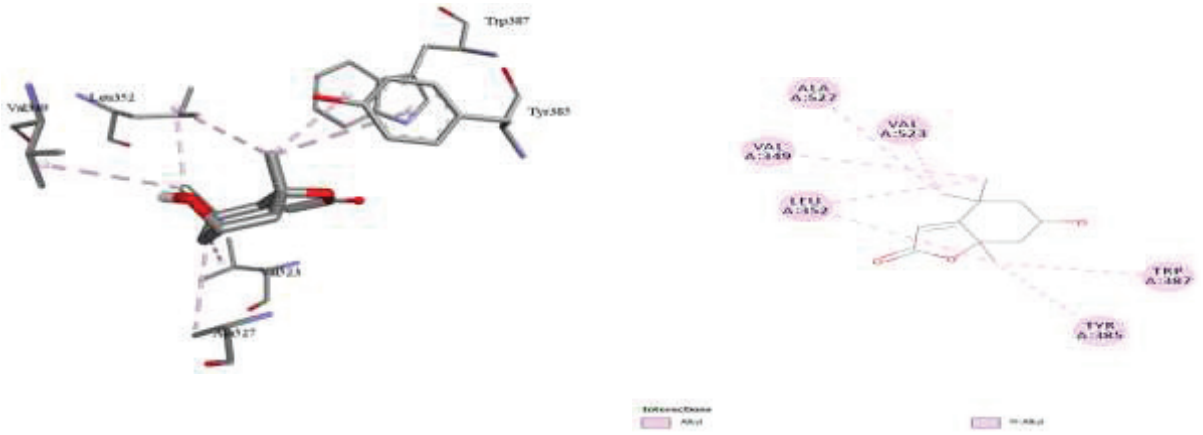
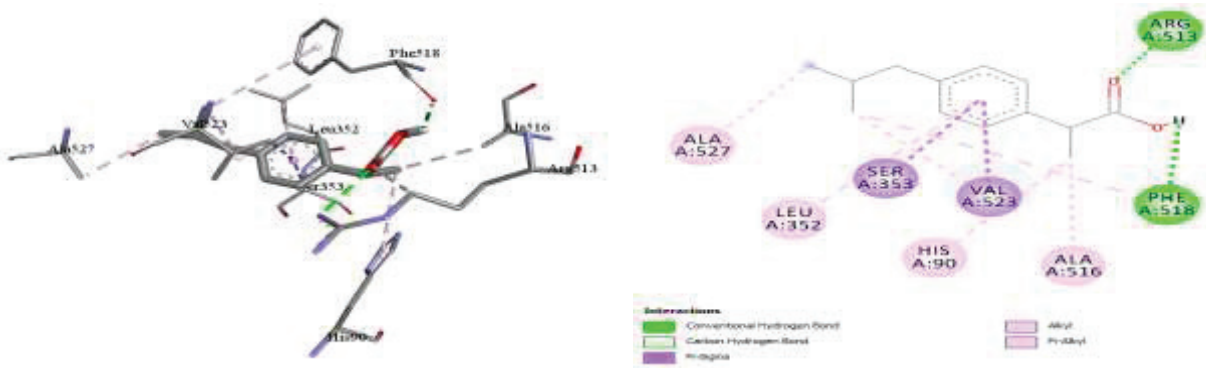


(-)-loliolide, E= -6.64 kcal/mol



Ibuprofen,E= -6.88kcal/mol

Figure 3. 3D and 2D visualization of (-)-loliolide and Ibuprofen in the active site of COX-2 (PDB: 1PXX)

ity of drugs based on a list of approximately 5,300 individual substructure fragments with related drug-likeness scores. A positive value suggests that the compound comprised fragments commonly found in commercial drugs. Nevertheless, it is not guaranteed that the compound will be considered a drug because of the possibility that these fragments may not possess the optimal balance of properties required for such classification. Drug scores (DS) take into account drug-likeness, Log P, log S, molecular weight, and toxicity risk to evaluate the overall potential of a compound to meet drug requirements. Compounds that did not have predicted toxicity risks and had a drug score DS of 0.4 or higher were deemed promising candidates. Based on the information provided, it can be inferred that (-)-loliolide holds potential as a drug or drug lead because of its lack of negative effects, such as mutagenicity, tumorigenicity, irritation, and reproductive toxicity, in addition to its DS of 0.49 (as shown in Table 3).

Evaluation of the pharmacokinetic properties of (−)-loliolide was carried out using the rule of five established by Lipinski, which asserts that a compound displaying drug-like behavior should not fail more than one of the following criteria: (i) a molecular weight of less than 500; (ii) a maximum of five hydrogen-bond donors; (iii) a maximum of ten hydrogen-bond acceptors; (iv) a lipophilicity value of less than five; and (v) a molar refractivity within the range of 40–130 (Adnan et al, 2019). In this study, (-)-loliolide showed acceptable physicochemical profiles, was less likely to have solubility problems, and had suitable MW values (MW = 196.24 g/mol), which are essential for successful penetration through biological membranes. The total surface

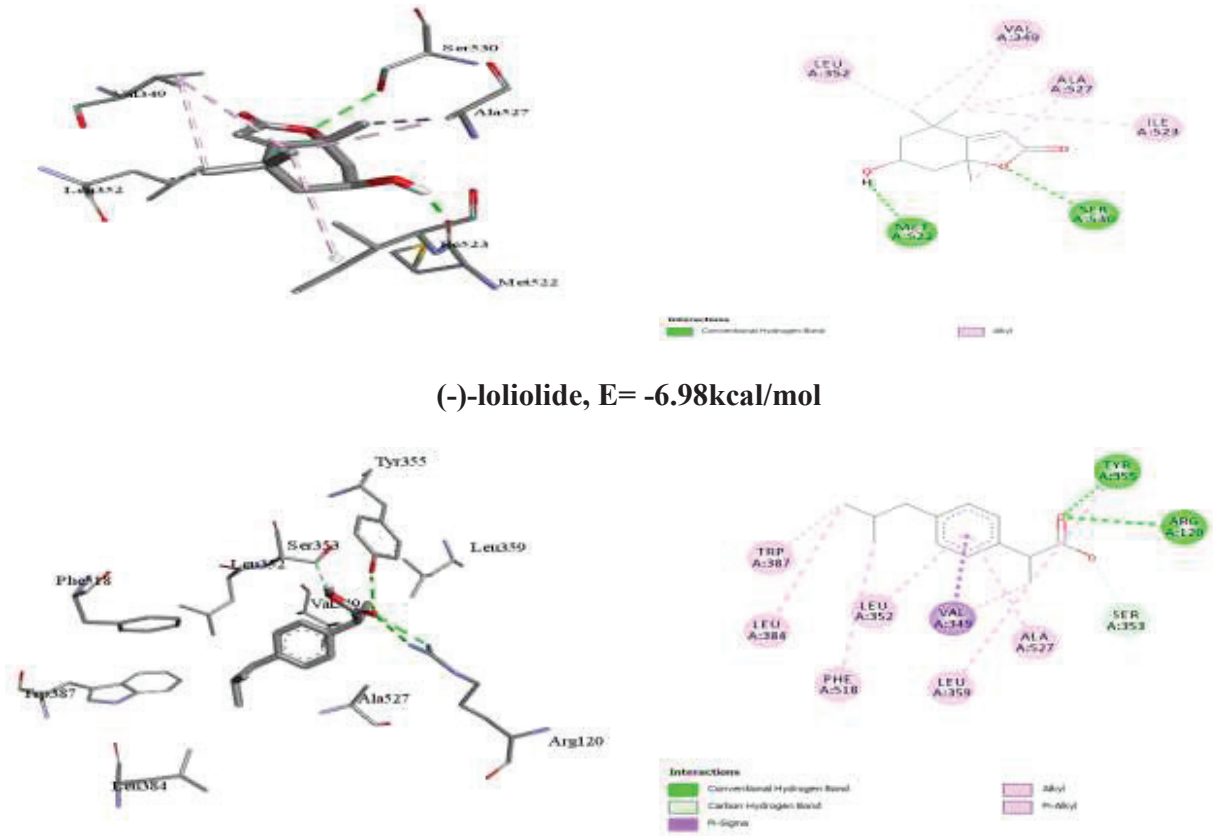


Figure 4. 3D and 2D visualization of (-)-loliolide and Ibuprofen in the active site of COX-1 (PDB: 6Y3C)

area of the polar atoms, primarily oxygen and nitrogen, in small molecules is referred to as the topological surface area (TPSA). A higher TPSA value is associated with a greater number of compounds that are not permeable or bioavailable. It has been suggested that an upper limit of 140 Å2 is indicative of low blood-brain-barrier penetration and poor oral bioavailability [21]. The suggested maximum value of PSA for a molecule to cross the blood-brain barrier is approximately 70 $Å^2$. [22], which is lower than that associated with an increased risk of adverse events due to non-specific toxicity, particularly when combined with high lipophilicity. Our research demonstrated that (-)-loliolide has a TPSA within a range that permits it to cross the blood-brain barrier (BBB) with high absorption after being taken orally, as shown in Table 4.

The permeability glycoprotein substrate (Pgp substrate) is an essential cell membrane protein that pumps many foreign substances and can be considered as a defense mechanism against harmful substances [23]. Active transport by P-gp can pose a significant challenge for pharmaceuticals, often leading to lower bioavailability after oral dosing and an impaired ability to penetrate the BBB. In addition, drugs are often affected by P-gp efflux, which can affect their distribution, absorption, and elimination. This is particularly relevant in the presence of potent P-gp inhibitors. However, it is important to note that (-)-loliolide is not expected to be a P-gp substrate [24].

Cytochrome P450 enzymes, also known as CYPs, are a group of essential enzymes in mammals that play a vital role in the removal of various substances and synthesis of hormones. The inhibition of these enzymes can result in drug-drug interactions and severe adverse effects due to the accumulation of the drug or its metabolite. These interactions can have

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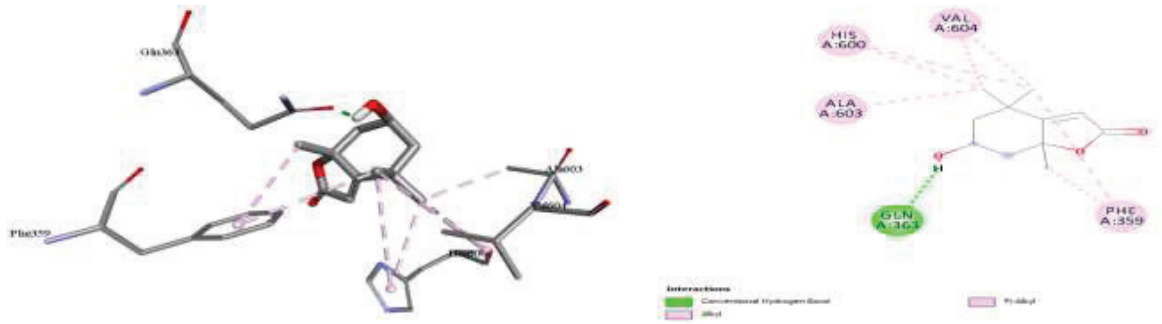
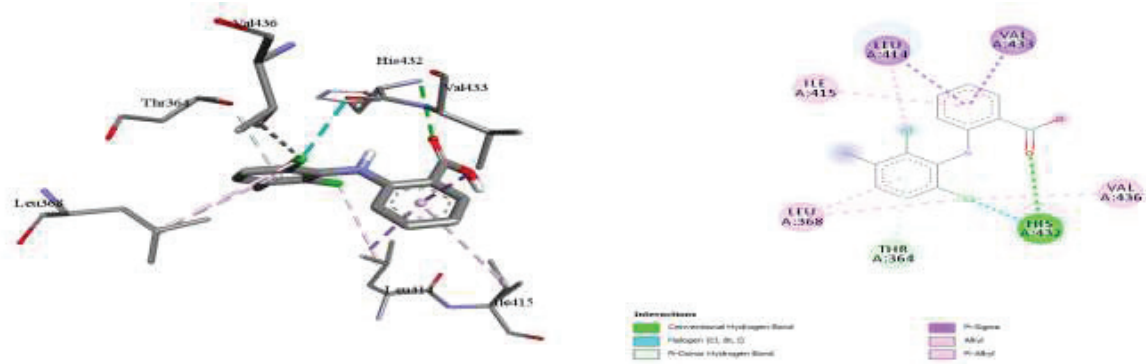


(-)-loliolide, E= -5.25kcal/mol



Meclofenamate sodium,E= -6.89kcal/mol

Figure 5. 3D and 2D visualization (-)-loliolide and Meclofenamate sodium in the active site of 5-LOX (PDB: 6N2W)

serious consequences for patients and may lead to complications, such as organ toxicity and even death. Therefore, it is crucial to understand the mechanisms of CYP enzymes and how they interact with drugs to prevent adverse effects and ensure patient safety [25]. (−)-Loliolide was predicted to be a non-inhibitor of any of the five CYP isoforms, making it an ideal anti-inflammatory candidate in addition to its good bioavailability (Table 5).

Molecular docking has become a widely used method to predict ligand-target interactions and gain insights into the biological activity of natural products. This approach also offers additional information about potential mechanisms and binding modes at different enzyme binding sites [26]. Cyclooxygenases (COX-1 and COX-2) and lipooxygenases (5-LOX) have been identified as three key proinflammatory enzymes [27]. COX isoenzymes are found in the cell membrane and have varying expressions, modes of regulation, and functions. COX-1 is typically seen as an enzyme that helps maintain balance in the body as it is always present in several tissues. In contrast, COX-2 is more sensitive to changes and can be activated in many tissues by various physical and pathological stimuli. Additionally, the two isozymes have different amino acid sequences at their active sites [28]. The exchange of the relatively bulky Ile-523 residue in COX-1 with val-523 in COX- 2 tends to result in an additional side pocket in COX-2. In addition, the exchange of Ile-434 with valine-434 in COX-2 allows a neighboring residue, Phe-518, to swing out of the way and increase its access to the side cavity. The other difference between the two isoforms, which does not alter the shape of the drug-binding site but rather changes its chemical environment, is Arg-513 within the side pocket of COX-2, which was replaced by His-513 in COX-1. These differences between the COX active sites have major implications for the selectivity profiles of inhibitors [29]. The cyclooxygenase active site consists of highly conserved residues (Arg-120, Tyr-355, Tyr-385, Ser-530, and Glu-524) [30]. (-)-Loliol-

ide showed significant interactions with most of the important binding pocket residues of these isozymes, with greater similarity in binding energies to that of the standard drug ibuprofen. It showed minimum binding energy of -6.98 kcal/mole with COX-1 compared to -6.83 kcal/mole for standard, and -6.64 kcal/mole for COX-2 compared to -6.88 kcal/mole for standard. (-)-Loliolide showed hydrogen (H) bonding and hydrophobic interactions with amino acids at the cyclooxygenase active site, which revealed 2H bonding with Ser-530 and Met-522, π-π stacking, and π-σ interactions with Val-349, Leu-352, Ile-523, and Ala-527 at COX-1; Val-349, Leu-352, Tyr-385, Trp-387, Val-523, and Ala-527 at the COX-2 active site (Figures 3-4).

5-lipoxygenase (5-LOX) is also an essential enzyme required for the synthesis of both pro-inflammatory leukotrienes and anti-inflammatory lipoxins. The crystal structure of the stable enzyme shows five invariant amino acids (Tyr-181, Ala-603, Ala-606, His-600, and Thr-364) that provide context for the development of 5-LOX–specific inhibitors [31]. (-)-loliolide revealed significant interactions with most of the important binding pocket residues of 5-LOX with binding energy of -5.25 kcal/mole compared to -6.89 kcal/mole for Meclofenamate sodium standard drug. It exhibited one hydrogen bond with Gln-363 and π-σ interactions with Phe-359, His-600, Ala-603, and Val-604 (Figure 5).

4. Conclusions

Natural products have played and continue to play an important part in the development of effective drugs. This study demonstrates that *T. terrestris* possesses anti-inflammatory effects that support and contribute to its traditional use (Ethics approval; November 14, 2016; FP/DO/RC/3-2016). In addition, (-)-loliolide was detected through GC-MS analysis as a possible bioactive component that displayed promising docking affinity towards various important inflammatory enzymes in molecular docking tests, and its drug-like properties were validated through ADME/T analysis. Additional research is necessary to separate (−)-loliolide in its pure form for both *in vitro* and *in vivo* anti-inflammatory evaluations.

Funding

The authors did not receive any external support.

Conflict of Interest

The authors declare no conflict of interest.

Statement of Contribution of Researchers

Concept; Design; Data Collection and/or Processing – W.O., S.S., M.M.; Analysis and/or Interpretation – W.O., S.S., M.M.; Literature Search – S.S., M.M.; Writing – S.S., M.M.; Critical Reviews – W.O., S.S., M.M.

Data Availability

The datasets supporting this article are fully included within the article itself.

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