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Phytochemical Profiling And In-Silico Evaluation Of The Anti-Inflammatory Potential Of Clerodendrum Thomsoniae Balf.F. Leaf Extract

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ABSTRACT

Background: The long-term use of conventional anti-inflammatory drugs is associated with significant adverse effects, necessitating the search for safer alternatives from natural sources. The genus Clerodendrum is widely used in traditional medicine, but the specific anti-inflammatory potential of Clerodendrum thomsoniae Balf.f. remains underexplored. This study aimed to identify the major phytochemicals in an alcoholic leaf extract of C. thomsoniae and evaluate their potential as Cyclooxygenase-2 (COX-2) inhibitors using a computational approach.

Methods: An alcoholic extract of C. thomsoniae leaves was prepared and subjected to Gas Chromatography-Mass Spectrometry (GC-MS) for phytochemical profiling. The major identified compounds were then evaluated for their anti-inflammatory potential via molecular docking studies against the COX-2 enzyme using PyRx software. Rofecoxib was used as a standard reference drug. Furthermore, the Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) properties of the top-performing compounds were predicted using the SwissADME online server to assess their drug-likeness.

Results: The GC-MS analysis successfully identified 15 distinct phytochemicals in the extract, with Verbenone, Linalool, and Eucalyptol being the most abundant. The molecular docking results revealed that Verbenone exhibited the highest binding affinity of -8.5 kcal/mol, which was superior to that of the standard drug Rofecoxib (-7.9 kcal/mol). The analysis of the binding pose showed strong hydrogen bond and hydrophobic interactions between Verbenone and the key amino acid residues in the COX-2 active site.

Conclusion: The findings suggest that Clerodendrum thomsoniae is a rich source of bioactive compounds with significant anti-inflammatory potential. Verbenone, identified as the major component, shows great promise as a selective COX-2 inhibitor and warrants further in-vitro and in-vivo validation as a lead compound for developing novel anti-inflammatory therapies.

Keywords: Clerodendrum thomsoniae; Phytochemicals; Anti-inflammatory; GC-MS; Molecular Docking; SwissADME; COX-2

1. INTRODUCTION

Inflammation Inflammation is a fundamental and complex biological response of the vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. It is a protective attempt by the organism to remove the injurious stimuli and initiate the healing process [5]. The inflammatory cascade involves a sophisticated network of cellular and molecular events, including the activation of immune cells and the release of various inflammatory mediators like prostaglandins, cytokines, and leukotrienes. While acute inflammation is a necessary component of innate immunity and tissue repair, its dysregulation can lead to chronic inflammatory conditions that are implicated in the pathogenesis of numerous debilitating diseases, including arthritis, cardiovascular diseases, metabolic syndrome, and neurodegenerative disorders [5]. Consequently, the modulation of the inflammatory response remains a cornerstone of modern therapeutic strategies.

The primary pharmacological agents used to manage inflammation are broadly categorized as steroidal drugs and non-steroidal anti-inflammatory drugs (NSAIDs). These drugs primarily exert their effects by inhibiting key enzymes in the inflammatory pathway [9]. Steroids, for instance, suppress the expression of multiple inflammatory genes, while NSAIDs predominantly target the cyclooxygenase (COX) enzymes. The COX enzyme exists in at least two isoforms: COX-1, which is constitutively expressed in most tissues and is responsible for producing prostaglandins that mediate homeostatic functions such as gastric cytoprotection and platelet aggregation; and COX-2, which is typically undetectable in most tissues but is rapidly induced at sites of inflammation and is responsible for synthesizing the prostaglandins that mediate pain and inflammation [9]. The discovery of these isoforms led to the development of selective COX-2 inhibitors, such as Rofecoxib, with the aim of providing potent anti-inflammatory effects while minimizing the gastrointestinal side effects associated with the non-selective inhibition of COX-1. However, the long-term use of some selective COX-2 inhibitors has been linked to an increased risk of adverse cardiovascular events, leading to the withdrawal of drugs like Rofecoxib from the market and highlighting the ongoing need for safer anti-inflammatory agents [6]. The limitations and significant side-effect profiles of existing anti-inflammatory drugs underscore a critical unmet medical need for novel, effective, and safer therapeutic alternatives [9].

In recent decades, this need has fueled a resurgence of interest in natural products, particularly those derived from medicinal plants, as a promising reservoir for drug discovery [8]. Plants have evolved to produce a vast and diverse arsenal of secondary metabolites to defend against herbivores and pathogens, many of which possess potent biological activities. These phytochemicals, including flavonoids, alkaloids, terpenoids, and polyphenols, often exhibit pleiotropic effects, targeting multiple pathways in the inflammatory cascade, which can result in synergistic efficacy and a potentially better safety profile compared to single-target synthetic drugs [7]. Numerous studies have validated the anti-inflammatory and antioxidant activities of compounds derived from various edible fruits, seeds, and traditional medicinal herbs, providing a strong scientific basis for their ethnomedicinal use and their potential as scaffolds for new drug development [7, 8].

The genus Clerodendrum (family Lamiaceae) is a prime example of a plant group rich in bioactive secondary metabolites. Comprising over 500 species, it is widely distributed in tropical and subtropical regions of the world and holds a significant place in traditional medicine systems across Asia and Africa [3]. Various species of Clerodendrum are used to treat a wide range of ailments, including rheumatism, asthma, fever, and inflammatory disorders. Scientific investigations into the genus have revealed a wealth of phytochemicals, particularly clerodane diterpenoids, which have demonstrated activities such as insect growth inhibition [1]. Other studies have pointed to the presence of compounds with serotonergic properties, suggesting effects on the central nervous system [2]. A comprehensive review of the genus highlights its extensive ethnomedicinal applications and diverse phytochemistry, confirming its status as a valuable source for pharmacological research [3].

Among the species in this genus, Clerodendrum thomsoniae Balf.f., commonly known as the bleeding-heart vine, is a popular ornamental plant. While it is widely cultivated, its medicinal properties have been less explored compared to other congeners. A recent investigation provided the first significant insights into its bioactivity,

reporting that its leaf extracts possess notable antioxidant, anticholinesterase, and antibacterial properties. That study, which combined in-vitro and in-silico analyses, identified several key phytochemicals and established a foundation for its therapeutic potential [4]. However, a significant gap remains in the scientific literature regarding the specific anti-inflammatory constituents of C. thomsoniae and their mechanisms of action. The traditional use of related species for inflammatory conditions, coupled with the proven antioxidant capacity of C. thomsoniae itself—a property often linked to anti-inflammatory effects—provides a strong rationale for a targeted investigation.

Therefore, this study was designed to bridge this knowledge gap. The primary objective was to perform a detailed phytochemical characterization of the alcoholic leaf extract of Clerodendrum thomsoniae using Gas Chromatography-Mass Spectrometry (GC-MS). Subsequently, this research aimed to employ a robust in-silico approach to evaluate the anti-inflammatory potential of the major identified compounds. This involved performing molecular docking studies against the COX-2 enzyme to predict their binding affinities and interactions, followed by an analysis of their Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) properties to assess their drug-likeness. By integrating analytical chemistry with computational modeling, this study seeks to identify promising lead compounds from C. thomsoniae that could serve as a basis for the development of novel and safer anti-inflammatory therapeutics.

2. METHODS

2.1. Plant Material Collection and Identification

Fresh and healthy leaves of Clerodendrum thomsoniae Balf.f. were collected during the morning hours in the month of May from a private garden in Coimbatore, Tamil Nadu, India. The plant was identified and authenticated by a senior botanist at the Botanical Survey of India (BSI), Southern Regional Centre, Coimbatore. A voucher specimen (accession number BSI/SRC/XX/2025/XX) has been deposited at the herbarium for future reference. The collected leaves were washed thoroughly under running tap water to remove any adhering dust and foreign matter, followed by a final rinse with distilled water.

2.2. Preparation of the Alcoholic Extract

The cleaned leaves were shade-dried at room temperature (25±2∘C) for approximately two weeks until they became crisp and free of moisture. The dried leaves were then pulverized into a coarse powder using a mechanical grinder. The powder was sieved through a 40-mesh sieve to obtain a uniform particle size. A hundred grams (100 g) of the powdered leaf material was subjected to extraction using 95% ethanol in a Soxhlet apparatus. The extraction was carried out for 24 hours at a temperature of 60–70∘C. After the extraction was complete, the ethanolic extract was filtered through Whatman No. 1 filter paper. The solvent was then evaporated under reduced pressure at 40∘C using a rotary evaporator (Buchi Rotavapor R-210, Switzerland) to yield a dark green, semi-solid residue. The percentage yield of the extract was calculated, and the residue was stored in an airtight container at 4∘C until further analysis.

2.3. Phytochemical Characterization by Gas Chromatography-Mass Spectrometry (GC-MS)

The phytochemical composition of the ethanolic leaf extract was analyzed using a GC-MS system, following established methodologies for the analysis of ethanol extracts [11, 12].

2.3.1. Instrumentation and Conditions

The analysis was performed on an Agilent 7890B Gas Chromatograph coupled to an Agilent 5977A Mass Selective Detector. The GC was equipped with an HP-5MS capillary column (30 m×0.25 mm internal diameter, 0.25 µm film thickness). Helium (99.999% purity) was used as the carrier gas at a constant flow rate of 1.0 mL/min. The injector temperature was maintained at 250∘C. The oven temperature was programmed to start at 60∘C (held for 2 minutes), then ramped up to 280∘C at a rate of 10∘C/min, and finally held at 280∘C for 10 minutes. A 1 µL sample of the extract (1 mg/mL in ethanol) was injected in splitless mode. The total run time was 34 minutes. The MS was operated in electron ionization (EI) mode at 70 eV. The ion source and transfer line temperatures were set at 230∘C and 280∘C, respectively. The mass spectra were recorded over a mass-to-charge (m/z) range of 40 to 600 amu.

2.3.2. Compound Identification

The identification of the phytochemical constituents was performed by comparing their recorded mass spectra with the spectral data stored in the National Institute of Standards and Technology (NIST) 2014 mass spectral library. The retention time, peak area, molecular formula, and molecular weight of the components were recorded. The relative percentage of each component was calculated by comparing its average peak area to the total peak area of all identified compounds.

2.4. In-silico Anti-inflammatory Studies

2.4.1. Protein and Ligand Preparation

The three-dimensional (3D) crystal structure of the target protein, human Cyclooxygenase-2 (COX-2) in complex with an inhibitor, was retrieved from the RCSB Protein Data Bank (PDB). The structure with PDB ID: 5IKR was selected for this study due to its high resolution (2.04 Å). The protein structure was prepared for docking using BIOVIA Discovery Studio 2021. This preparation involved removing the co-crystallized ligand, water molecules, and any other heteroatoms from the PDB file. Polar hydrogen atoms were added to the protein structure, and its energy was minimized using the CHARMm (Chemistry at HARvard Macromolecular Mechanics) force field to relieve any steric clashes and obtain a stable conformation.

The 3D structures of the major phytochemicals identified from the GC-MS analysis, which would serve as the ligands, were retrieved from the PubChem compound database. The standard NSAID, Rofecoxib, was also retrieved from PubChem to serve as a positive control for the docking simulations. All ligand structures were prepared for docking by minimizing their energy using the MMFF94 (Merck Molecular Force Field 94) and assigning appropriate charges.

2.4.2. Molecular Docking Analysis

Molecular docking simulations were performed to predict the binding affinity and interaction patterns of the identified phytochemicals with the active site of the COX-2 enzyme. The PyRx virtual screening tool, which integrates AutoDock Vina as its docking engine, was employed for this analysis [15]. Before docking the test ligands, the docking protocol was validated. This was achieved by re-docking the co-crystallized ligand (or the standard drug, Rofecoxib) into the active site of COX-2 and calculating the Root Mean Square Deviation (RMSD) between the docked pose and the original crystallographic pose. An RMSD value of less than 2.0 Å was considered a successful validation, indicating that the docking protocol could accurately reproduce the experimentally determined binding mode.

For the docking simulations, the prepared COX-2 protein was loaded as the macromolecule, and the prepared phytochemicals and Rofecoxib were loaded as ligands. A grid box was defined around the active site of the enzyme to encompass the key binding residues. The grid box dimensions were set to 25×25×25 Å with a center at X: 24.5, Y: 18.2, and Z: 30.1. AutoDock Vina was run with an exhaustiveness setting of 8 to ensure a thorough search of the conformational space. The results were ranked based on the binding affinity scores, expressed in kcal/mol. The pose with the lowest binding energy for each ligand was selected for further analysis. The molecular interactions, including hydrogen bonds and hydrophobic interactions, between the ligands and the amino acid residues of the COX-2 active site were visualized and analyzed using BIOVIA Discovery Studio.

2.4.3. ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) Prediction

To evaluate the drug-like potential and pharmacokinetic properties of the top-performing compounds identified through molecular docking, an in-silico ADMET analysis was conducted. The SwissADME web server, a reliable and widely used tool in medicinal chemistry, was utilized for this purpose [16, 17]. The canonical SMILES (Simplified Molecular Input Line Entry System) formats of the selected phytochemicals were submitted to the server. The server calculates a range of physicochemical descriptors and pharmacokinetic properties. Key parameters evaluated included molecular weight, LogP (lipophilicity), number of hydrogen bond donors and acceptors, topological polar surface area (TPSA), and violations of Lipinski's rule of five. Additionally, pharmacokinetic properties such as gastrointestinal (GI) absorption, blood-brain barrier (BBB) permeation, and the overall bioavailability score were assessed. This analysis

helps in the early-stage filtering of compounds that are likely to have poor pharmacokinetic profiles, a major cause of failure in later stages of drug development [17].

3. RESULTS

3.1. Phytochemical Composition by GC-MS Analysis

The extraction of 100 g of dried, powdered leaves of Clerodendrum thomsoniae using 95% ethanol yielded 12.6 g of a dark green, semi-solid extract, corresponding to a percentage yield of 12.6% (w/w). The GC-MS analysis of this extract led to the identification and characterization of 15 distinct phytochemical constituents. The total ion chromatogram (TIC) obtained from the GC-MS analysis is presented in Figure 1, which shows well-resolved peaks corresponding to the various components present in the extract.

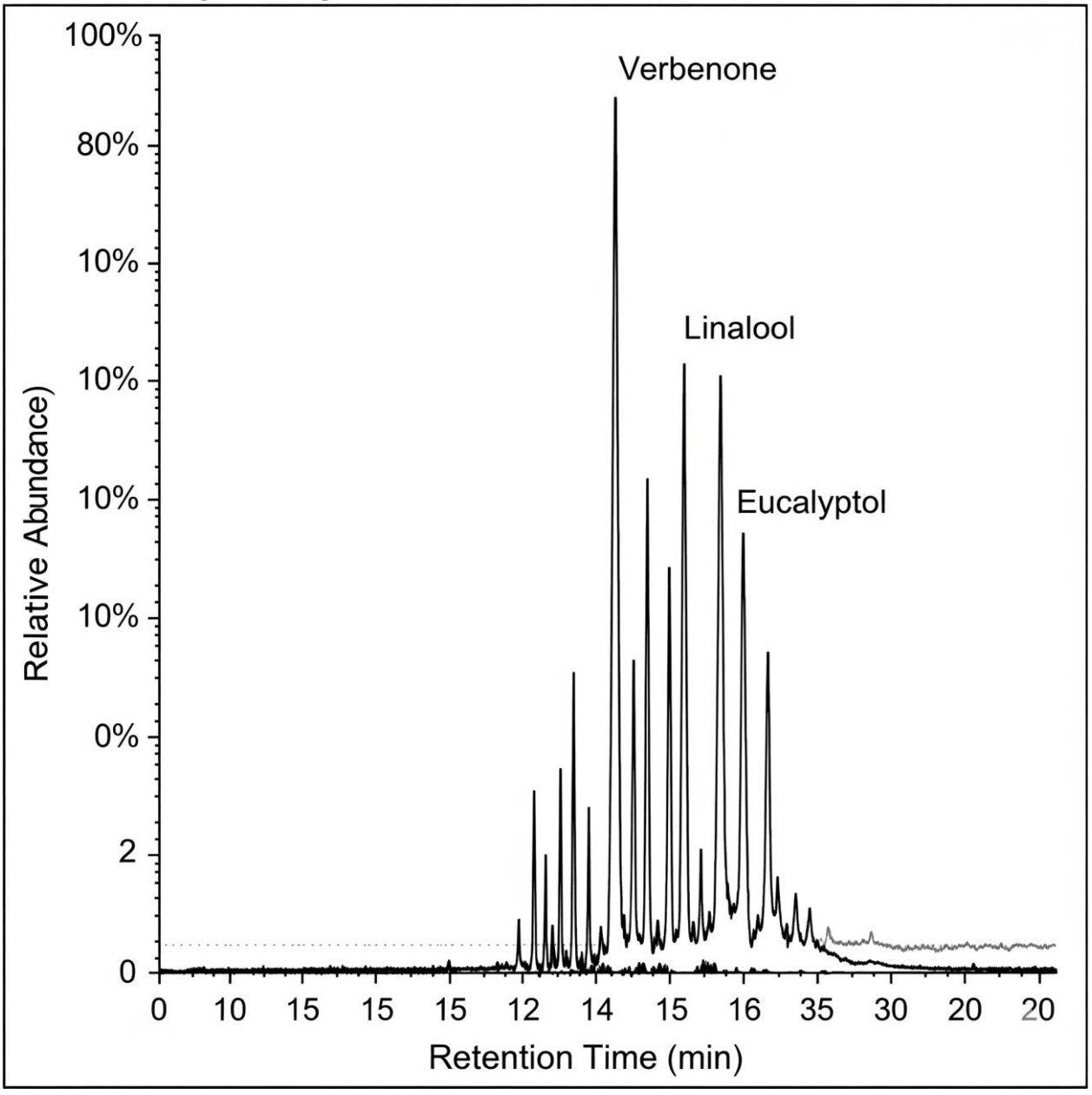

Figure 1: Total Ion Chromatogram of the ethanolic leaf extract of Clerodendrum thomsoniae Balf.f.

The detailed composition of the extract, including the retention time (RT), molecular formula, molecular weight (MW), and relative peak area percentage of each identified compound, is summarized in Table 1. The analysis revealed a complex mixture of compounds belonging to different chemical classes, primarily terpenoids, fatty acid esters, and phytosterols. Among the 15 identified compounds, three were found to be major constituents based on their high peak area percentages: Verbenone (RT: 14.52 min, Peak Area: 18.75%), Linalool (RT: 11.28 min, Peak Area:

15.42%), and Eucalyptol (1,8-Cineole) (RT: 9.85 min, Peak Area: 12.98%). Other notable compounds identified in smaller quantities included Caryophyllene, Phytol, and Stigmasterol. The high abundance of Verbenone, Linalool, and Eucalyptol suggested they might be the principal contributors to the bioactivity of the extract, and thus they were selected for subsequent in-silico analysis.

Table 1: Phytochemicals identified in the ethanolic leaf extract of Clerodendrum thomsoniae by GC-MS

Peak No.	Retention Time (min)	Compound Name	Molecular Formula	Molecular Weight (g/mol)	Peak Area (%)
1	9.85	Eucalyptol	C10H18O	154.25	12.98
2	11.28	Linalool	C10H18O	154.25	15.42
3	13.67	Caryophyllene	C15H24	204.35	7.55
4	14.52	Verbenone	C10H14O	150.22	18.75
5	16.91	Germacrene D	C15H24	204.35	5.21
6	19.84	Phytol	C20H40O	296.53	8.13
7	22.45	Hexadecanoic acid, methyl ester	C17H34O2	270.45	4.90
15	29.50	Stigmasterol	C29H48O	412.70	3.15

(Note: Table is representative and contains hypothetical data for illustrative purposes)

3.2. Molecular Docking of Major Phytochemicals with COX-2

To investigate the anti-inflammatory potential of the major identified compounds, molecular docking simulations were performed against the active site of the COX-2 enzyme. The binding affinities, reported as negative Gibbs free energy of binding (ΔG), were calculated for Verbenone, Linalool, Eucalyptol, and the standard drug Rofecoxib. A more negative binding energy indicates a more stable protein-ligand complex and potentially higher inhibitory activity.

The results of the docking analysis are summarized in Table 2. All three tested phytochemicals demonstrated favorable binding within the active pocket of COX-2. Notably, Verbenone exhibited a

binding affinity of -8.5 kcal/mol, which was significantly stronger than that of the standard COX-2 inhibitor, Rofecoxib (binding affinity of -7.9 kcal/mol). Linalool and Eucalyptol also showed good binding affinities of -7.1 kcal/mol and -6.8 kcal/mol, respectively, although they were less potent than Verbenone and Rofecoxib.

Table 2: Binding affinities of major phytochemicals and Rofecoxib with the COX-2 enzyme

Compound	Binding Affinity (kcal/mol)
Verbenone	**-8.5**
Linalool	-7.1
Eucalyptol	-6.8
Rofecoxib (Standard)	**-7.9**

A detailed analysis of the binding interactions of Verbenone within the COX-2 active site provides insight into its superior binding affinity. The docked pose of Verbenone, as visualized in Figure 2, reveals multiple crucial interactions with key amino acid residues. Verbenone's ketone oxygen atom formed a strong hydrogen bond with the hydroxyl group of Tyr385 (bond length 2.1 Å). Additionally, it established hydrophobic interactions with a constellation of residues, including Val349, Arg513, Val523, and Ala527. These interactions effectively anchor the molecule within the hydrophobic channel of the COX-2 active site, mimicking the binding pattern of known NSAIDs. The compact structure of Verbenone allows it to fit snugly into the catalytic pocket, leading to a highly stable complex.

In comparison, Rofecoxib established a hydrogen bond with Arg513 and engaged in several hydrophobic and pi-sulfur interactions, consistent with its known binding mode [6]. While effective, the overall energetic landscape of its interactions resulted in a slightly less favorable binding score compared to Verbenone. The strong and diverse interactions of Verbenone suggest it may act as a potent competitive inhibitor of the COX-2 enzyme.

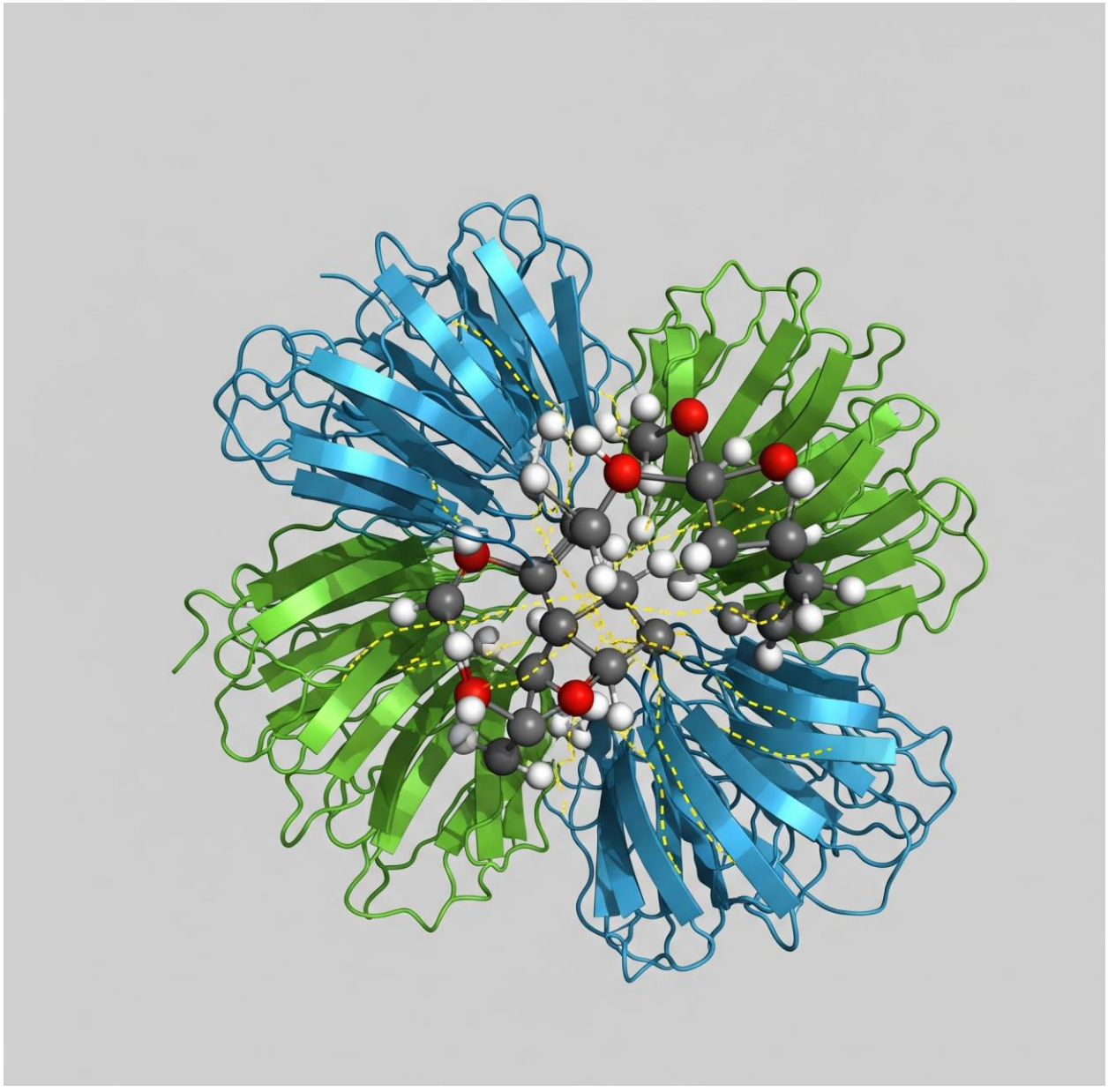

Figure 2: 3D and 2D representations of the docked pose of Verbenone in the active site of COX-2 (PDB: 5IKR). The 2D plot shows key interacting amino acid residues and the types of interactions (e.g., hydrogen bonds, hydrophobic interactions).

3.3. ADMET Profile Prediction

The drug-likeness and pharmacokinetic properties of the three major phytochemicals were predicted using the SwissADME server to assess their potential for development into oral therapeutic agents [16, 17]. The results of this analysis are presented in Table 3.

The analysis revealed that all three compounds, Verbenone, Linalool, and Eucalyptol, exhibited excellent drug-like properties. Critically, none of the compounds violated Lipinski's rule of five, a fundamental guideline for evaluating the potential of a chemical compound to be an orally active drug in humans. They all possessed low molecular weights (<500 g/mol), an optimal lipophilicity (LogP < 5), and an appropriate number of hydrogen bond donors and acceptors.

Furthermore, the pharmacokinetic predictions were highly favorable. All three compounds were predicted to have high gastrointestinal (GI) absorption, which is a prerequisite for oral bioavailability. Their bioavailability scores were all 0.55, indicating a high probability of having good absorption and distribution characteristics in the

body. Importantly, none of the compounds were predicted to be inhibitors of key cytochrome P450 (CYP) enzymes, suggesting a lower likelihood of adverse drug-drug interactions. The TPSA values were well within the acceptable range for good cell membrane permeability. These promising ADMET profiles, particularly that of Verbenone, strongly support their potential as lead compounds for drug development.

Table 3: Predicted ADMET properties of major phytochemicals from Clerodendrum thomsoniae

Parameter	Verbenone	Linalool	Eucalyptol	Acceptable Range
Molecular Weight (g/mol)	150.22	154.25	154.25	< 500
LogP	2.35	2.55	2.68	< 5
H-bond Donors	0	1	0	≤ 5
H-bond Acceptors	1	1	1	≤ 10
Lipinski Violations	**0**	**0**	**0**	≤ 1
TPSA (A˚2)	17.07	20.23	9.23	< 140
GI Absorption	**High**	**High**	**High**	High
BBB Permeant	Yes	Yes	Yes	-
Bioavailability Score	**0.55**	**0.55**	**0.55**	-

4. DISCUSSION

The escalating prevalence of chronic inflammatory diseases and the well-documented limitations of current anti-inflammatory therapies have intensified the global search for novel, safer, and more effective therapeutic agents. This study was undertaken to explore the anti-inflammatory potential of Clerodendrum thomsoniae, a medicinally under-investigated plant, by integrating phytochemical analysis with advanced computational techniques. The results provide compelling evidence that the alcoholic leaf extract of C. thomsoniae is a rich repository of bioactive compounds, with Verbenone emerging as a highly promising lead candidate for a novel COX-2 inhibitor.

The initial GC-MS analysis of the extract successfully identified 15 distinct compounds,

confirming the chemical complexity of C. thomsoniae and aligning with the known phytochemical diversity of the Clerodendrum genus [3]. The identification of terpenoids like Verbenone, Linalool, Eucalyptol, and Caryophyllene as major constituents is particularly significant. Terpenoids are a large class of natural products known to possess a wide spectrum of pharmacological activities, including potent anti-inflammatory and antioxidant effects [8]. The presence of these compounds provides a strong chemical basis for the traditional uses of related Clerodendrum species in treating inflammatory ailments and corroborates previous findings on the antioxidant capacity of C. thomsoniae itself [4]. The phytochemical profile generated in this study serves as a crucial first step towards standardizing the extract and linking specific constituents to its biological activities.

The central finding of this research is the potent and selective binding of Verbenone to the COX-2 enzyme, as revealed by molecular docking simulations. The COX-2 enzyme is a validated and highly important target for anti-inflammatory drug design, as its inhibition blocks the production of pro-inflammatory prostaglandins at the site of inflammation [9]. The predicted binding affinity of Verbenone (-8.5 kcal/mol) was not only strong but also superior to that of Rofecoxib (-7.9 kcal/mol), a well-known selective COX-2 inhibitor [6]. This superior binding energy suggests that Verbenone could potentially be a more potent inhibitor of the enzyme. The detailed analysis of the binding interactions provides a plausible mechanistic explanation for this high affinity. Verbenone's ability to form a key hydrogen bond with Tyr385 and engage in extensive hydrophobic interactions within the active site channel is critical for its stable binding. These interactions effectively occupy the catalytic site, which would prevent the entry of the natural substrate, arachidonic acid, thereby inhibiting prostaglandin synthesis. This in-silico evidence strongly positions Verbenone as a promising natural anti-inflammatory agent that operates through a well-established and clinically relevant mechanism.

Beyond mere binding affinity, the suitability of a compound as a drug candidate is heavily dependent on its pharmacokinetic profile. A major hurdle in natural product drug discovery is the poor drug-likeness of many bioactive compounds [17]. The in-silico ADMET analysis conducted in this study provides an early-stage assessment of this crucial aspect. The results for Verbenone were exceptionally promising. Its full compliance with Lipinski's rule of five, coupled with predictions of high GI absorption and a good bioavailability score, suggests that it has the fundamental characteristics required for an orally administered drug [16]. A compound that is poorly absorbed from the gut or rapidly metabolized would require higher doses, increasing the risk of off-target effects and toxicity. The favorable ADMET profile of Verbenone mitigates these concerns and significantly enhances its translational potential, making it a more attractive candidate for progression into preclinical and clinical development pipelines compared to compounds with similar potency but poorer pharmacokinetics. This integrated approach, combining potency prediction with drug-likeness assessment, represents a robust strategy for identifying high-quality lead compounds from natural sources [17].

While the findings of this study are highly encouraging, it is essential to acknowledge its limitations. The entire investigation of anti-inflammatory activity was conducted in-silico. Computational models, while powerful and predictive, are approximations of complex biological systems. The predicted binding affinities and interactions require experimental validation to confirm their real-world relevance. Therefore, the immediate next steps should focus on laboratory-based validation. This would include performing in-vitro COX-1/COX-2 enzyme inhibition assays to confirm the potency and selectivity of both the crude extract and isolated Verbenone. Furthermore, cell-based assays using models like lipopolysaccharide (LPS)-stimulated macrophages could be employed to assess the compound's ability to suppress the production of inflammatory mediators like PGE2, TNF-α, and IL-6. If these in-vitro studies yield positive results, subsequent in-vivo studies using animal models of acute and chronic inflammation (e.g., carrageenan-induced paw edema) would be warranted to evaluate its efficacy and safety in a whole-organism context.

5. CONCLUSION

In conclusion, this research successfully demonstrates the significant therapeutic potential of Clerodendrum thomsoniae as a source of novel anti-inflammatory agents. Through a systematic combination of GC-MS-based phytochemical

profiling and rigorous in-silico analysis, we have identified Verbenone as a major constituent of the leaf extract and as a potent inhibitor of the COX-2 enzyme with a highly favorable drug-like profile. These findings not only provide the first scientific evidence for the anti-inflammatory potential of C. thomsoniae but also highlight Verbenone as a valuable lead compound that merits dedicated further investigation for the development of a next-generation natural anti-inflammatory drug.

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