Phytochemical Profiling, In Silico Screening And Anti-Inflammatory Evaluation Of Bioactive Compounds From *Larrea Tridentata*

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Abstract

Enzymatic pathways and pro-inflammatory cytokines including COX-2, TNF-α, IL-1β, NF-κB, and PLA2 drive the complicated biological reaction known as inflammation. The hunt for safer plant-derived substitutes is necessary since long-term usage of non-steroidal anti-inflammatory medicines (NSAIDs) is linked to serious side effects. The current work used pharmacognostical standardization, phytochemical profiling, GC-MS analysis, in silico molecular docking, and ADMET prediction to assess the anti-inflammatory potential of Larrea tridentata. L. tridentata whole plant part powder purchased, verified, and examined macroscopically, microscopically, physicochemically, and phytochemically. Secondary metabolites such as flavonoids, tannins, alkaloids, coumarins, saponins, and phenolic chemicals were most abundant in methanolic extract. The results showed that the total phenolic and flavonoid contents were 53.37 ± 1.20 mg GAE/g and 43.20 ± 0.76 mg QE/g, respectively. Nordihydroguaiaretic acid (33.80%) was the main component of the 57 phytocompounds found by GC-MS analysis. 27 compounds were selected by drug-likeness screening and docked against important inflammatory targets (COX-2, IL-1β, NF-κB, PLA2, TNF-α). The greatest binding affinities for COX-2 were shown by terameprocol and nordihydroguaiaretic acid (−9.5 and −8.8 kcal/mol), outperforming diclofenac (−7.7 kcal/mol). Strong binding to PLA2 and TNF-α was shown by totarol and larreantin, and ADMET analysis showed that the hit compounds had good pharmacokinetic and safety profiles. Larrea tridentata is a rich source of anti-inflammatory phytochemicals, and the work offers compelling computational evidence for its traditional therapeutic usage. These results show that the plant is a viable option for the creation of safer natural anti-inflammatory medications.

Keywords: Larrea tridentata, NDGA, molecular docking, ADMET, anti-inflammatory, network pharmacology

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Introduction

Cytokines, prostaglandins, and transcription factors including COX-2, TNF-α, IL-1β, and NF-κB mediate the complicated biological reaction known as inflammation. Diabetes, cancer, cardiovascular disease, and arthritis are all associated with chronic inflammation. There is a need for safer plant-based substitutes since synthetic anti-inflammatory medications like NSAIDs often have negative side effects [1]. Plants are a key source of many goods that are essential to human needs. There are several uses for plant products, such as food, medicine, and lumber. Plant-based products have been used in medicine since ancient times. The use of plant-based treatments is documented in a number of old manuscripts. The current pharmaceutical business has also used the information from ancient systems of plant-based medicines. Based on the ethno-medical data, there is thus a huge potential for the development of novel medications from plants. Approximately one-third of pharmaceuticals on the market today are derived from plant-based natural compounds [2-3].

Research on plant-based cures is still lagging behind, despite the fact that they have a lot of promise to advance contemporary medical therapies (particularly when contrasted to the interest in generating synthetic pharmaceuticals for commercial usage) [4]. This might be partially due to the delayed and costly nature of traditional plant drug development techniques. However, further study in the field of medicinal plants could be beneficial. The dispersed

nature of the literature and resources in this field makes it difficult to easily use the knowledge that is currently accessible on medicinal plants. The diversity of chemicals may be analyzed using a variety of computer methods. Computer-aided drug design has benefited greatly from these methods [5-6]. In order to meet the ever-increasing demands of the pharmaceutical industry, the area of drug design and discovery from medicinal plants must use such methodologies for faster and more effective advancement [7]. For the analysis and interpretation of massive amounts of data produced by molecular biology-based methods, bioinformatics provides a set of crucial methods. These methods are increasingly crucial for evaluating and integrating data to infer information from a whole systems perspective due to the development of high-throughput techniques [8]. A thorough examination of genomic, proteomic, and metabolomic data is necessary to improve our comprehension of plant cellular processes. The identification of genes and pathways that may be connected to significant bioactive secondary metabolites from medicinal plants is made possible by bioinformatics techniques [9]. A unified platform is needed for consolidated and integrated access to the vast amount of knowledge on medicinal plants that has been gathered throughout time and that is being produced by modern approaches. Numerous databases have been created to catalog information on one or more elements of medicinal plants, including pharmacological applications, bioactive metabolites, ethnobotany, genomic or transcript-based data, molecular targets of active compounds, etc. [10-11] The National Center for Biotechnology Information, the Kyoto Encyclopedia of Genes and Genomes (KEGG), and KNApSAcK are just a few examples of general taxonomic, chemical, and molecular data sources that contain information about medicinal plants. Here are a few instances of databases that provide helpful data on medicinal plants [12]. A free ethnobotanical data repository with multilingual capabilities is the International Ethnobotany Database (ebDB) (http://ebdb.org). Designed primarily for ethnobotanical study, this database offers a wide range of features, including comprehensive location information, robust searching, and data export capabilities. A database called NAPALERT was created to identify and analyze experimental data pertaining to natural products, including those derived from plants [13]. Diclofenac and other non-steroidal anti-inflammatory medications (NSAIDs) are still often used to treat inflammation. However, long-term NSAID use is linked to cardiovascular risks, hepatotoxicity, renal toxicity, and gastrointestinal bleeding. Due of these restrictions, scientists are investigating natural substances produced from plants as safer substitutes with better therapeutic profiles and fewer adverse effects [14].

Historically, medicinal plants have been important sources of bioactive compounds with immunomodulatory, anti-inflammatory, and antioxidant qualities. Larrea tridentata, sometimes known as creosote bush, is a member of the Zygophyllaceae family and is found in many dry areas of North America [15]. The herb has long been used to treat inflammatory diseases, diabetes, kidney stones, rheumatism, arthritis, and skin ailments. Flavonoids, tannins, lignans, terpenes, and phenolic compounds particularly nordihydroguaiaretic acid (NDGA), which is known for its strong anti-inflammatory and antioxidant properties have been found in earlier phytochemical studies [16]. The identification of bioactive compounds using in silico screening techniques has been expedited by recent developments in computational drug discovery. Today drug discovery takes less time and money because of the quick assessment of ligand-protein interactions, pharmacokinetics, and toxicity profiles made possible by molecular docking and ADMET prediction. Important inflammatory targets that are interesting therapeutic targets include COX-2, TNF-α, IL-1β, NF-κB, and PLA2 [17]. Creosote bush, or Larrea tridentata (Zygophyllaceae), has long been utilized to treat metabolic illnesses, infections, and inflammatory conditions. Nordihydroguaiaretic acid (NDGA), a strong antioxidant and lipoxygenase inhibitor, is one of the plant's several lignans [22]. Multi-target plant-derived molecules may now be identified thanks to recent developments in network pharmacology and in-silico drug discovery. Drug-likeness, ADMET characteristics, and molecular interactions with disease targets may all be evaluated simultaneously using this integrated method [18]. Therefore, by combining pharmacognostical standardization, phytochemical screening, GC-MS profiling, and in silico molecular docking against important inflammatory targets, the current research was created to provide a thorough evaluation of Larrea tridentata. In order to find possible lead molecules for the creation of anti-inflammatory drugs, the research also sought to assess the pharmacokinetic and toxicity profiles of the found phytocompounds using ADMET prediction methods [19].

Material and methods

Macroscopical studyof the whole plant powder:

Because of its high resin content, the dried powder of the Larrea tridentata whole plant appears as a coarse, heterogeneous, olive-green to brownish-green

powder with a very sticky texture. Tiny fibrous particles from the woody stems, pieces of brittle, yellowish-green leaf laminas, and soft, fuzzy white tufts from the shredded fruit capsules are all visible upon visual scanning. Crushed flower petals are indicated by scattered pale-yellow flakes. The persistent resinous coating makes it difficult for the powder to flow smoothly since the particles readily clump together. When tasted, it has a very bitter, astringent, and somewhat acrid taste along with the distinctive, pungent, resinous, and creosote-like scent of the plant [20].

Microscopical study:

Whole plant powder asa crude drug: A tiny pinch of Larrea tridentata whole plant powder is put onto a clean glass slide and pre-treated with a few drops of either 5% sodium hydroxide (NaOH) or 95% ethanol to remove the sticky, hydrophobic resin coatings covering the particles in preparation for microscopic examination. A few drops of an 80% w/v chloral hydrate solution are added to the washed powder in order to dissolve the dense cellular contents and dark amber resins that mask the structural details. The tissue bits are then cleared and made entirely transparent by passing the slide slowly over a small Bunsen burner flame until it barely boils. The cleared sample is treated with Safranin O to stain lignified structures (like stem fibers and vessels) bright red for differential visualization of cell types, counterstained with Fast Green FCF to dye parenchyma cells blue-green, and then mounted under a coverslip with a drop of diluted glycerol to keep the preparation from drying out.

A highly diverse diagnostic matrix comprising vegetative, structural, and reproductive fragments can be seen under a microscope in the cleared and stained whole plant powder. Thick-walled wood fibers and thin xylem vessels with bordered pitting that stain a clear bright red make it easy to identify the severely lignified stem parts. Fragments of isobilateral mesophyll tissue with thick, amber-colored resin pockets and epidermal cells with anomocytic stomata validate leaf architecture.Fragments of thin-walled, parenchymatous petals, multilayered staminal scales, and innumerable released, spherical tricolporate pollen grains with three different germination apertures are all contributed by the floral and reproductive organs. Numerous translucent, highly refractive calcium oxalate crystals (mainly large druses and sharp prisms) and numerous unicellular, thick-walled, non-glandular trichomes from the fuzzy fruit capsules are scattered throughout the entire field; under a polarized light microscope, both elements stand out with a brilliant, glowing white birefringence.

Extraction and GCMS Analysis Method:

A homogeneous whole-plant powder was extracted using analytical-grade methanol (1:10 w/v) using room-temperature ultrasonication for 40 minutes inside an amber glass tank in order to profile Larrea tridentata. After centrifuging the crude slurry for ten minutes at 4,000 rpm, the supernatant was filtered through a 0.22 µm PTFE membrane and divided into two aliquots. A 200 µL aliquot was fully dried under nitrogen gas to avoid reagent hydrolysis, and the underivatized part was saved for direct volatile measurement.Before cooling and injecting, this dry residue was reconstituted in 100 µL of anhydrous pyridine, reacted with 100 µL of hexamethyldisilazane (HMDS) and 20 µL of trifluoroacetic acid (TFA) catalyst, and incubated at 70 °C for 45 minutes to transform polar phenolic and carboxylic groups into volatile trimethylsilyl (TMS) ethers.

Using an HP-5MS UI column (30 m × 0.25 mm, 0.25 µm), a helium flow of 1.2 mL/min, and an automated injection of 1.0 µL in a 1:20 split mode at 250 °C, chromatographic separation was carried out on an Agilent 7890B GC with a 5977 MSD. With a 4.2-minute solvent delay and a total run period of 41.25 minutes, the oven program began at 70 °C (2 min hold), ramped at 8 °C/min to 200 °C, and then climbed at 10 °C/min to 300 °C (8 min hold).With temperatures set to 280 °C (transfer line), 230 °C (ion source), and 150 °C (quadrupole), mass detection ran in full scan mode (m/z 50–550) at 70 eV. While a C7-C40 n-alkane calibration provided Retention Index (RI) filtering to remove structural isomer false positives, built-in Agilent MassHunter and AMDIS software deconvoluted co-eluting peaks, filtered baseline noise, and cross-referenced mass spectra against NIST/Wiley databases (>80% match factor).

Larrea tridentata was thoroughly profiled on an Agilent GC-MS platform utilizing an affordable HMDS/TFA procedure to silylate polar fractions. Both native terpenes and heavy silylated lignans were resolved across an optimized temperature gradient that reached 300 °C on an HP-5MS UI column. Agilent MassHunter deconvolution and NIST/Wiley library screening were used to handle automated identification natively. Retention Index (RI) filtering was used to cleanly resolve complicated structural plant isomers. Total 57 compound peaks were identified using the NIST/Wiley library.

In Silico study by Ligand Preparation

The smiles 57 compounds were downloaded from the NCBI PubChem Compounds database (https://www.pubchem.ncbi.nm.nih.gov/). The 57 compounds were subjected to drug-likeness screening based on the Lipinski, Ghose, Veber, Egan, and Muegge rules, and the bioavailability of the compounds was determined using the admetSAR web platform (https://lmmd.ecust.edu.cn/admetsar2/) using respective compound smiles. The 3D structures of the 27 compounds after ADMET screening and the reference drug diclofenac were downloaded from the NCBI PubChem Compounds database (https://www.pubchem.ncbi.nm.nih.gov/) in SDF format. Using Discovery Studio, the SDF files were saved in PDB format.

In Silico study by Protein Preparation: The target proteins, COX-2 (5F19), IL-1β (4G6M), NF-κB (1NFI), PLA2 (1DCY), and TNF-α (2AZ5), with crystallographic resolutions of 2.04, 1.81, 2.70, 2.70, and 2.10 Å, respectively, were downloaded from the Protein Data Bank (http://rcsb.org) [112]. The target proteins were minimized and constructed using UCSF Chimera software (v 1.16). The binding sites of proteins were predicted using BIOVIA Discovery Studio and published studies [21].

In Silico study by Molecular Docking Analysis: Molecular docking analysis was performed and binding affinity was determined using Auto Dock v. 4.2. The protein targets and ligands in pdbqt format were dragged into their respective columns. All ligands were docked, and a maximum exhaustiveness of 8 was computed for all of them. All other parameters in the software were in default mode. The binding affinity of compounds for the protein targets was recorded. The compounds were then ranked by their docking scores. Also, the molecular interactions between protein targets and compounds were viewed using BIOVIA Discovery Studio. Amino acids engaging with the ligand, hydrogen bonds (H-bonds), hydrophobic interactions, and the individual atoms involved were examined in each ligand cluster [22].

In Silico Pharmacokinetic Profile and Drug-Likeness Prediction: The admetSAR web platform (http://lmmd.ecust.edu.cn/admetstar2 (accessed on 29 January 2026)) was used to predict the absorption, distribution, metabolism, and toxicity (ADMET) of the reference drug (DCF) and the hit compounds. The 57 compounds of MMEx were subjected to drug-likeness testing based on the Lipinski, Ghose, Veber, Egan, and Muegge rules and their bioavailability. A total of 30 compounds were screened out, leaving 27 compounds.

Data Analysis: Data obtained from the laboratory experiment were subjected to one-way analysis of variance (ANOVA) followed by a post hoc LSD test, with a level of significance of $p < 0.05$, using GraphPad Prism 8. All of the results were presented as the mean ± standard deviation [23].

Results and Discussion:

Macroscopic evaluation: Larrea tridentata is a woody shrub with many branches that ranges in height from 0.5 to 3.5 meters, according to macroscopic analysis of the entire herb. A glossy, varnished resin layer covers the prominent, swelling nodes on the thin, dark gray-to-blackish stems, which are home to oppositely joined, asymmetrical, V-shaped compound leaves. The reproductive organs include a central, densely woolly ovary, ten prominent stamens with scale-like appendages, five appressed-pubescent sepals, and pale-to-golden yellow flowers (1 to 2.5 cm in diameter) with five petals twisted at their bases into a propeller-like look.

Figure 1: Larrea tridentata

The dried Larrea tridentata entire plant powder is described as a coarse, heterogeneous, olive-green to brownish-green powder by organoleptic and visual inspection. Because of the high resin content of the plant, the substance has a very sticky feel that makes the particles clump together and prevent smooth flow. Tiny fibrous particles from the heavily lignified woody stems, brittle pieces of yellowish-green leaf laminas, scattered pale-yellow flakes from the crushed flower petals, and soft, fuzzy white tufts from the shredded fruit capsules are all visible when scanned visually in ambient light. Sampling of the bulk powder reveals a highly distinct, pungent, creosote-like scent paired with a strongly bitter, astringent, and slightly acrid taste.

Microscopic evaluation: A highly varied diagnostic matrix of vegetative and reproductive pieces was found when the prepared Larrea tridentata whole

plant powder was examined under a microscope. The stem's structural components were narrow xylem vessels with bordered pitting and bright red, lignified, thick-walled wood fibers. Epidermal cells with anomocytic stomata and pieces of isobilateral mesophyll tissue with prominent amber-colored resin pockets made it easy to identify the leaf architecture. The presence of multi-layered staminal scales, thin-walled parenchymatous petal tissue, and a large number of released, spherical tricolporate pollen grains with three different germination apertures indicated the reproductive components.The entire field also displayed a large number of unicellular, thick-walled, non-glandular trichomes that were derived from the fruit capsules and sepals, as well as translucent, highly refractive calcium oxalate crystals that appeared as both large druses and sharp prisms. These crystals were unstained and had a brilliant, glowing white birefringence under polarized light.

An Agilent GC-MS system with an HP-5MS UI column (30 m × 0.25 mm, 0.25 μm) was used to profile Larrea tridentata after an optimized whole-plant methanolic extraction (1:10 w/v) and without and with derivatization workflow using affordable HMDS/TFA silylation (stable trimethylsilyl (TMS)) derivatives. Altogether 57 compounds were resolved, mapped, and identified using built-in Agilent MassHunter deconvolution software combined with NIST/Wiley spectral library matching (>80% match factor) and multi-dimensional Retention Index (RI) filtering to remove false positives from structural isomers.

In Silico Study: An acute inflammatory cascade is a protective process characterized by a spatiotemporal orchestration of enzymatic reactions and cellular activation. It starts abruptly and generally resolves quickly, resulting in tissues being returned to functional homeostasis with an endogenous programmed resolution. It progresses through the generation of a large pool of arachidonic acid (AA) from membrane phospholipids by the action of phospholipase A2 (PLA2). Subsequently, AA is metabolized by cyclooxygenase (COX), resulting in the production of eicosanoids such as leukotrienes (LTs), prostaglandins (PGs), and thromboxane (TX). The primary inflammatory stimuli, including interleukin-1β (IL-1β) and tumor necrosis factor alpha (TNF-α), which are cell-signaling proteins (cytokines) responsible for the acute phase reaction by activating typical NF-κB signaling and related receptors, are closely linked to oxidative stress mechanisms. If acute inflammation is not resolved, it becomes destructive, more complex, and sophisticated. This scenario is likely to amplify drastically due to the prevalence of uncontrollable increased cytokine release (cytokine storm), leading to long-lasting inflammation. Chronic inflammation also produces free radicals, which ultimately aggravate the inflammatory status.

In the pharmaceutical industry, archetypal non-steroidal anti-inflammatory drugs (NSAIDs) have been extremely popular for their certified ability to prevent inflammation; they do this by extinguishing COXs, which in turn inhibit the formation of PGs and PLA2 to suppress the production of other pro-inflammatory mediators. The unrestrained increase in the use of NSAIDs over the years is still controversial, since there are an escalating incidences of toxicity. Unexpectedly, adverse gastrointestinal reactions, including occult bleeding, so-called enteropathy, kidney failure, and liver damage, were reported to be the most pervasive and unavoidable side effects of the prolonged consumption of NSAIDs, putting people all over the world in jeopardy. Notably, the alarming spread of adverse toxicity in certain segments of the population, the high cost, and the upsurge in drug resistance are three specific hindrances to increasing the use of NSAIDs, which are becoming less supportive with a narrow safety window. With an increased collective consciousness among people, a belief has taken root that everything “natural” is undoubtedly healthy. Against the backdrop of these contrasting circumstances, a number of research teams are eagerly searching for a prophylactic therapeutic strategy from nature that has a significant safety margin when applied to both experimental animals and humans.

The exceptional advancements in computational approaches represent an opportunity to identify and design pharmacologically active natural molecules that can target proteins of interest. The use of in silico tools has recently opened new avenues for research in the realm of pharmacotoxicology. Consequently, there is currently an increased focus on developing effective, safe, and low-cost therapeutic modalities with the help of cheminformatics tools, including pharmacokinetic and molecular docking, in order to provide valuable insights into complex and experimentally difficult phenomena such as enzyme reaction mechanics and ligand-receptor linkages.

Larrea tridentata, often known as chaparral or gobernadora, is a medicinal shrub with many branches that is indigenous to desert regions of North America. Growing up to 3.5 meters, it is traditionally used in industrial and forage uses as well as to treat a variety of illnesses, including as rheumatism,

infertility, diabetes, inflammation, gall and kidney stones, and skin problems. Its rich phytochemical composition, which includes tannins, flavonoids, terpenes, and important bioactive compounds including ellagic acid, gallic acid, catechins, methyl gallate, cinnamic acid, luteolin, apigenin, resorcinol, kaempferol, quercetin, nordihydroguaiaretic acid (NDGA), thymol and carvacrol, powers its powerful anti-inflammatory, antibacterial, antioxidant, and neuroprotective qualities.

The objective of this study is to provide a holistic and comprehensive view of the pharmacological potential of Larrea tridentata first by deciphering and identifying the phytochemical composition, and secondly by evaluating in silico anti-inflammatory activities involved. Furthermore, the ADMET profiling and drug-likeness of the identified phytochemicals are rationalized using molecular docking tools.

The binding energy between molecules determines the effect of molecular docking. Lower molecular docking-binding energy represents higher binding affinity. When the binding energy is <5 kcal/mol, the receptor and ligand have relatively good binding properties.

The anti-inflammatory mechanism of the extract as determined by in vivo and in vitro experiments was demonstrated by the in silico analysis.

Drug-likeness of Compounds: The compounds of the extract were evaluated for their ability to serve as active oral drugs. Out of the 57 compounds from Larrea tridentata, 27 were considered to be drug-like compounds while the remaining 30 were not included in further analyses, since they failed the drug-likeness test.

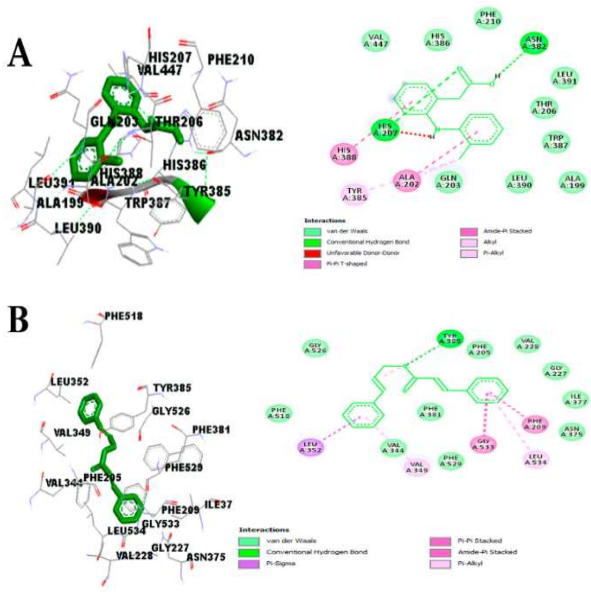
Effect on pro-inflammatory targets: Cox-2, IL-1β, NF-κB, PLA2, and TNF-α: The 27 compounds were virtually screened against five key anti-inflammatory proteins: Cox-2, IL-1β, NF-κB, PLA2, and TNF-α. Among the 27 compounds, 5 were observed to have good binding affinity based on the docking scores: Nordihydroguaiaretic acid, terameprocol, hedycaryol, totarol, and larreantin.

Table 1: Docking scores of compounds and standard drugs within binding pocket of pro-inflammatory receptors.

Ligand	Docking Scores (kcal/mol)				
	COX-2	IL-1β	NF-κB	PLA2	TNF-α
Diclofenac	−7.7	−6.1	−6.1	−7.7	−5.7
Nordihydroguaiaretic acid	−8.8	−5.7	−5.7	−7.4	−6.2
Terameprocol	−9.5	−5.5	−6.5	−8.0	−6.1
Hedycaryol	−7.3	−6.2	−5.8	−7.2	−6.2
Totarol	−8.0	−6.2	−7.1	−8.1	−7.0
Larreantin	−8.5	−6.1	−6.4	−8.5	−6.5

Nordihydroguaiaretic acid(−8.8 kcal/mol) and terameprocol (−9.5 kcal/mol) were found to have higher binding scores than the other compounds and diclofenac when docked against the active site of Cox-2. For IL-1β, hedycaryol (−6.2 kcal/mol) and totarol (−6.2 kcal/mol) stood out from the other compounds after docking. Terameprocol(−6.5 kcal/mol) and totarol (−7.1 kcal/mol) were found to have good binding scores with NF-κB. However, totarol (−8.1 and −7.0 kcal/mol) and Larreantin(−8.5 and −6.5 kcal/mol) were found to be good binders of PLA2 and TNF-α, respectively.

The molecular interaction analysis of compounds with diclofenac that were found to have good binding with the five targets is presented in below figure 2. The analysis shows how the functional groups of the compounds interact. These interactions are critical to the stability and binding of a given compound in the binding pocket of a given protein.



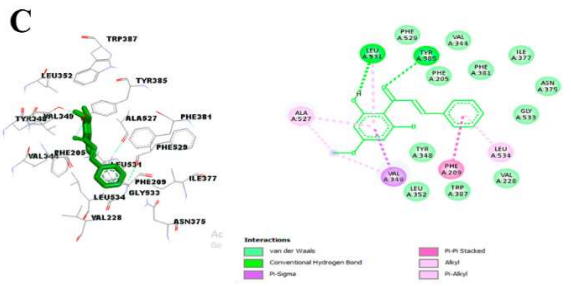


Figure 2. Binding interaction of COX-2 with (A) Diclofenac, (B) Terameprocol and (C) Nordihydroguaiaretic acid. Purple indicates pi-sigma, violet indicates pi-pi stacked, and light pink indicates alkyl/pi-alkyl.

The type of interaction observed with diclofenac, terameprocol, and nordihydroguaiaretic acidafter visualization were mainly hydrogen bonds and hydrophobic and pi-interactions with the binding site of Cox-2. Hydrogen bonds were established between the atoms of terameprocol and nordihydroguaiaretic acidand side chains of Tyr385 and Leu531 while those of diclofenac were with His207 and Asn382.

The predominant amino acids interacting with the atoms of the two hit compounds and diclofenac via hydrophobic and pi-interactions are shown in light green (van der Waals). Following visualization, hydrogen bonds, hydrophobic interactions, and pi-interactions with the IL-1β active site were the main types of molecular interaction seen with diclofenac, hedycaryol, and totarol. No hydrogen bonds formed between the atoms of totarol and the amino acid side chains in the active site of IL-1β. Hydrogen bonds were observed between the atoms of diclofenac, hedycaryol, totarol, and side chains of Asn66 and Leu62. The predominant amino acids interacting with the atoms of the two hit compounds and diclofenac via hydrophobic and pi-interactions are shown in light green (van der Waals), purple (pi-sigma), and violet (pi-pi stacked) and light pink (alkyl/pi-alkyl).

The molecular interactions observed with diclofenac, terameprocol, and totarol after visualization were mainly hydrogen bonds (diclofenac, Ile298) and hydrophobic and pi-interactions with the active site of NF-κB. No hydrogen bonds formed between the atoms of terameprocol, totarol, and the amino acid side chains in the active site of NF-κB. The prominent amino acids interacting with the atoms of the two hit compounds and diclofenac via hydrophobic and pi-interactions are shown in light green (van der Waals), purple (pi-sigma), violet (pi-pi stacked), and light pink (alkyl/pi-alkyl).

The chemical interactions with diclofenac, larreantin, and totarol were primarily hydrogen bonds and hydrophobic and pi-interactions with the phospholipase A2 active site. Hydrogen bonds formed between the atoms of larreantin, totarol, and the amino acid side chains of Gly29, Asp48, His27, and Glu55 in the active site of phospholipase A2. The predominant amino acids interacting with the atoms of the two hit compounds and diclofenac via hydrophobic and pi-interactions are shown in light green (van der Waals), purple (pi-sigma), violet (pi-pi stacked), orange (pi-charges), and light pink (alkyl/pi-alkyl).

The molecular interaction fingerprints observed with diclofenac, larreantin, and totarol after visualization were majorly hydrogen bonds, hydrophobic and pi-interactions with the active site of TNF-α. The hydrogen bonds formed between the atoms of larreantin, totarol, and the amino acid side chains of Gly122, Gly121, Leu120, and Tyr59 in the active site of TNF-α. The predominant amino acids interacting with the atoms of the two hit compounds and diclofenac via hydrophobic and pi-interactions are shown in light green (van der Waals), purple (pi-sigma), violet (pi-pi stacked), and light pink (alkyl/pi-alkyl)

ADMET Results

The admetSAR web platform (http://lmmd.ecust.edu.cn/admetsar2) was used to predict the absorption, distribution, metabolism, and toxicity (ADMET) of the reference drug (DCF) and the hit compounds (terameprocol, nordihydroguaiaretic acid, hedycaryol, totarol, and larreantin. In the absorption class, caco-2 permeability, human intestinal absorption, human oral availability, p-glycoprotein inhibition, and substrates were predicted. All were predicted to permeate caco-2, which is absorbed by the human intestine. Only DCF and hedycaryol are orally bioavailable. Larreantinwas predicted to inhibit p-glycoprotein, and none of the compounds can serve as a substrate for p-glycoprotein.

The distribution of these molecules was predicted via two properties, PPB and BBB. DCF, nordihydroguaiaretic acid, and totarol might be able to reach target sites in high to moderate doses, as their ability to bind to plasma proteins was predicted to be high. Only nordihydroguaiaretic acid cannot permeate the blood-brain barrier (BBB). All compounds were predicted to be localized in the mitochondria except hedycaryol, which is localized in the lysosome.

The metabolism of these molecules via the human liver was predicted in order to ascertain whether they can cause harm or not. Diclofenac, terameprocol,

nordihydroguaiaretic acid, totarol, and larreantinwere predicted to inhibit cytochrome P450 1A2. Also, terameprocol and nordihydroguaiaretic acidwere predicted to inhibit cytochrome P450 2C19. The inhibitors of cytochrome P450 2C9 were predicted to be diclofenac and nordihydroguaiaretic acid. Only nordihydroguaiaretic acidwas predicted to inhibit cytochrome P450 3A4.

DCF and totarol were predicted to be substrates for cytochrome P450 2C9. Totarol was also predicted to be a substrate for cytochrome P450 2D6 and 3A4. Larreantinwas likewise predicted to be a substrate for cytochrome P450 3A4.

All compounds were catalyzed by UGT except nordihydroguaiaretic acid, as predicted by admetSAR. Toxicity was evaluated based on acute oral toxicity, Ames mutagenesis, carcinogenicity, and human hepatotoxicity. None of the compounds were predicted to be carcinogenic, although diclofenac, nordihydroguaiaretic acid, hedycaryol, and larreantin were predicted to be hepatotoxic. Terameprocoland hedycaryol were predicted to have Ames mutagenicity. The acute oral toxicity (kg/mol) was also predicted.

Prior to the molecular docking run, 57 compounds from the plant flower extract with anti-inflammatory properties were subjected to drug-likeness analysis. This analysis ensured the removal of phytocompounds with poor pharmacokinetic parameters from the drug pipeline, thus saving time and cost. Using the SwissADME web tool, we found that 27 of the 57 screened compounds could potentially be used as drugs based on multiple scoring schemes.

The binding affinity between the docked compounds (ligands) and target proteins in this study were influenced by non-covalent interactions such as hydrogen bonds, hydrophobic bonds, and pie-stack interactions. Many researchers have confirmed that binding affinity will be higher (binding energy will be lower) if ligands are able to form hydrophobic interactions with appreciable numbers of hydrophobic amino acid residues in the binding site of a target protein. This may be responsible for the appreciable binding affinity of the active compounds docked against the selected proteins in this study. Notably, the impact of hydrogen bonds on the stabilization of molecular interactions between ligands and proteins should not be downplayed due their critical role in enzyme catalysis and protein substrate and protein-inhibitor complexes, including the structural stability of many biological molecules. To this end, the analysis of molecular interactions exhibited by the active compounds assessed in this study as judged by their binding energy, hydrogen bonding, and hydrophobic interactions with the surrounding amino acids in the selected protein targets revealed the positive impact of their ligand-protein complex status, which may enhance the mechanism of action of the essential oil in ameliorating chronic inflammation. The logic behind performing the absorption, distribution, metabolism, excretion, and toxicity (ADMET) screening of any chemical compound within the human body is that it can determine the pharmacological and pharmacodynamic properties of candidate drug compounds within the biological system.

Adsorption, distribution, metabolism, excretion, and toxicity (ADME/Tox) screening is an increasingly necessary technique for the therapeutic evaluation of small molecules for further processing. Based on this in silico approach, more small molecules have been discovered as potential therapeutic candidates. The increasing failure rate of drug molecules at the clinical trial stage has been attributed to poor ADME/Tox profiles. Hence, predictions of good ADME/Toxprofiles of natural compounds can help to eliminate compounds with unacceptable side effects. Therefore, the in silico approach remains the cheapest and most time-efficient model for screening bioactive molecules. In this study, the ADME/Tox and drug-likeness analysis of the phytochemicals from *Larrea tridentata* was performed to screen out compounds with potential toxicity that violate the drug-likeness rules. Out of the 57 phytochemicals obtained from *Larrea tridentata*via GC-MS analysis, 27 were predicted to have good pharmacokinetic profiles and obey the Lipinski, Vebers, and Egan rules of drug-likeness.

While several rules have been developed and used to predict the drug-likeness of bioactive compounds from plants, the most prominently used is Lipinski's rule of five, which was considered in this study. According to this rule, drug-like compounds should not violate more than one of the following rules: a number of H-bond acceptors less than 10, a number of H-bond donors less than 5, a molecular weight less than 500, a partition coefficient less than or equal to 5, and a polar surface area less than or equal to 14 Å. The results from this study reveal that all the hit compounds were positive for human intestinal absorption and Caco-2 and were negative for the P-glycoprotein substrate, CYP2D6 inhibition, and carcinogenicity, while nordihydroguaiaretic acidwas the only compound that was negative for blood-brain barrier penetration. Most orally administered drugs exhibit their potency when they are absorbed into the bloodstream for circulation. The ability of bioactive molecules to cross the blood-brain barrier is a

significant consideration for the management of neurodegenerative disorders. Human intestinal absorption and Caco-2 remain as the tools for screening the intestinal permeability of therapeutic agents. Hence, the hit compounds in this study can effectively permeate the intestine for metabolism. The membrane P-glycoprotein (P-gp) is known to inhibit the absorption, distribution, and bioavailability of its substrate as small molecules, thereby eliminating them from circulation [93]. The negative result predicted for the hit compounds revealed that they have high bioavailability in the system. The hit compounds were also revealed to be non-carcinogenic, indicating the safety of the plant.

Virtual screening via molecular docking is an in silico model that employs a computer-based tool that has been used in structure-based drug development. It relies on the principle of predicting the binding orientation and evaluating the binding energy of small molecules interacting at the binding pocket of the target. The hit compounds showed better binding affinity against COX-2 and PLA-2 compared with the standard drug (diclofenac). Hence, these proteins might be predicted as the mechanism of anti-inflammatory action, which corroborates with the in vitro and in vivo anti-inflammatory results obtained in this study.

Conclusion

The present study provides a comprehensive pharmacognostical, phytochemical and computational evaluation of *Larrea tridentata* as a potential source of anti-inflammatory agents. Pharmacognostical and physicochemical standardization confirmed the authenticity and purity of the plant powder material. Preliminary phytochemical screening revealed the presence of diverse bioactive secondary metabolites, while GC-MS analysis identified 57 phytocompounds with nordihydroguaiaretic acid as the predominant constituent.

Drug-likeness and ADMET screening shortlisted 27 compounds with favorable pharmacokinetic profiles. Molecular docking studies demonstrated strong binding affinity of terameprocol, nordihydroguaiaretic acid, totarol, hedycaryol and larreantintoward key inflammatory targets. Notably, terameprocol and nordihydroguaiaretic acid showed higher binding affinity toward COX-2 compared with the standard drug diclofenac, suggesting a promising mechanism of anti-inflammatory action. ADMET predictions indicated acceptable safety and pharmacokinetic profiles for most hit compounds.

Overall, the findings support the traditional medicinal use of *Larrea tridentata* and highlight its phytochemicals as promising candidates for the development of novel, safe and effective anti-inflammatory drugs. Further studies involving in vitro, in vivo and clinical evaluations are recommended to validate these findings and facilitate drug development.

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