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Elucidation of *Chromolaena odorata* Extract's Pharmacological Potential Through Integration of Network Pharmacology and Molecular Docking Study

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ABSTRACT

Network pharmacology, an interdisciplinary field that combines principles from pharmacology, systems biology, and network science, provides a robust framework for exploring the intricate relationship between biological systems and pharmacologically active compounds. This study focuses on the herbal medicine *Chromolaena odorata*, known as "*Daun kapal terbang*" in Malaysia. This plant, renowned for its diverse medicinal properties, underwent thorough analysis, revealing its anti-inflammatory, antimicrobial, anticancer, antidiabetic, and wound-healing attributes. However, a deeper understanding of its pharmacological mechanism of action remains unclear. This study addresses this gap by conducting network pharmacology analysis and molecular docking studies on *C. odorata*. In this current work, three identified compounds from *C. odorata,* namely squalene, linolenic acid and hexadecanoic acid, were subjected to compound-target identification via SwissTargetPrediction and Cytoscape 3.10.1 visualization tools. Subsequently, Gene Ontology enrichment was performed to analyze gene clusters within the network. Finally, AutoDOCK tools were employed to elucidate the protein-ligand interaction among selected targets. PPARA was identified as the most important target among all the key proteins based on the binding affinity and GO enrichment

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analysis. PPARA displayed the strongest binding affinities: -9.6 kcal/mole for squalene, -7.6 kcal/mole for linolenic acid, and -7.0 kcal/mole for hexadecanoic acid, surpassing the affinities observed for PGR and RORA. This comprehensive study not only emphasizes the significance of network pharmacology in delineating herbal remedy potentials but also underscores its implications for advancing drug development, particularly in designing novel therapeutics based on targeted mechanisms.

Keywords: Chromolaena odorata, gene ontology, herbal medicine, KEGG, molecular docking, network pharmacology

INTRODUCTION

Network pharmacology is an increasingly interdisciplinary field integrating principles from pharmacology, systems biology, and network science. It offers a powerful framework for comprehensively searching the complex relationship between biological systems and pharmacologically active compounds, thereby shedding light on the complex mechanisms of drug actions. Within the field of herbal medicine and pharmaceutical development, network pharmacology emerges as a pivotal player, presenting a systematic and data-driven approach to unveil the pharmacological potential inherent in herbal remedies (Hopkins, 2008). Its value in modern drug discovery has been demonstrated through case studies where herbal medicine findings have been successfully translated into therapeutics. For example, the application of network pharmacology in the study of traditional Chinese medicine has led to significant advancements in identifying multi-target drug candidates (Zhou et al., 2020). Notably, compounds such as imatinib, a tyrosine kinase inhibitor for chronic myeloid leukemia, and zanamivir, an antiviral drug for influenza, have been successfully developed from Computer-Aided Drug Design (CADD), showcasing the synergy of computational approaches with network pharmacology in modern drug discovery (Andrews et al., 2000; Sliwoski et al., 2014).

Herbal medicines, often composed of a collection of bioactive compounds, possess the capacity to interact with diverse targets within the human body. The compound-target network empowers researchers to recognize and characterize these multi-target effects, providing profound insights into the involved mechanisms that underscore the efficacy of herbal remedies. Such insights, in turn, promise to inform the design of more effective and safer drugs. Through the detailed mapping of interactions between herbal compounds and their molecular targets, researchers can identify key proteins and pathways essential to the therapeutic effects of these phytochemicals (Li & Zhang, 2013).

Throughout history, small molecular compounds derived from natural sources have played a significant role in combating diseases and serving as valuable starting points for drug development. Natural products possess the unique ability to interact with multiple

targets within these intricate disease systems, aligning with this multifaceted therapeutic approach. As the utilization of natural products continues to evolve as promising candidates for drug discovery, it has become increasingly imperative to thoroughly assess the range of interactions these small molecules have with multiple biological targets. Nevertheless, the lack of a comprehensive understanding of the pharmacological mechanisms governing the actions of these drugs has hindered their broader integration into the field of drug development (Sun et al., 2022).

In this study, we aimed to evaluate the pharmacological potential of *C. odorata*, a traditional herbal medicine locally known as "Daun kapal terbang" in Malaysia, using a network pharmacology-based approach. This botanical species has a well-documented array of medicinal properties, encompassing anti-inflammatory, antimicrobial (Olawale et al., 2022), anticancer, antidiabetic, and wound-healing (Sirinthipaporn & Jiraungkoorskul, 2017). Our study contributes to the understanding of the key target proteins and therapeutic potential of this plant.

In our study, we conducted network pharmacology analysis and molecular docking studies on *C. odorata*, verifying and enhancing the findings. These approaches highlight network pharmacology's significance in identifying this herbal remedy's therapeutic potential. Our research sheds light on the compound-target network of *C. odorata.* It contributes valuable insights to herbal medicine and drug development, with potential implications for developing novel therapeutics.

MATERIALS AND METHODS

Plant Verification, Plant Extraction and GS-MS Analysis

The plant was collected, verified, extracted, and submitted for GC-MS analysis as described in our prior work (Mokhtar et al., 2023). In brief, GCMS analysis was performed on an Agilent 7890B gas chromatography system (Agilent, CA, USA) coupled with Agilent 5975C (Agilent, CA, USA) mass selective detector and fitted with DB-1MS column (30 m x 0.25mm x 0.25 μm). Helium was used as the carrier gas at a flow rate of 1.0 mL/min under the following operating conditions: 2ml initial injection volume, split injection ratio of 1:10, initial oven temperature stabilization at 60°C for 4 min and ramping to 230°C at a rate of 6°C/min, detector temperature of 260°C, injector temperature of 230°C and ionization voltage of 70 eV. The chemical components in the extract were identified and quantified by comparing the mass spectra to the NIST library database.

Construction of Compound-target Network

The bioactive compounds of *C. odorata* (squalene, linolenic acid, and hexadecanoic acid) were analyzed to establish a compound-target network. Potential target proteins for each compound were identified using SwissTargetPrediction (https://www.swisstargetprediction.

ch/) (Daina et al., 2019). The identified targets were then visualized using Cytoscape 3.10.1, a software tool for constructing interaction networks (Shannon et al., 2003). To further understand the protein-protein interactions (PPI) within the network, the targets were imported into the STRING database (https://string-db.org/) with the interaction confidence threshold set to "medium confidence," restricted to the "Homo sapiens" species. Nodes without network connections were excluded. This methodology enables a detailed exploration of the molecular interactions and pathways associated with the pharmacological potential of *C. odorata*.

Gene Ontology (GO) Enrichment Analysis

Gene Ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were conducted to investigate the potential mechanisms of *C. odorata* in therapeutic applications using ClueGO, an additional Cytoscape plug-in. The analyses were performed to elucidate insights into the biological processes, molecular functions, and pathways associated with the identified targets. This approach facilitated a deeper understanding of the pathways and mechanisms through which the bioactive compounds of *C. odorata* may contribute to its pharmacological activities, particularly in wound healing (Thomas, 2017).

Molecular Docking on Selected Target Protein

Protein Data Bank (PDB) (https://www.rcsb.org/) was used to retrieve the 3D structure of all protein targets. Subsequently, AutoDock Tools was employed to prepare the docking input files and parameters at the binding pocket of PGR (PDB ID: 1SQN), PPARA (PDB ID:5HYK) (Madauss et al., 2004) and RORA (PDB ID:1N83) (Kallen et al., 2002). Then, the grid box dimensions of the active site were constructed at 28 Å on each side. A docking procedure was carried out to find the affinity of the squalene, linolenic acid and hexadecanoic acid by docking them against the receptors PGR, PPARA and RORA using Assisted Molecular Docking AMDock (Valdés-Tresanco et al., 2020), a software integrated with AutoDock Vina. Finally, complex details of the protein-ligand interactions were visualized using tools such as PyMOL and Discovery Studio Visualizer.

RESULTS

Compound Analysis of *Chromolaena odorata*

Based on Mokhtar et al. (2023), the ethanolic extract of *C. odorata* contains a diverse array of secondary metabolites, encompassing terpenoids, steroids, flavonoids, alkaloids, saponins, and tannins. The presence of these significant phytocompounds was substantiated through gas chromatography-mass spectrometry (GC-MS) analysis, confirming the

prominence of linolenic acid, squalene, and hexadecanoic acids as the primary compounds, as shown in Table 1. Therefore, in this current work, we explored the therapeutic potential of *C. odorata* based on these identified compounds.

Table 1
Major compound of ethanolic extract of Chromolaena odorata based on GC-MS based on prior work (Mokhtar et al., 2023)

Compound Name	Molecular Formula	Area (%)	Quality (%)
Squalene	$C_{30}H_{50}$	3.53	99
Linolenic acid	$C_{20}H_{34}O_2$	8.07	99
Hexadecanoic acid	$C_{18}H_{36}O_2$	9.40	98

Construction of Compound-target Network

Identification of the target protein of these compounds was conducted through SwissTargetPrediction, which gave a total of 98, 99 and 99 targets for squalene, linolenic acid and hexadecanoic, respectively. These protein targets were further used to construct a compound-target network using Cytoscape 3.10, consisting of three compounds (squalene, linolenic acid and hexadecanoic acid) and 296 interactive target proteins (Figure 1). Based on this network analysis, a total of 10 targets shown overlapping among the three

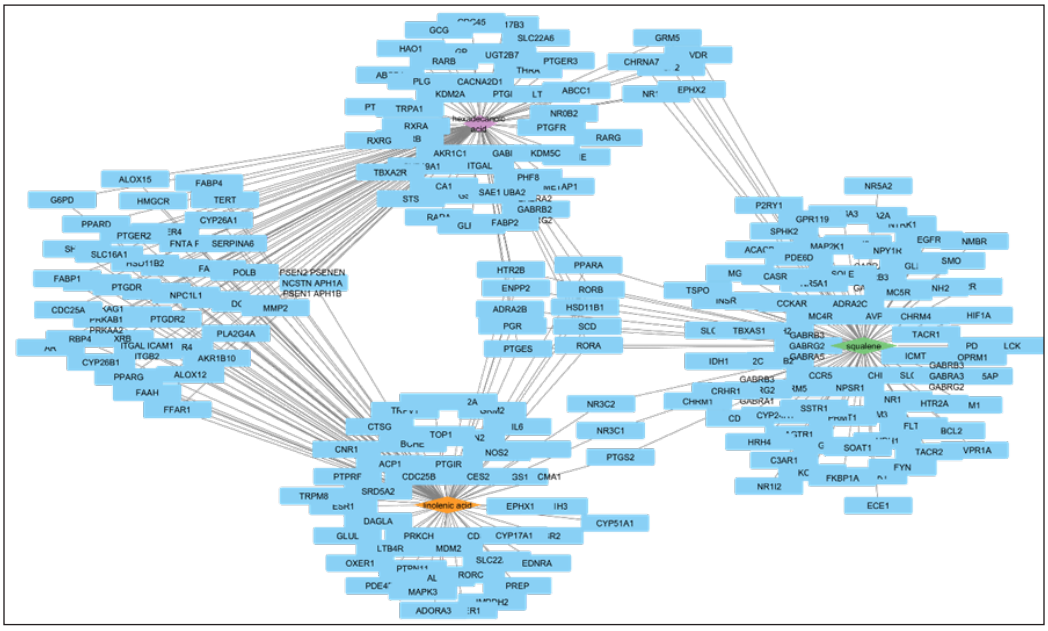

Figure 1. Compound-targets network of squalene, linolenic acid and hexadecanoic acid. The compounds were represented in a diamond shape, and each compound was differentiated using color coding: squalene (green), linolenic acid (orange), and hexadecanoic acid (purple)

compounds, which are identified as HTR2B, ENPP2, ADRA2B, PGR, PPARA, PTGES, RORB, HSD11B1, SCD and RORA. This means that the highest-degree genes are greatly linked to each other; thus, all of these genes might be hub targets (Batool et al., 2022).

Gene Ontology and KEGG Pathway Analysis

GO enrichment analysis was carried out to analyze these 10 hit target proteins. The selection of the 10 overlapping targets was based on their central roles within the constructed compound-target network, as these targets represent shared nodes with high connectivity among the three studied compounds, as depicted in Figure 1. Focusing on these targets enables a network-driven approach to understanding the pharmacological mechanisms of *C. odorata*. The analysis was performed using the ClueGO plugin according to KEGG pathways based on biological processes, cellular processes, and molecular function. The GO term fusion was restricted to $pV \leq 0.005$, which is based on the false discovery rate (Bindea et al., 2009). Based on the GO enrichment analysis, nuclear receptor activity and regulatory activity of Small Ubiquitin-like Modifier (SUMO) protein pathways were found to be associated with studied compounds (Figure 2).

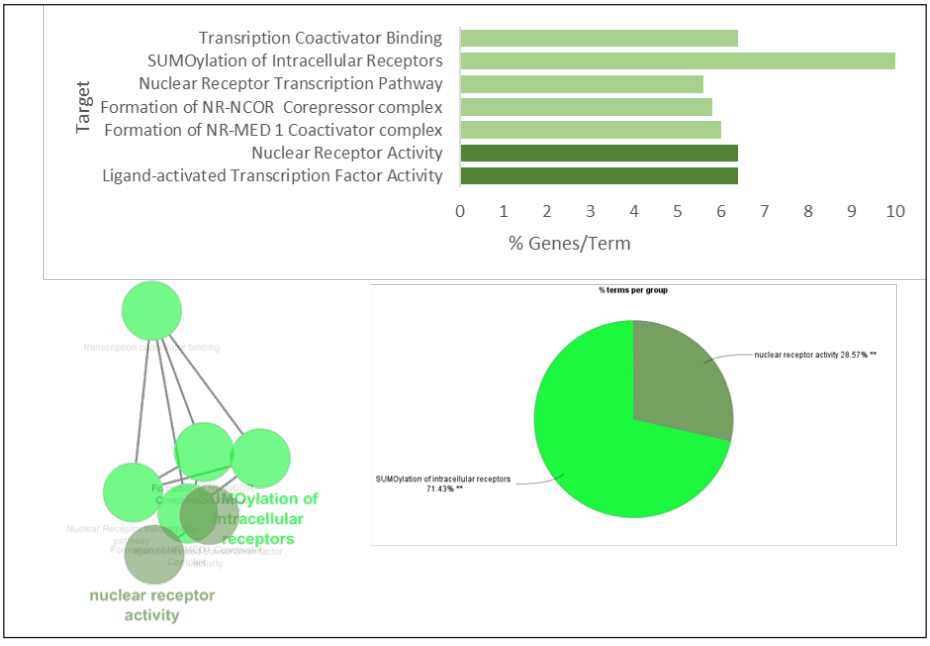

Figure 2. GO enrichment analysis is based on ten selected target genes

Through GO enrichment analysis, it was found that these associated genes (PGR, PPARA and RORA) are the nuclear receptors that have main roles in regulating gene expression and various physiological processes, as shown in Table 2.

Table 2
GO functional group analysis with associated genes

GO ID	GO Term	Associated Genes Found
GO:0098531	Ligand-activated transcription factor activity	[PGR, PPARA, RORA, RORB]
GO:0004879	Nuclear receptor activity	[PGR, PPARA, RORA, RORB]
R-HSA:376419	Formation of NR-MED1 Coactivator Complex	[PGR, PPARA, RORA]
R-HSA:382096	Formation of NR-NCOR Corepressor Complex	[PGR, PPARA, RORA]
R-HSA:383280	Nuclear Receptor transcription pathway	[PGR, PPARA, RORA]
R-HSA:4090294	SUMOylation of intracellular receptors	[PGR, PPARA, RORA]
GO:0001223	Transcription coactivator binding	[PGR, PPARA, RORA]

Molecular Docking Evaluation

Table 3 and Figure 3 provide details on the docking scores and binding poses of specific compounds (squalene, linolenic acid, and hexadecanoic acid) against three distinct proteins, which are PGR (PDB ID: 1SQN), PPARA (PDB ID: 5HYK), and RORA (PDB ID: 1N83).

Table 3
Docking score of target protein and compounds

Receptor Target	Compounds	Binding Affinity (kcal/mol)	Hydrogen Bonding	Hydrophobic Bond
PGR	Squalene	-6.6	-	Val 379, Met 368, Val 403, Ala 330, Ala 371, Phe 391, Ile 400, Val 364, Arg 367, Tyr 380, Cys 288, Leu 295
	Linolenic Acid	-6.6	Gln 277, Val 270	Ile 447, Phe 278, Val 4444, Ile 354 His 440 Leu 460, Leu 456 and Ala 454
	Hexadecanoic acid	-6.2	Leu 718 and Arg 370	Leu 887, Leu 797, Cys 891, Met 759, Phe 778, Met 801, Val 760, Leu 763, and Tyr 890
PPARA	Squalene	-9.6	-	Cys 891, Met 801, Leu 763, Leu 797, Leu 887, Leu 218, Leu 721, Met 759, Phe 778, and Val 760.
	Linolenic Acid	-7.6	Arg 766, Phe 778	Met 759, Leu 718, Met 801, Leu 797, Cys 891, Leu 715, Tyr 890, Met 756, and Leu 887
	Hexadecanoic acid	-7.0	-	Val 274, Phe 273, Val 444, Ile 447, Ile 354, and Leu 456
RORA	Squalene	-9.0	-	Ala 454, Val 270, Ile 447, Ile 354, Phe 273, Cys 276, Phe 318, Leu 321, Met 220, Val 324, Phe 218 amd Met 320
	Linolenic Acid	-6.6	-	Leu 394, Phe 273, Val 444, Ile 354, His 440, Leu 460, Leu 456, and Ala 454
	Hexadecanoic acid	-5.8	Tyr 290 and Gln 289	Arg 367, Ala 371, Val 364, Ala 330, and Ala 454,

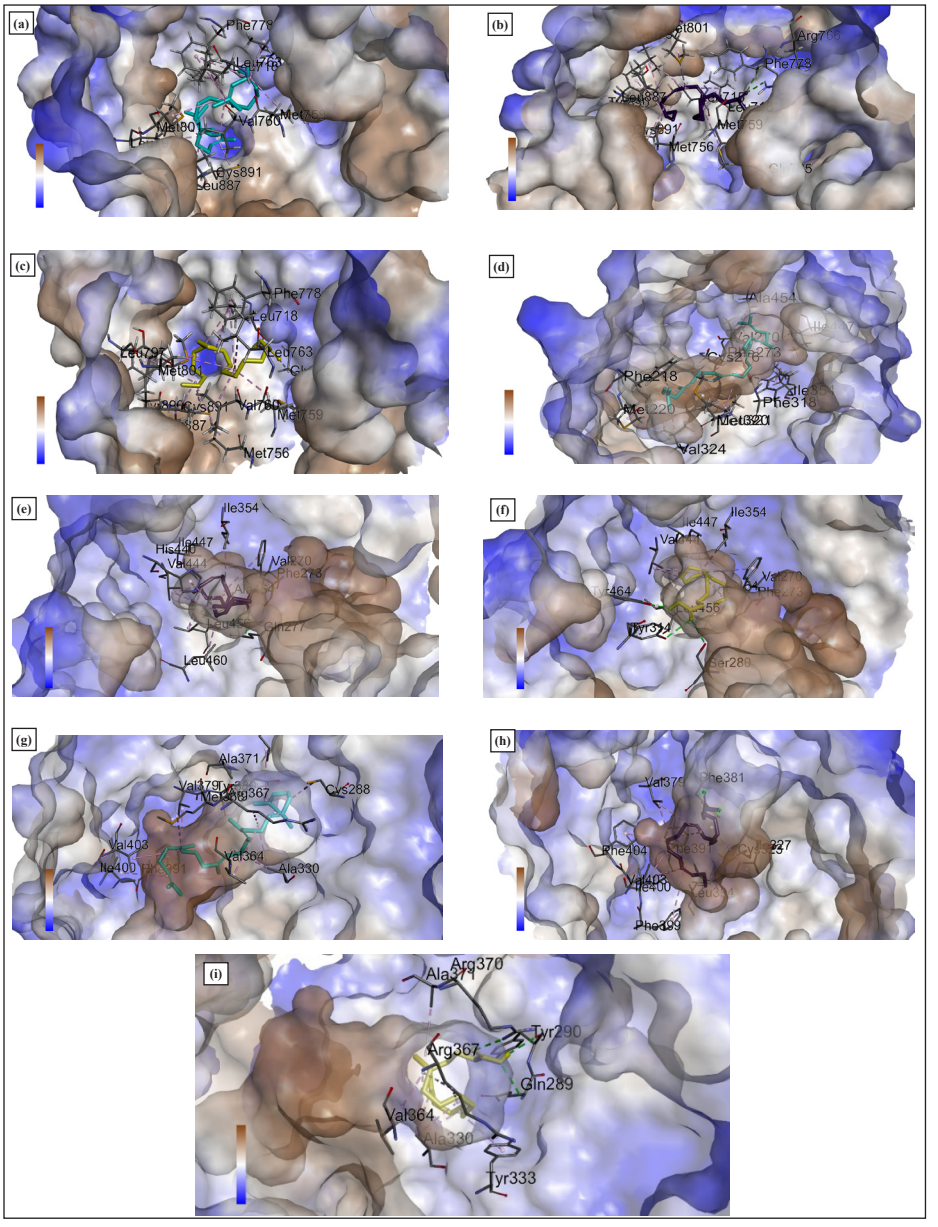

Figure 3. The docking of squalene (cyan), linolenic acid (purple) and hexadecanoic acid (yellow) in the binding site of key target proteins (a) (b) (c) PGR, (d) (e) (f) PPARA and (g) (h) (i) RORA. The representation of amino acid residues is the interactions in the binding site. The representation of brown and blue showed high hydrophobicity and less hydrophobicity, respectively

The results from docking simulations elucidate the proficient binding capabilities of these compounds within the active sites of their respective target proteins, underscoring their potential as either inhibitors or agonists. Notably, among these three proteins investigated,

PPARA was shown to exhibit the most distinct binding affinities: -9.6 kcal/mole for squalene, -7.6 kcal/mole for linolenic acid, and -7.0 kcal/mole for hexadecanoic acid, surpassing the affinities observed for PGR and RORA. Also, visualized 2D molecular interactions between the docked protein-ligand complexes are depicted in Figure 4.

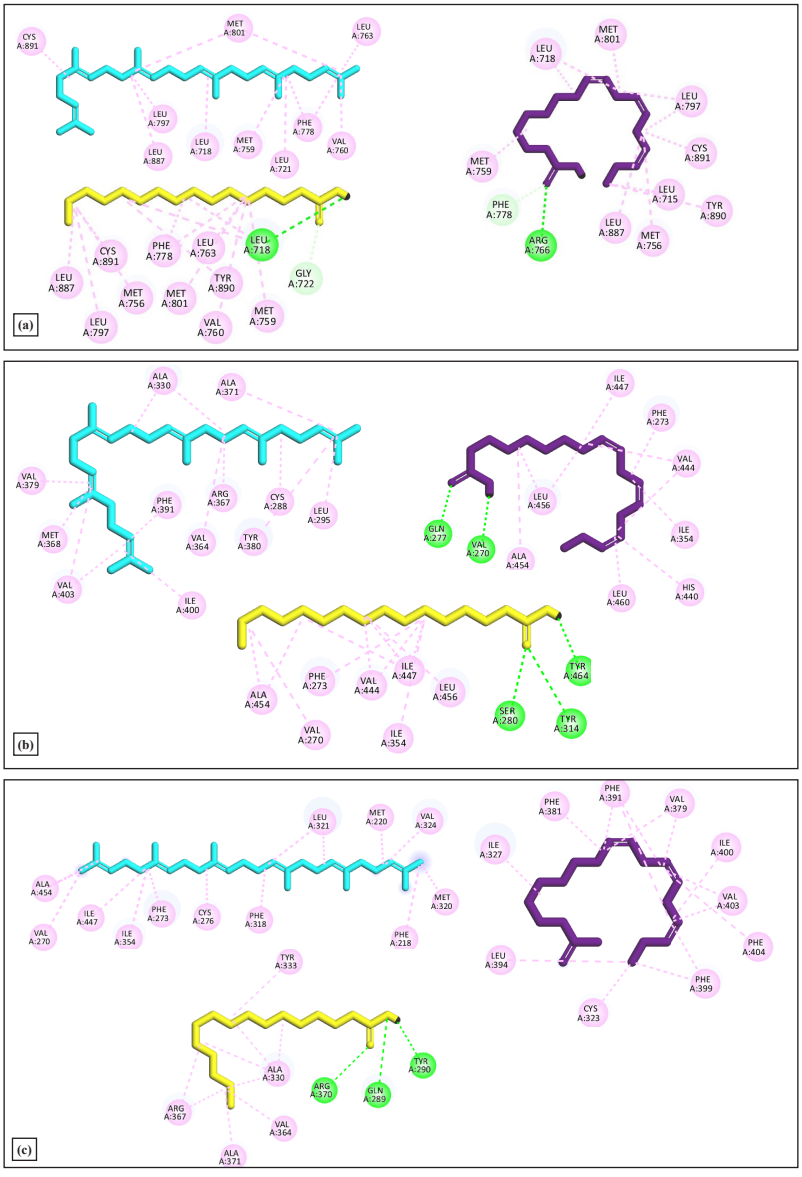

Figure 4. The 2D representations (a) illustrate molecular interactions between squalene, linolenic acid, hexadecanoic acid, and PGR. Meanwhile, representations (b) depict the 2D interactions of these three studies' compounds with PPARA. Additionally, the 2D interactions (c) showed interactions RORA. In these 2D diagrams, pink dashed lines indicate hydrophobic contacts, while green dashed lines represent hydrogen bonds

Based on this molecular docking prediction, the binding affinity of these compounds is mostly attributed to hydrophobic interactions rather than hydrogen bonding between the compounds and residues within the active site vicinity of the targeted protein.

DISCUSSION

Many studies have been conducted to evaluate the medicinal potential of *C. odorata* despite its status as an invasive weed. Its notable pharmacological activity, such as wound healing, anti-inflammatory, anticancer, antidiabetic, and antifungal properties, has been well documented (Olawale et al., 2022). However, a deeper understanding of its pharmacological mechanism of action remains unclear. Hence, this study aims to address this gap by conducting network pharmacology analysis and molecular docking studies on *C. odorata*. Specifically, we are interested in defining which protein target *C. odorata* might have an interaction with that greatly influenced its therapeutic activity. The network pharmacological approach offers a comprehensive overview of uncovering novel therapeutic applications by mapping connections between its properties and diseases. Moreover, understanding its pharmacological network could facilitate the development of new drugs, considering its wide-ranging medicinal activities, while providing essential insights into the safety and efficacy crucial for its integration into modern medicine.

We begin by selecting three prominent compounds of ethanolic extract of *C. odorata,* which are squalene, linolenic acid and hexadecanoic acid, as these three compounds were shown to be abundantly present through GCMS analysis. Then, the gene targets associated with these compounds were retrieved using the compound target database tool SwissTargetPrediction (https://www.swisstargetprediction.ch/). To further understand how these gene targets relate to particular pathways or target diseases, which might further be used to unravel their pharmacological potential, gene ontology (GO) was carried out. GO follows what could be called the "molecular biology paradigm," in which a gene encodes a gene product, and that gene product carries out a molecular-level process or activity (molecular function) in a specific location relative to the cell (cellular component). This molecular process contributes to a larger biological objective (biological process) comprised of multiple molecular-level processes (Thomas, 2017). In this context, gene ontology analysis aims to identify those biological processes, cellular locations and molecular functions impacted by the condition studied. Based on the GO enrichment analysis of this current work, we have found that PGR, PPARA, and RORA are nuclear receptors that mainly regulate gene expression and various physiological processes associated with *C. odorata's* prominent compounds.

The progesterone receptor, encoded by the PGR gene, is found in various tissues in the body, including the uterus, breast, and other reproductive organs. Its main function is to bind to progesterone, which triggers a series of cellular responses essential for various

physiological processes (Grimm et al., 2016). On the other hand, Peroxisome Proliferator-Activated Receptor Alpha (PPARA) plays an important role in regulating lipid metabolism, particularly fatty acid oxidation in the liver and other tissues (Kersten & Stienstra, 2017). A recent study indicates that downregulation of PPARA plays an important role in the impaired mitochondrial function in the corneal epithelium and delayed corneal wound healing in diabetes (Liang et al., 2023). The study also suggested that based on their finding, PPARA agonists might have therapeutic potential for treating diabetic keratopathy. In our work, we found that all three ligands tested (squalene, linolenic acid and hexadecanoic acid) have shown the most stable binding affinity towards PPARA, which gives the idea that *C. odorata's* prominent phytoconstituents possess the potential to be one of the PPARA agonists candidates.

Additionally, Retinoic Acid Receptor-Related Orphan Receptor Alpha (RORA) has been implicated in lipid metabolism regulation, immune system modulation, and inflammatory responses, making it a crucial player in various physiological processes and potentially affecting multiple aspects of health and disease. Its role in inflammatory responses means that RORA can affect how the body responds to injury and stress, which can have implications for conditions like heart disease and arthritis (Franczyk et al., 2022). In a study by Jiang et al. (2020), RORA overexpression inhibited the proliferation and tumorigenesis of glioma cell lines and glioma stem cells (GSCs) by inhibiting the TNF-α mediated NF-κB signaling pathway. This suggested that RORA inhibition would be a potential treatment target for tumor disease. In this context, it could be suggested that *C. odorata* anticancer and antitumoral activity might be driven by the regulation of RORA in the same manner.

After analyzing the pathways related to these genes, a molecular docking study was conducted to further support the finding through structure-based analysis, including predicting the ability of squalene, linolenic acid and hexadecanoic acid to bind at the appropriate target binding site of PGR, PPARA and RORA. The docking results demonstrated that all the compounds effectively bound to the active site of their target proteins. PPARA exhibited the highest binding affinity among these three key proteins studied compared to PGR and RORA. These findings accentuate the preferential interaction and stronger affinity of the studied compounds towards PPARA, signifying its pivotal role as a primary target for these ligands and suggesting their potential therapeutic relevance in modulating PPARA-associated pathways, including metabolic syndrome, neurodegenerative and heart disease (Lin et al., 2022).

Using a molecular docking approach, we further elucidate the structure-based interactions potentially established between specific gene targets and the compounds under investigation. This step holds significant importance as it substantiates the preliminary predictions derived from the gene ontology analysis. According to the findings of the

molecular docking study, it was determined that squalene primarily engages with PGR, PPARA, and RORA through hydrophobic interactions, with the lack of any observed hydrogen bonding with these gene targets. The specific amino acid residues implicated in these hydrophobic interactions were identified as follows: Val 379, Met 368, Val 403, Ala 330, Ala 371, Phe 391, Ile 400, Val 364, Arg 367, Tyr 380, Cys 288, and Leu 295 for PGR; Cys 891, Met 801, Leu 763, Leu 797, Leu 887, Leu 218, Leu 721, Met 759, Phe 778, and Val 760 for PPARA; and residues Ala 454, Val 270, Ile 447, Ile 354, Phe 273, Cys 276, Phe 318, Leu 321, Met 220, Val 324, Phe 218, and Met 320 for RORA. Structurally, squalene is composed of six double bonds, suggesting that this feature may contribute significantly to the observed hydrophobic interactions.

Conversely, the second compound under investigation, namely linolenic acid, demonstrated the formation of two hydrogen bonds with residues Gln 277 and Val 270 on the PGR target protein while interacting with residues Arg 766 and Phe 778 through hydrogen bonding in the PPARA protein. Linolenic acid also engaged in hydrophobic interactions with all target proteins. Specifically, residues Ile 447, Phe 278, Val 444, Ile 354, His 440, Leu 460, Leu 456, and Ala 454 formed hydrophobic bonds with the PGR protein, whereas Leu 394, Phe 273, Val 444, Ile 354, His 440, Leu 460, Leu 456, and Ala 454 interacted in a hydrophobic manner with RORA. In the case of the PPARA target protein, hydrophobic interactions were observed with residues Met 759, Leu 718, Met 801, Leu 797, Cys 891, Leu 715, Tyr 890, Met 756, and Leu 887. Considering the structural attributes of linolenic acid, probable interactions with the proteins PPARA, PGR, and RORA through hydrogen bond formation were due to functional groups such as hydroxyl (-OH) or carboxyl (-COOH) groups. Furthermore, due to its lengthy hydrocarbon chain, its hydrophobic nature could potentially cause hydrophobic interactions with the nonpolar domains of the target proteins, which is crucial in stabilizing the complexes formed.

Last, in the sequence, hexadecanoic acid formed hydrogen bonds with Leu 718 and Arg 370 within the PGR target protein, along with Tyr 290 and Gln 289 within the RORA target protein. Additionally, hexadecanoic acid was used to establish hydrophobic contacts. Specifically, residues that are Leu 887, Leu 797, Cys 891, Met 759, Phe 778, Met 801, Val 760, Leu 763, and Tyr 890 participated in hydrophobic interactions with the PGR gene. In the context of hydrophobic interactions with the RORA and PPARA target proteins, distinct residues of Arg 367, Ala 371, Val 364, Ala 330, and Ala 454 for RORA, and Val 274, Phe 273, Val 444, Ile 447, Ile 354, and Leu 456 for PPARA were found to be involved.

The interconnected roles of PPARA, PGR, and RORA in wound healing, as obtained in this study, suggest a multifaceted mechanism through which *C. odorata* exerts its therapeutic potential, particularly in wound healing. GO enrichment analysis identified these nuclear receptors as central to the regulatory network of *C. odorata*'s bioactive compounds. PPARA contributes significantly by regulating lipid metabolism and mitochondrial function,

which are essential for energy production during keratinocyte migration and fibroblast proliferation in tissue regeneration (Briganti et al., 2024). Furthermore, PPARA mitigates oxidative stress through fatty acid oxidation, reducing the accumulation of reactive oxygen species (ROS) and fostering an optimal environment for cellular repair (Lin et al., 2022). RORA complements this role by modulating immune responses via the NF-κB signaling pathway, suppressing pro-inflammatory cytokines such as TNF-α and IL-6, thereby facilitating inflammation resolution and promoting angiogenesis (Franczyk et al., 2022). This angiogenic role is critical for delivering oxygen and nutrients to the regenerating tissue (Ham et al., 2010). PGR enhances wound healing by mediating progesterone signaling, which supports collagen deposition and angiogenesis during the proliferation phase and by influencing keratinocyte proliferation and differentiation for epidermal layer formation (Barrientos et al., 2008).

These pathways are interconnected through their shared involvement in inflammation resolution, energy metabolism, and oxidative stress reduction. The coordinated actions of PPARA and RORA ensure a balanced inflammatory response and an oxidative stress-free environment, while PGR drives structural tissue repair and vascularization. Together, these findings demonstrate how *C. odorata* bioactive compounds target these pathways to create a multifaceted approach to wound healing, integrating metabolic, inflammatory, and structural processes.

CONCLUSION

This study successfully conducted network pharmacology and docking study of squalene, linolenic acid and hexadecanoic acid against the selected target proteins. Our compound-network interaction revealed that these three studied compounds were associated with nuclear receptor key proteins. Out of the three gene target proteins studied, PPARA stood out as the most crucial target according to the GO enrichment analysis and molecular docking assessment. Specifically, among these three proteins, PPARA displayed the strongest binding affinities: -9.6 kcal/mole for squalene, -7.6 kcal/mole for linolenic acid, and -7.0 kcal/mole for hexadecanoic acid, surpassing the affinities observed for PGR and RORA. This finding demonstrated that at the molecular level, C. odorata is most likely modulating the nuclear receptors key protein exerting its pharmacological activities. However, to address the limitations in the computational study, further analysis, such as molecular dynamics simulations and experimental validations, is essential to confirm the active components and elucidate the therapeutic mechanisms of *C. odorata*. These next steps will provide credibility to the findings and further our understanding of their pharmacological potential.

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