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In Vitro and In Silico Anticyclooxygenase and Antitopoisomerase Activity of *Anonna cherimola* Ent-Kaurenes

Carlos Eduardo Camacho-González [1], Alejandro Pérez-Larios [2,*], Sonia G. Sáyago-Ayerdi [1], Jasmin Salazar-Mendoza [1,2] and Jorge A. Sánchez-Burgos [1,*]

[1] Food Research Laboratory, Technological Institute of Tepic, National Technological Institute of Mexico, Instituto Tecnológico Avenue No 2595, Lagos del Country, Tepic 63175, Nayarit, Mexico; charly_cecg@hotmail.com (C.E.C.-G.); jasmin.salazar@academicos.udg.mx (J.S.-M.)

[2] Laboratorio de Investigación en Nanocatalisis, Centro de Estudios para la Agricultura, la Alimentación y la Crisis Climática (CEAACC), Centro Universitario de los Altos, Universidad de Guadalajara, Av. Rafael Casillas Aceves 1200, Tepatitlán de Morelos 47620, Jalisco, Mexico

* Correspondence: alarios@cualtos.udg.mx (A.P.-L.); jsanchezb@ittepic.edu.mx (J.A.S.-B.)

Abstract: *Annona cherimola* is noted for its bioactive compounds, particularly diterpenes called ent-kaurenes, which exhibit various biological activities. This study focused on evaluating the ability of ent-kaurenes from *Annona cherimola* to inhibit cyclooxygenase (COX) and topoisomerase (TOP) enzymes. Researchers used solvent-free lipophilic eluates (SFLEs) from the plant in enzymatic assays and a yeast model. The major compounds in SFLE were identified using gas chromatography–mass spectrometry (GC-MS), and in silico studies explored their inhibition mechanisms. SFLE showed significant inhibition of COX-II (95.44%) and COX-I (75.78%) enzymes and fully inhibited the yeast strain. The effectiveness of inhibition is attributed to the compounds' structural diversity, lipophilicity, and molecular weight. Two main compounds, kauran-16-ol and isopimaral, were identified, with in silico results suggesting that they inhibit COX-II by blocking peroxidase activity and COX-I by interacting with the membrane-binding region. Additionally, these compounds allosterically and synergistically inhibit TOP-II and potentially sensitize cancer cells by interacting with key amino acids. This research is the first to identify and evaluate kauran-16-ol and isopimaral in silico, suggesting their potential as anti-inflammatory and anticancer agents.

Keywords: *Anonna cherimola*; anticyclooxygenase; antitopoisomerase; ent-kaurenes

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1. Introduction

Annona is the second largest genus of flowering plants in the family Annonaceae after *Guatteria*. *Annona cherimola*, commonly called "chirimoya", is a subtropical fruit native to South America and the Antilles, with Spain, Peru, and Chile as the main producers worldwide [1]. It is recognized the high bioactivity in this plant, which is traditionally used for the treatment of many ailments, is attributed to the presence of numerous bioactive compounds in various parts of the plant, such as alkaloids, acetogenins, polyphenols, and terpenoids [1]. The diterpenes known as ent-kaurenes, found in greater proportion in lipophilic extracts of this plant matrix, have generated a growing interest since the last century due to their diversity and structural complexity, together with their extensive bioactivity profiles and a particular interest in the attractive anti-inflammatory and antiproliferative pharmacological profiles of this class of natural compounds [2].

Inflammation is known to be related to physiological and pathological processes such as cancer, neurodegenerative, and cardiovascular diseases by activating the immune system and several cells within the damaged tissue [3]. Prostaglandins (PGs) are one of the biosynthetic inflammatory mediators of cyclooxygenases (COX) that are associated with inflammatory disorders. The production of prostaglandin E2 (PGE_2), catalyzed by COX-II, plays a key role in inflammation and its associated diseases [4]. Traditional nonsteroidal

anti-inflammatory drugs (NSAIDs) block the COX pathway that is associated with cell-mediated PGE_2 production. However, its prolonged use may cause serious adverse side effects [4]. Therefore, there is an urgent need for the discovery or development of new anti-inflammatory agents with minimal side effects, which are currently of interest. Other targets of pharmacological interest are topoisomerase enzymes (TOP) because they catalyze topological rearrangement processes in DNA during cell replication.

It is known that many conditions, mainly cancer, present with high rates of over-expression and enzymatic activity of these chemical entities, so their inhibition at the post-translational level has generated great interest in the scientific community. Inhibition is based on a mechanism called "TOP poisons", which involves the stabilization of the excision complex by creating a ternary complex (DNA–protein–drug) that accumulates and causes a cytotoxic effect [5]. However, to assert that the inhibitor is governed by this system, it is necessary to generate an in silico study in order to evaluate possible inhibitor–enzyme interactions and then establish the probable inhibition mechanism. In recent years, there has been an increased interest in evaluating the interaction of small molecules with proteins that are related to the understanding of medicine, chemistry, food science, toxicology, and biology [6]. Therefore, studies on interactions with small molecules are of great importance in sizing the level and form of inhibition of this molecule. Several reports have been published on interactions between potential inhibitors in COX [7–10], as well as for TOP [9–12], each of which stands out as potential inhibitors. However, so far, there are no reports on the possible mechanisms of inhibition based on the interactions between small molecules and the enzymes evaluated.

While in vitro and in vivo studies provide an effective approach to the biochemical phenomena under study, in silico analyses are key to current research because of their ability to accelerate scientific discovery by rapidly performing simulations and predictions and to reduce costs by minimizing the need for extensive physical assays. In that sense, today, there are reports of the potential activity of COX [7–10] and TOP [9–12] inhibitory agents, which dictate opportunities to search for new agents of natural origin that, based on in vitro and in vivo results, can validate their viability, thus optimizing the experimental design and saving resources.

Thus, the objective of the present study was to evaluate in vitro the anti-cyclooxygenase and antitopoisomerase activity of ent-kaurenes from an extract of *Annona cherimola* besides carrying out an in silico study where potential interactions were elucidated alongside possible inhibition mechanisms.

2. Materials and Methods

2.1. Chemicals and Solvents

All the chemicals used in this study were of analytical reactive grade and most of them were from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Biological Material

Genetically modified *Saccharomyces cerevisiae* strains were donated by Dr. John Nitiss of St. Jude Children's Research Hospital, Memphis, Tennessee; JN394 is deficient in DNA repair and membrane permeability is altered (Matα ura3-52, leu2, trp1, his7, ade1-2, ISE2, rad52:LEU2), so it increases the sensitivity of these cells to drugs and JN362a is exacerbated in relation to the mechanisms of DNA repair (Matα, ura3-52, leu2, trp1, his7, ade1-2, ISE2) [13,14].

2.3. Collection and Treatment of Anonna cherimola

Anonna cherimola was donated by producers from Tumbiscatio, Michoacán, Mexico. The fruit was washed and disinfected, then pulped and packed in 1 Kg sealed bags and frozen (−56 °C). Once the fruit was frozen, the pulp was freeze-dried (−50 °C and 0.12 mbar, Labconco, FreeZone 6.0, Kansas City, MO, USA). The sample was ground (Nutribullet,

NB-101-B, 600 w, Los Angeles, CA, USA) and then sieved using an ASTM No. 500. The powder obtained was stored for further analysis.

2.4. Preparation of the Plant Extract

For the extraction of the diterpenes, 5 g of lyophilized powder was taken and re-suspended in 20 mL of methanol (MetOH) and subsequently subjected to an extraction process for 20 min (incubating orbital shaker, VWR, Radnor, PA, USA). After this time, the samples were centrifuged at 32 °C at 9000 rpm for 30 min. The residue was recovered, and the extraction process was repeated twice more, collecting the residues along with those of the first extraction. After this stage, the same extraction procedure was carried out with the residue, ethyl acetate (EtOAc), collecting the supernatants from the 3 extraction cycles. Finally, these were concentrated by rotaevaporation (Büchi Labortechnik R-100, B-100 HB, I-100, F-105, Flawil, Suiza), and the extracts obtained were collected and stored for further analysis.

2.5. Isolation of Ent-Kaurenes and Qualitative Identification

The soluble portion in EtOAc was chromatographed in a silica gel column (1600 g, 200–300 mesh) by elution with EtOAc and an increasing polar MetOH mixture. For the qualitative identification of each fraction, they were analyzed by thin layer chromatography (TLC) based on the Liebermann–Burchard (LB) reaction that consists of a mixture of the Liebermann–Burchard reactive [20:1:10 volumes of acetic anhydride (Ac_2O): sulfuric acid (H_2SO_4): acetic acid (HOAc)] sprinkled on TLC plates (normal phase). The red coloration obtained indicated the presence of diterpenes. Once the different fractions were identified, they were gathered and concentrated by rotating evaporation until SFLEs were obtained.

2.6. Anticyclooxygenase Activity

SFLE was evaluated for COX inhibitory activity using an Enzyme Immunoassay (EIA) kit (catalog no. 560131, Cayman Chemical, Ann Arbor, MI, USA). The EIA assay was performed with a heat-treated enzyme (100 °C/3 min) for 100% inhibition and acetylsalicylic acid [1 mg/mL] (ASA) as positive controls. According to the directions, the reaction mixtures were prepared with COX and Tris-HCl buffer, and the addition of arachidonic acid (AA) initiated the reaction. The reaction was terminated by adding HCl after 2 min, and PG was quantitated by the ELISA method. SFLE was dissolved in *Dimethyl sulfoxide* (DMSO) [1 mg/mL], following transfer to a 96-well plate coated with a mouse anti-rabbit IgG; the tracer prostaglandin acetylcholine esterase and primary antibody (mouse anti PGE2) were added. Then, plates were incubated at room temperature overnight, reaction mixtures were removed, and wells were washed. Ellman's reagent was added to each well, and the plate was incubated for 1 h, where at the end of that time, a reading was made at 415 nm in a microplate reader (Biotek, Synergy HT, Winooski, VT, USA), the Gen5 version 2.0 software. The inhibition of COX was calculated based on the quantification of PG, considering a sample not treated as having 100% prostanoid generation; for this, PG was first quantified with a calibration curve [250, 125, 62.5, 31.25, 15.63, and 0 pg/mL].

2.7. Antitopoisomerase Activity

The antitopoisomerase assay was evaluated according to the methodology of Nitiss and Wang [13]. SFLE and Camptothecin (CPT) were suspended in DMSO [1 mg/mL]. The JN362a and JN394 mutant strains were incubated in YPD medium with 0.07% adenine (30 °C/18 h); each was incubated at a volume of a 3 mL medium with suspended cells and 50 µL treatment (30 °C/24 h) in an orbital incubator (MaxQ4450, Thermo Scientific, Marietta, OH, USA). To control the effect of DMSO, vehicular treatment was added as a negative control (100% growth), and the positive control was CPT, known as a topoisomerase 1 inhibitor. At the end of the incubation period, an aliquot of 100 µL of the treatment and controls was transferred to be inoculated in Petri dishes with YPDA medium and incubated at (30 °C/48 h). Antitopoisomerase activity was determined based on the number of

colonies present in each plate and the negative control that was considered as representing 100% growth.

2.8. Identification of Ent-Kaurenes

The identification of ent-kaurenos was carried out according to the methodology described by [1,15], with some modifications. SFLE (1 mg) was weighed and resuspended in 2 mL EtOAc (HPLC grade) and placed in vials for subsequent injection into the GC/MS Agilent 7890 with a 5977 AMSD mass detector. The analysis started at 70 °C, and the temperature was maintained for 3 min. At the end of this period, the temperature was taken up to 300 °C with increments of 10 °C per min and maintained for 9 min. The temperature of the injection port was 250 °C with a helium flow at 1.5 mL/min and with an ionization voltage of 70 eV. Samples were injected in the splitless mode, and the ion search was performed in the scan mode at a range of 300 to 500 m/z. For the identification of the present compounds, an integration of the present peaks was generated and compared with the NIST library version 2016.

2.9. In Silico Study

The molecular docking study was carried out among the identified ent-kaurenes to determine the possible interactions that may occur between these compounds and the proteins evaluated; for this purpose, methodologies were proposed [16]. The ligands were obtained from PubChem when energy minimization was performed with molecular mechanics using the Hartree–Fock algorithm (UCSF Chimera 1.14 software). Once the calculation was completed, the final structure was stored in the mol2 format for later manipulation. The 3D structure of COX-I and COX-II in pdb format was obtained from the Swiss Institute of Bioinformatics (SIBs). The enzymatic identification for the rat COX-I of the National Centre for Biotechnological Information (NCBI) was 10116, and for the Universal Protein Resource (UniProt) was Q63921, while for the human COX-II, NCBI, it was 9606 and UniProt was P354. The 3D structure of TOP-I and TOP-II in pdb format was acquired from the Protein Data Bank (PDB). The enzymatic identification for the human TOP-I is 1A36, and for the TOP-II of *Saccharomyces cerevisiae* is 1BJT. Finally, after obtaining the ligands and target proteins, the SIB website service performed and optimized the docking procedure. Molecular docking results were visualized in USCF Chimera and evaluated using the FullFitness parameter (FF) calculated by the average of 30% of the most favorable "n" energies of a cluster, used to reduce the risk of a few complexes penalizing an entire cluster. Finally, a thermodynamically more favorable FF cluster was used to determine the residues involved in the generation of the complex.

2.10. Statistical Analysis

All analyses were performed in triplicate. Analytical data were expressed as the mean ± standard deviation.

3. Results

3.1. In Vitro Anticyclooxygenase Activity

Table 1 shows the results obtained from the anti-inflammatory effect in the COX enzymes of the SFLE as well as from the positive and negative controls, exhibiting an inhibition for COX-I and COX-II in 95% and 75%, respectively. The effects of PGE_2 are diverse, but ultimately, the process leads to the loss of inhibition of cell contact as well as an increase in cell proliferation: crucial characteristics of malignant cell transformation [17]. It is for this reason that numerous terpenoids have been proposed and analyzed as potential inhibitors of this enzyme [18–21], with maximum inhibition rates close to 60% for COX-I and COX-II, which is an event that enhances SFLE obtained as a potential inhibitor of COX enzymes.

Table 1. Percentage of inhibition and concentration of prostaglandins COX-I and II of SFLE and controls compared to ASA.

Enzyme	Treatment	Prostaglandin (pg/mL) *	Inhibition (%)
COX-II	SFLE	7.92 ± 0.73	95.44 ± 9.51
	ASA	0.08 ± 0.08	99.86 ± 8.12
	Inactive	0.07 ± 0.01	99.95 ± 5.44
	100% active	173.88 ± 9.72	-
COX-I	SFLE	3.90 ± 0.30	75.78 ± 4.22
	ASA	0.02 ± 0.01	99.83 ± 8.43
	Inactive	0.06 ± 0.01	99.61 ± 7.75
	100% active	16.06 ± 1.42	-

* Results are expressed ± the standard deviation. Acetylsalicylic acid (ASA), thermal stress enzyme (inactive), and stress-free enzyme (100% active).

3.2. In Vitro Antitopoisomerase Activity

Because this trial is sensitive to antimicrobial compounds, SFLE was analyzed in the strain JN362a, which is a strain resistant to DNA repair and sensitive to antimicrobial agents [22]. The JN394 strain was used to determine possible antitopoisomerase activity. It presents mutations (*Rad52* and *Ise2*) promoting a deficiency in DNA regeneration and greater permeability in the cell membrane [13]. Therefore, SFLEs can present antitopoisomerase activity by achieving significant inhibition in the growth of this yeast after treatment with eluate. Figure 1 shows the results of the antitopoisomerase activity of the SFLE and the positive control (CPT), in which a possible antimicrobial effect was ruled out due to the minimal growth exhibited in JN362a strain: an event that gives way to evaluate antitopoisomerase activity in the JN394 strain. Moreover, the action carried out by the SFLE to achieve 100% inhibition of the JN394 strain is noteworthy. Numerous extracts with possible antitopoisomerase activity from different plant matrices have been proposed [22–24], although the percentages of inhibition to the JN394 strain have not been remarkable compared to the results obtained with SFLE, which leads to the conclusion that it is postulated as a potential antitopoisomerase agent.

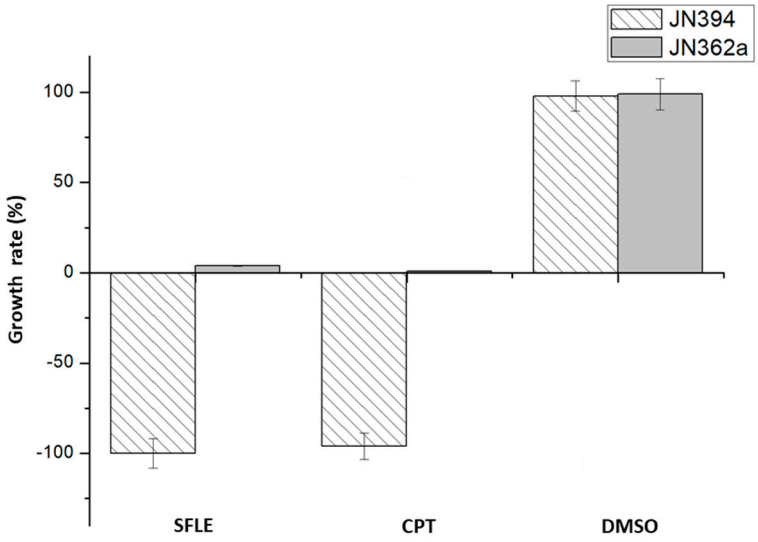

Figure 1. The growth rate of modified strains of *Saccharomyces cereviseae* JN362a and JN394 on SFLE, CPT, and DMSO.

3.3. Identification of Ent-Kaurenes by GC/MS

In SFLE (40 mg), we obtained the response of two peaks (Supplementary Material Figure S1) under the conditions of injection, from which we identified an ent-kaurene diterpene known as kauran-16-ol that, according to the library consulted, presented a similarity of 86%, as well as a precursor called isopimaral with a similarity of 83% by

GC-MS, obtaining percentage of accumulated area for kauran-16-ol close to 97. The presence of these compounds is associated with the gibberellic acid pathway in which a number of compounds characteristic of the mevalonate pathway are generated, particularly substances related to cyclic diterpenes such as ent-kaurenes, ent-kaurenols, ent-kaurenals, and ent-kaurenic acids alongside gibberellic acid precursors [25].

3.4. In Silico Anticyclooxygenase Activity

In the in silico study, it was important to know the total energy of the enzyme–ligand complex for the main endogenous ligand of the enzymes COX and AA because, from this, a comparative analysis of the two ligands was carried out. In addition to three anti-inflammatory drugs already known as reference standards. The energies of the compounds that interact with the COX enzymes are shown in Table 2.

Table 2. Molecular docking for COX enzymes expressed as having total binding complex energy (full fitness).

Ligand	COX-I	COX-II	Reference
	(Kcal/mol)		
Isopimaral	−2233.4333	−2398.0186	-
Arachidonic acid (endogenous substrate)	−2380.8158	−2385.5303	[16]
NS-398 (selective to COX-II)	−2383.8495	−2385.4913	[16]
Kauran-16-ol	−2221.6966	−2380.2690	-
Meloxicam (selective to COX-II)	−2347.0044	−2349.0139	[16]
Indomethacin (selective to COX-I)	−2310.1476	−2313.8915	[16]

Kauran-16-ol and isopimaral have a high affinity for COX-II by presenting energies similar to and higher than those of the endogenous substrate; the same situation was observed for the standards in analyses, where their affinity was already corroborated in numerous trials. However, this behavior was completely different with COX-I, displaying lower energies from the ligands. The main interactions for kauran-16-ol and isopimaral at their lowest energy position with residues of surrounding amino acids of COX-II are shown in Figure 2 where the residues include kauran-16-ol: Gly203, His207, His388, Leu391 and Val447; isopimaral: Gly203, His207, His388 and Val447 (residues that share these terpenoids are underlined for better appreciation).

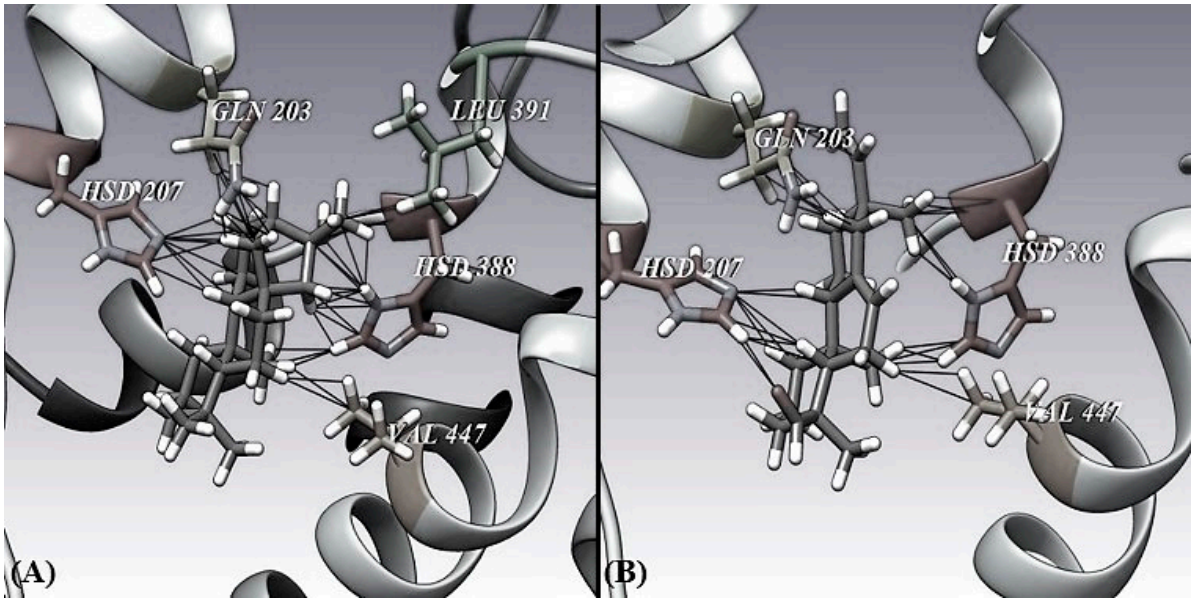

Figure 2. Molecular docking simulation obtained with the lowest energy conformation for COX-II. (**A**) Kauran-16-ol; (**B**) isopimaral.

The case of interactions with COX-I residues is shown in Figure 3, where the residues are as follows: kauran-16-ol: His43, Tyr80, Arg83, and Gly471; isopimaral: Glu347, Gly350, Gly351, and His581.

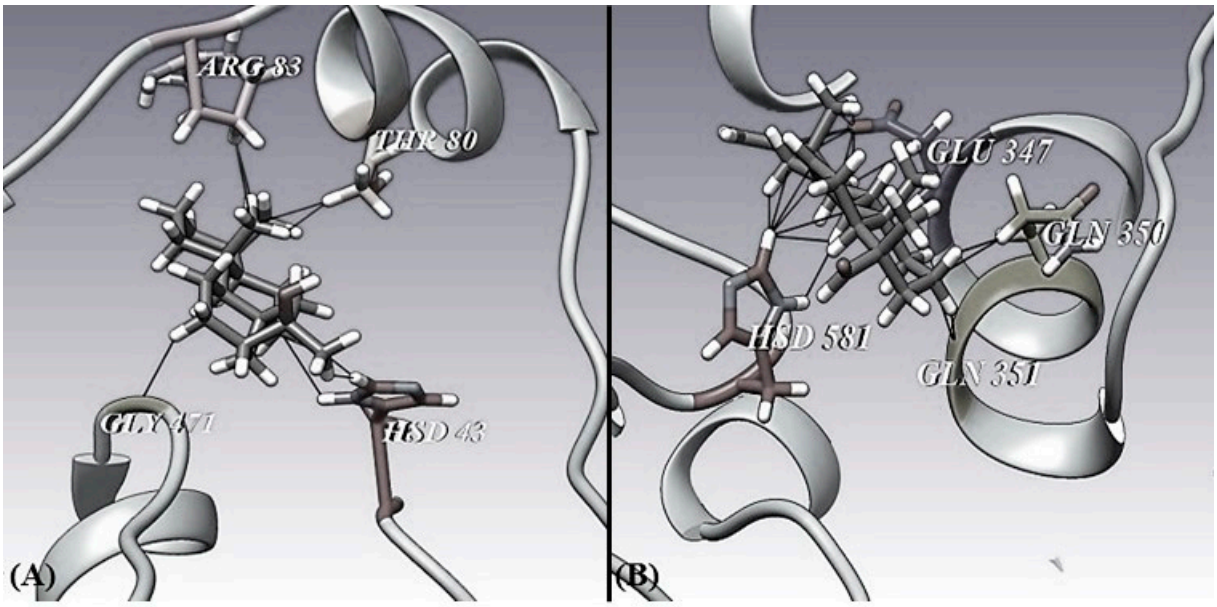

Figure 3. Molecular docking simulation obtained with the lowest energy conformation for COX-I. (**A**) Kauran-16-ol; (**B**) isopimaral.

Unlike numerous anti-inflammatory synthetic drugs, terpenoids, mainly diterpenes, have been classified as biologically active for these enzymes due to the various reactive moieties present in their structure; however, studies have revealed that due to their structural diversity, moderately high molecular weight, substantial lipophilia (the high affinity of COX with lipophilic compounds), and minimized polarity, they correspond well with the chemical characteristics of functional groups of the amino acid residues of these enzymes [16,26] and physicochemical molecular attributes, exhibiting both kauran-16-ol and isopimaral.

3.5. In Silico Antitopoisomerase Activity

It is essential to know the spontaneity of the enzyme–ligand complex towards endogenous ligands where its action on chemical entities in question has been corroborated. CPT and amsacrine were postulated as the main inhibitors due to their high affinity of interaction with the catalytic site of these enzymes. The comparative analysis of the two ligands was made, and the energies of the compounds that interacted with the TOP enzymes are shown in Table 3.

Table 3. Molecular docking for TOP enzymes expressed as total binding complex energy (full fitness).

Ligand	TOP-I	TOP-II
	(Kcal/mol)	
Isopimaral	−3784.4620	−4518.8753
Camptothecin (selective to TOP-I)	−3741.6567	-
Kauran-16-ol	−4564.1074	−4501.5980
Amsacrine (selective to TOP-II)	−3666.9740	−4537.3400

Kauran-16-ol and isopimaral exhibited a high affinity for TOP-I by presenting this attribute in a superior and similar form, respectively, in contrast to the analyzed standards. However, the same approach was slightly different with TOP-II, displaying energies from ligands relatively similar to the standards. The main interactions for kauran-16-ol and

isopimaral at their lowest energy position with the surrounding amino acid residues for the interaction of TOP-I with kauran-16-ol and isopimaral residues are shown in Figure 4, where the residues are as follows: kauran-16-ol: Phe227, Pro230, Arg376, Met378, Pro396, and His399; isopimaral: Met305, Arg321, Ala498, and Ser502.

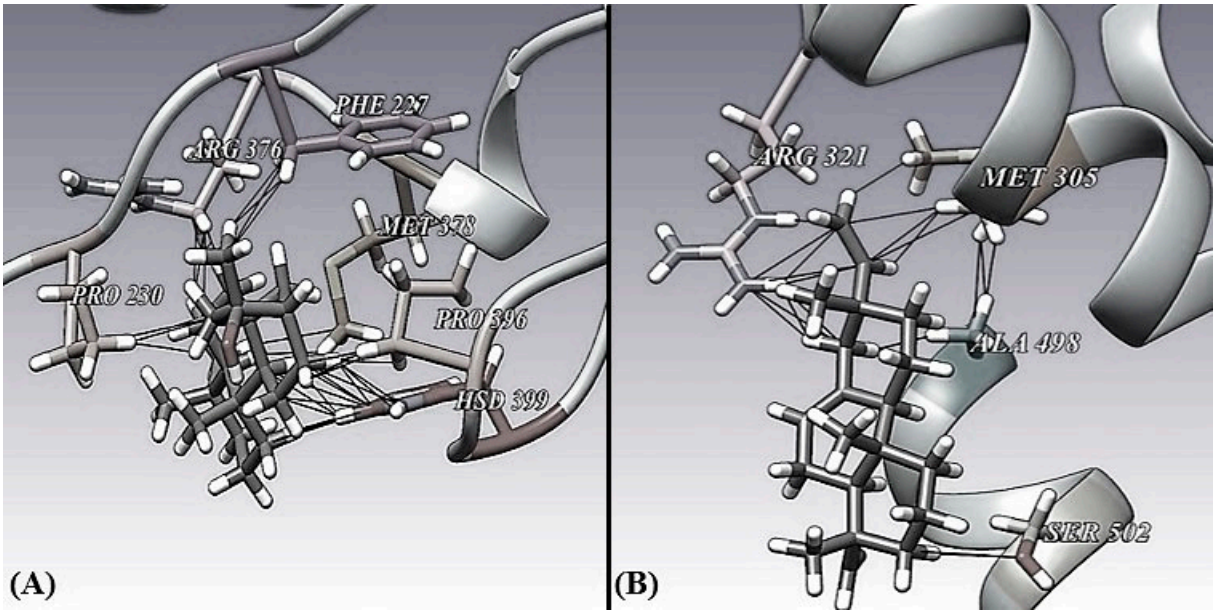

Figure 4. Molecular docking simulation obtained with the lowest energy conformation for TOP-I. (**A**) Kauran-16-ol; (**B**) isopimaral.

For the interaction of TOP-II with kauran-16-ol and isopimaral, the residues are represented in Figure 5, where the residues are as follows: kauran-16-ol: Met1119, Ser1123, Arg1128, Leu1132, and Gln1135; isopimaral: Leu685, Ala686, Ile689, Arg690, and His735.

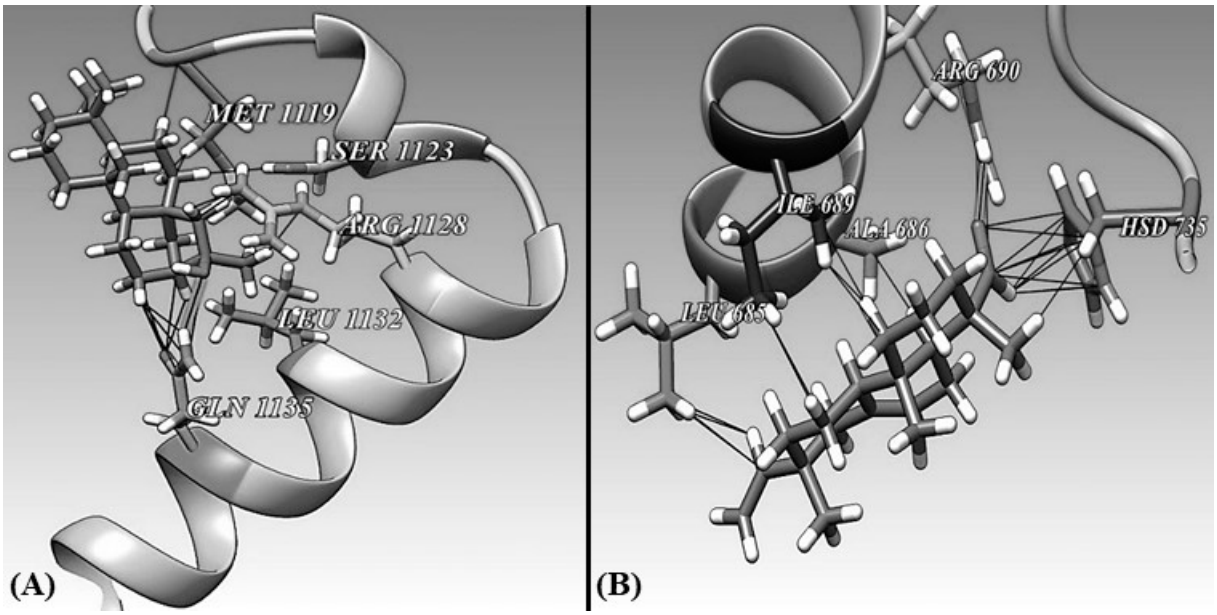

Figure 5. Molecular coupling simulation obtained with the lowest energy conformation for TOP-II. (**A**) Kauran-16-ol; (**B**) isopimaral.

Unlike COX, TOP does not have a highly hydrophobic region, so the role of conformational isomeric is the main phenomenon by which the marked difference in the spontaneity of the generation of the complex is governed; therefore, it can be asserted

that the polarity, the molecular rigidity, the number of conformations, as well as the size and the steric impediment of some of the rings of the analyzed terpenoids correspond efficiently with the chemical characteristics of functional radicals of amino acid residues in TOP interaction zones.

4. Discussion

As far as we know, there are no reports in which we have identified any matrix of plant origin kauran-16-ol or isopimaral, and therefore, there is no information on the biological activity of both. Therefore, in addition to the fact that the compounds identified in SFLE comply with the Lipinski rule, it is promising to enter the pharmacological field and to achieve new perspectives of this class of compounds in the health sector focused on inflammation and cell proliferation. Prostaglandin G/H synthase II (PGHS-II) has a catalytic domain where, in turn, it exhibits two catalytic segments, including cyclooxygenase action that converts AA to prostaglandin G_2 (PGG_2) and peroxidase activity by reducing PGG_2 to PGH_2, the latter of which is responsible for the training of different types of prostanoids [17]. The COX catalysis requires the enzyme to be activated, which is a process that depends on COX activity, mainly by reducing two electrons of a peroxide substrate, and resulting in the oxidation of the heme group in a radical oxo-ferril porphyrin cation, which subsequently dissociates the C_{13} hydrogen from AA, initiating the COX reaction; here, the said activation is coordinated by specific amino acids such as His207, His374, and His388 [27]. The ligands exposed in the molecular coupling simulation interacted strongly with His207 and His388 both through hydrophobic interactions and the hydrogen bridge interaction, and the latter by the hydroxyl group (acting as a nucleophile in the prototropic histidine tautomer), thus achieving an inhibition of enzyme activity as visualized with in vitro results.

PGHS-I has a membrane-binding domain commonly known as a monotopic membrane (MBD), where the enzyme is associated with one side of the lipid bilayer [28]. It is formed by four consecutive α-propellers that promote hydrophobicity to the enzyme to facilitate its interaction with the membrane. However, studies have revealed the importance and functionality of this amino acid sequence in the process, which involves, in addition to the function of anchoring to the membrane through hydrophobic interactions, creating a continuous hydrophobic tunnel from the substrate release site to the catalytic domain; therefore, that the proper folding of the enzyme requires that each of the α-helices of this domain be structurally intact. Otherwise, direct access of the substrate to the active site can be prevented [28]. It is important to note that this domain for COX-I has been characterized in terms of amino acid sequences involving residues 25 to 166 [29].

From the above-mentioned facts and taking into consideration that kauran-16-ol interacts with three residues of this domain (His43, Tyr80, Arg83), it can be asserted that the considerable inhibition of COX-I visualized in the in vitro part is attributed to the lack of integrity of this boundary, resulting in the impairment of the direct access of the AA from the site of its release to the next catalytic site of the enzyme. At the beginning of the twenty-first century, the critical residues that interact directly between DNA and TOP-I, linking to Tyr723, causing the generation of the DNA-TOP-I complex, were revealed, where four basic amino acids such as Arg488, Lys532, Arg590, and His632 aligned and adapted to stabilize the transition state; so, these five residues are classified as critical for the activity of this enzyme [30]. Therefore, comparing this with the interactions obtained for the ligands analyzed, it is asserted that there was no significant enzymatic inhibition. In addition, a discrepancy between the two results was noted by comparing the results obtained in the in vitro phase.

To explain this issue, a mechanism based on forced intermolecular coupling was incorporated in which it was argued that the insertion of an atypical chemical agent into one or more amino acid residues in an environment of a catalytic reaction generates results in an alternative conformational structure of TOP-I, which, in turn, can potentially inhibit its binding with DNA either indirectly (through a chain reaction with the amino acid where it is close to the critical residue) or collaterally to a lesser extent (with cascading

intermolecular reactions until it reacts with the critical amino acid); this is a phenomenon known as allosteric enzyme inhibition [9]. In addition, it is important to mention that cancer has been strongly linked to the interaction of lysine residues present in the enzymes (Lys391 and Lys436) by means of SUMOylation analysis, where anchoring to the motive domain between the protein SUMO and TOP-I (SUMO-TOP-I covalent complex) leads to genome instability by inducing mutagenesis [31].

From the above and with the knowledge that the majority of compounds interact with residues near Lys391 (Pro396, His399), it can be asserted that kauran-16-ol manages to position itself as a potential candidate in the sensitization of cells during chemotherapy. On the other hand, the homodimer TOP-II of *Saccharomyces cerevisiae* exhibits a total of 1428 amino acid residues bound in three domains: the ATP-dependent or N-terminal segment (1–409), the catalytic or DNA-binding and excision site (410–1163), and a "tail" domain or C-terminal segment (1164–1428) [30]. As for its catalytic mechanisms, it has three alternative configurations: an open–closed and closed–closed door or clamp, where it remains one state or another depending on the absence (open–closed) or (closed–closed) presence of ATP in the ATPase domain; and a third conformation (closed–open) dependent on the process of homodimerization of the queue domain and part of the catalytic domain for the release of the DNA segment T (Transport) [31,32]. Therefore, this work, aimed at the inhibition of this enzyme, has been focused on these three aspects, in which each one has exhibited allosteric inhibition, achieving promising results [10–12].

For the evaluated compounds, it can be seen that they have an allosteric inhibition: kauran-16-ol is inclined to limit the change to the third state by its indirect interaction with the Lys1127 residue, which is critical for the opening process [30] and by isopimaral, affecting the state of the TOP-II DNA complex by interacting in the region involved in this mechanism. Concluding that both compounds again exert a synergistic effect by inhibiting different bounds of the chemical entity in question, the expected results contrast with what is visualized in the part in vitro. Based on the results obtained, it can be concluded that for both kauran-16-ol and isopimaral compounds identified in the SFLE of *Anonna cherimola*, it was possible to elucidate an inhibition mechanism in the in silico form that was well matched with the in vitro phase data in relation to the COX and TOP enzymes. Kauran-16-ol and isopimaral inhibit COX-II by blocking POX activity via direct interactions with critical residues in this boundary; in addition, they inhibit COX-I synergistically by modifying the membrane-binding region and interacting with residues indirectly involved (allosteric regulation) in the catalytic domain. Both compounds inhibit TOP-II allosterically and synergistically by limiting the dimerization process and by anchoring with expendable amino acids in the stereospecificity of DNA and TOP-II; in addition, kauran-16-ol is able to sensitize cancer cells by interacting indirectly with a critical activity modulation amino acid. Finally, these results provide the first precedent for different efforts aimed at the discovery of various methodologies for the identification, isolation, and purification of the two bioactive compounds identified, and their anticyclooxygenase and antitopoisomerase activity is potentially promising.

5. Conclusions

In silico, kauran-16-ol and isopimaral inhibit COX-II by blocking peroxidase activity through direct interactions with critical residues in this bound. In addition, they inhibit COX-I synergistically by modifying the membrane binding region and interacting with the residues indirectly involved (allosteric regulation) in the catalytic domain. Kauran-16-ol and isopimaral allosterically inhibit TOP-II synergistically by limiting dimerization and anchoring dispensable amino acids in DNA for stereospecificity and TOP-II. In addition, kauran-16-ol manages to sensitize cancer cells by interacting indirectly with a critical amino acid for modulating activity (Lys391). This study represents the first antecedent where information is made available regarding the biological activity of kauran-16-ol and isopimaral, corroborated by means of an in silico analysis.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/separations11090263/s1, Figure S1: Spectrum of SFLE from gas chromatography coupled with mass (GC-MS).

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