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Computational Insights into the Anti-Inflammatory Potential of *Ocimum americanum* Phytochemicals in Malaria-Associated Cytokine Dysregulation

Nur Balqis Maulydia[1], Syahputra Wibowo[1,*], Josephine Elizabeth Siregar[1], Rinaldi Idroes[2], Rony Abdi Syahputra[3], Putri Cahaya Situmorang[4,*]

[1]*Eijkman Research Center for Molecular Biology, National Research and Innovation Agency, Jalan Raya Bogor KM. 46, Cibinong, Bogor 16911, Indonesia*
[2]*Department of Pharmacy, Faculty Mathematics and Natural Science, Universitas Syiah Kuala, Banda Aceh, Indonesia*
[3]*Department of Pharmacology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia*
[4]*Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Medan, Indonesia*

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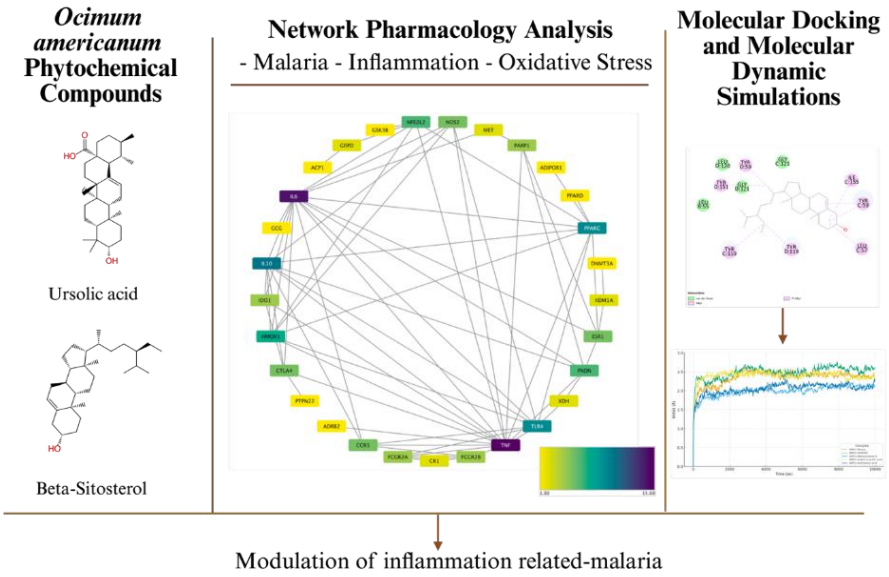
ABSTRACT

Ocimum americanum is a traditionally used medicinal plant that remains pharmacologically underexplored in the context of malaria-associated inflammation. In this study, an integrated chemical and computational approach was employed to investigate the potential molecular mechanisms underlying the anti-inflammatory relevance of *Ocimum americanum* –derived phytochemicals. A curated set of literature-reported secondary metabolites representative of *Ocimum americanum* was analyzed using network pharmacology to explore their interactions with malaria-, inflammation-, and oxidative stress–related targets. Network analysis identified a set of core regulatory targets shared across disease contexts, with functional enrichment highlighting pathways associated with cytokine-mediated signaling and redox homeostasis. Pro-inflammatory mediators, particularly interleukin-6 (IL-6) and tumor necrosis factor (TNF), emerged as central hub nodes within the protein–protein interaction network, suggesting their relevance as key molecular convergence points. To further assess mechanistic plausibility, molecular docking and molecular dynamics simulation were performed against selected hub targets. Among the evaluated phytochemicals, ursolic acid demonstrated the most favorable binding affinities toward TNF and IL-6, indicating a strong theoretical potential for modulating cytokine-driven inflammatory signaling. Collectively, these findings provide computational evidence supporting the multi-target anti-inflammatory potential of *Ocimum americanum* phytochemicals in malaria-associated hyperinflammation. This study positions ursolic acid as a promising lead compound and establishes a mechanistically informed *in silico* framework to guide future experimental validation and therapeutic exploration.

* Corresponding author: Putri Cahaya Situmorang and Syahputra Wibowo
E-mail: putri.cahaya@usu.ac.id and syah031@brin.go.id

GRAPHICAL ABSTRACT



Introduction

Malaria remains one of the most burdensome infectious diseases in tropical and subtropical regions. The world health organization (WHO) estimated more than 249 million malaria cases and 608,000 deaths globally in 2022, with *Plasmodium falciparum* responsible for the majority of severe and fatal infections [1,2]. In Indonesia, significant progress toward elimination has been made: by mid-2024, 398 of 514 districts had achieved malaria elimination certification, although the national annual incidence still reached approximately 400,000 cases [3].

Malaria is fundamentally both an inflammatory and oxidative disease, in which oxidative stress plays a pivotal role in its pathogenesis and severity. The infection profoundly disrupts the host's total antioxidant status, resulting in an imbalance between pro-oxidant and antioxidant systems that favors the accumulation of reactive oxygen and nitrogen species (ROS/RNS) [4,5]. The production of ROS by host phagocytes forms a crucial component of the immune response against *Plasmodium* infection; however, excessive or uncontrolled ROS generation can lead to oxidative damage in erythrocytes and host tissues, worsening disease outcomes. Upon infection, the activation of nitrogen oxide (NOX) drives the rapid uptake of oxygen and the formation of superoxide anion (O_2^-), which is subsequently converted to hydrogen peroxide (H_2O_2) by superoxide dismutase (SOD). This process, while essential for parasite clearance, simultaneously overwhelms the host's antioxidant defenses, resulting in lipid peroxidation, protein oxidation, and DNA damage [6].

Excessive inflammation is implicated in severe malaria and associated mortality. During the blood-stage infection, the host immune system responds to the parasite by releasing proinflammatory cytokines, including interleukins (IL-1β, IL-6, IL-8, IL-12), interferon gamma (IFN-γ), and TNF, which are critical for controlling parasite growth and elimination [7]. Notably, significantly elevated IL-6 levels have been observed in patients with severe malaria compared to those with non-severe cases, suggesting IL-6 as a potential biomarker for severe malaria [8]. Regulatory cytokines typically balance proinflammatory and anti-inflammatory responses. However, disruption of this balance can lead to an exaggerated proinflammatory response, contributing to the adverse effects of severe malaria and increased mortality [9].

The interconnection between oxidative stress, inflammation, and malaria represents a critical yet underexplored aspect of disease pathophysiology. During malaria infection, excessive production of

ROS by both the parasite and host immune response contributes to oxidative damage in red blood cells and tissues, thereby amplifying inflammatory cascades and worsening disease severity [4,5]. Conversely, chronic inflammation promotes further oxidative stress through cytokine-mediated pathways, leading to impaired antioxidant defences and multi-organ dysfunction. A study by Borges *et al.* demonstrated that in murine models susceptible to cerebral malaria, overexpression of eicosanoid-producing enzymes, namely cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX), and the transcriptional regulator peroxisome proliferator-activated receptor gamma (PPAR-γ) in vascular and brain tissues correlated with higher parasitemia, reduced survival, and diminished NO and H_2O_2 production [10]. These findings underscore the intertwined roles of oxidative stress and inflammatory dysregulation in malaria pathology, highlighting the urgent need for research on bioactive compounds capable of targeting both mechanisms to improve disease outcomes.

Plants have been used as sources of therapeutic agents for centuries, with *Ocimum americanum* L. synonyms: *Ocimum americanum* var. Americanum and *Ocimum americanum* var. Pilosum (Willd.) A.J. Paton, a member of the Lamiaceae family, standing out for its diverse pharmacological potential. It offers benefits as a cough medicine, treating bronchitis, immune disorders, toothache, and dysentery [11]. Traditionally, this species has been employed for its antimicrobial, analgesic, and anti-inflammatory properties, supported by its rich phytochemical profile of phenols, flavonoids, and terpenes [12]. Originating in Africa, *Ocimum americanum* is characterized by glabrous blossoms, glandular patches, broad, serrated leaves, and sub-rectangular striated stems. Mucilaginous fruits are produced by its tiny and pitted notelets [13].

A recent study on *Ocimum americanum* highlights its multifaceted pharmacological properties and ethnomedicinal significance. In Ethiopia, a detailed survey revealed its widespread traditional use for treating ailments such as headaches, stomach issues, and depression, as well as its role in food preservation and flavoring [14]. The current research's data demonstrate that *Ocimum americanum* L., a herbal medicinal plant, includes many secondary metabolites that cause its antimicrobial and cytotoxic actions [15]. Cytotoxic studies indicate that *Ocimum americanum* essential oils may have anticancer properties by targeting cancer cell lines like HepG2 without causing significant toxicity to normal cells. These findings support its use in folk medicine for managing tumor-related conditions [16].

The present study focuses on *Ocimum americanum* as a promising source of bioactive phytochemicals with the potential to modulate oxidative stress and inflammatory pathways that are frequently implicated in malaria and related inflammatory disorders. Phytochemicals from medicinal plants are increasingly recognized to exert their pharmacological effects through multi-target interactions, particularly by influencing cytokine signaling and redox-regulated processes that contribute to disease progression. Recent developments in network-based pharmacological approaches and structure-based computational methods have provided effective tools to investigate the molecular mechanisms underlying the biological relevance of medicinal plants. These approaches enable systematic exploration of compound–target–pathway relationships and have been successfully applied to *Ocimum* species to reveal mechanistic links between their phytochemical constituents and therapeutic potential. This study applies network pharmacology and molecular docking analyses to explore the potential molecular targets and signaling pathways associated with phytochemicals reported from *Ocimum americanum* . By integrating predicted compound–target interactions with malaria-, inflammation-, and oxidative stress-related biological networks, this work aims to elucidate putative mechanisms of action that may underlie the plant's relevance in malaria-associated inflammatory conditions. The findings provide a mechanistically informed *in silico* framework to support future experimental investigations and the rational exploration

of *Ocimum americanum* as a candidate source of adjunctive therapeutics.

Materials and Methods

Selection of Ocimum Americanum–Derived Phytochemicals

Phytochemical selection for this study was conducted using a database-driven curation strategy based on the Indian Medicinal Plants, Phytochemistry, and Therapeutics 2.0 (IMPPAT 2.0), a curated and manually annotated resource that integrates medicinal plants with experimentally reported phytochemical constituents and associated therapeutic information [17]. *Ocimum americanum* was queried in the IMPPAT database to retrieve compounds documented as constituents of the species or closely related *Ocimum* taxa. From the retrieved dataset, ten representative phytochemicals were selected to reflect the chemical diversity characteristic of the genus, while maintaining relevance to inflammation-associated molecular pathways. The final compound panel comprised the following molecules: ursolic acid, oleanolic acid, apigenin, camphor, camphene, betulinic acid, rosmarinic acid, humulene, eugenol, and *β*-sitosterol [18-21]. These compounds encompass multiple major phytochemical classes reported for *Ocimum* species, including pentacyclic triterpenoids, flavonoids, phenolic acids, phenylpropanoids, monoterpenes, sesquiterpenes, and phytosterols. Compound selection was guided by the following criteria: (i) documented association with *Ocimum americanum* or taxonomically related *Ocimum* species in the IMPPAT database; (ii) availability of well-defined chemical structures suitable for computational analysis; and (iii) prior implication in inflammation-, immune-, or oxidative stress–related biological processes (BP) based on existing pharmacological literature. This database-guided selection approach was employed to ensure chemical validity, transparency, and reproducibility. The curated phytochemical set was subsequently used for network pharmacology analysis and molecular docking to explore potential multi-target mechanisms relevant to malaria-associated inflammatory signaling.

Network Pharmacology

A network pharmacology analysis was employed to elucidate the molecular targets and signaling pathways potentially modulated by bioactive phytochemicals associated with *Ocimum americanum*. A curated panel of selected compounds was first prepared based on database-driven phytochemical selection. Canonical SMILES representations of each compound were retrieved from PubChem and submitted to SwissTargetPrediction for target identification, with only protein targets exhibiting a probability score greater than 0.5 retained for downstream analysis [22]. To contextualize the predicted targets within disease-relevant biological space, four target datasets were integrated: (i) malaria-associated targets, (ii) inflammation-related targets, (iii) oxidative stress–related targets (retrieved from OMIM database), and (iv) predicted targets associated with *Ocimum americanum* phytochemicals obtained from SwissTargetPrediction. A Venn diagram–based intersection analysis was conducted to identify shared and overlapping targets, and duplicate entries were removed to isolate core molecular targets potentially involved in malaria-associated inflammatory processes [23,24]. The identified core targets were subsequently imported into the STRING database to construct a protein–protein interaction (PPI) network, applying a high-confidence interaction threshold (confidence score ≥ 0.7). The resulting network was visualized and analyzed using cytoscape to evaluate key topological parameters, including degree centrality and betweenness centrality, enabling identification of hub proteins with potential regulatory significance [25,26]. Functional enrichment analysis was performed using STRING's built-in tools to investigate gene ontology (GO) categories, including BP, molecular functions (MF), and cellular components (CC). Enrichment results were filtered using a false discovery rate (FDR) threshold of **<** 0.05 to ensure

statistical robustness. The enriched pathways and functional associations were visualized using bar plots and network-based representations in Cytoscape to facilitate interpretation of the systems-level interactions [27].

Molecular Docking Analysis

A structure-based molecular docking workflow was employed to evaluate the binding affinity of chemical compounds from IMPPAT-curated *Ocimum americanum* against protein targets identified through network pharmacology analysis. Three-dimensional structures of the selected ligands were obtained from PubChem and subjected to geometry optimization using Open Babel. The optimized ligands were subsequently converted to pdbqt format for docking simulations. Protein structures corresponding to selected hub targets were retrieved from the RCSB Protein Data Bank. Before docking, receptor structures were prepared by removing crystallographic water molecules and co-crystallized ligands, followed by the addition of polar hydrogens and assignment of Kollman charges using AutoDock Tools. Potential ligand-binding pockets were identified using P2Rank, a machine-learning–based method that detects ligandable regions across the solvent-accessible surface of proteins. For each receptor, the binding pocket with the highest probability score was selected as the docking site. Docking simulations were then performed using AutoDock Vina, chosen for its balance of computational efficiency and reliable binding affinity estimation [28,29]. Grid boxes were centered on the predicted active sites and sized to fully encompass the binding pocket. Docking outputs included predicted binding affinities, ligand orientations, and interacting amino acid residues. Compounds exhibiting consistent binding modes and favorable interaction profiles across key inflammatory targets were prioritized for subsequent dynamic evaluation [30].

Molecular Dynamics Simulations (MDs)

MDs were conducted to investigate the conformational stability and dynamic activity of selected protein–ligand complexes using CABS-flex 3.0, a coarse-grained simulation platform suitable for efficient exploration of protein flexibility [31]. Each simulation corresponded to an approximate 10,000 ps dynamic trajectory, generated through 50 Monte Carlo cycles, under a temperature of 300 K to approximate physiological conditions [32,33]. Following simulation, root mean square deviation (RMSD) analyses were performed to assess overall structural stability of the protein–ligand complexes, while root mean square fluctuation (RMSF) analyses were used to identify residue-level flexibility and dynamic regions within the proteins. These metrics enabled evaluation of the persistence of ligand interactions and the influence of binding on protein conformational dynamics throughout the simulated timeframe [34-36].

Results and Discussion

Ocimum americanum L. (syn. *O. canum* Sims.), commonly known as hairy basil, is a widely distributed aromatic medicinal herb across tropical Africa and South Asia, traditionally used for treating fever, infections, and inflammatory disorders. Phytochemical investigations have confirmed that *Ocimum americanum* contains a rich repertoire of secondary metabolites, including alkaloids, flavonoids, terpenoids, tannins, and phenolic compounds that contribute to its broad pharmacological activities [15]. The essential oil of *Ocimum americanum* is reported to be dominated by bioactive terpenoids such as camphor, limonene, caryophyllene, longifolene, and 1,8-cineole, which are known for their antimicrobial, antioxidant, and insecticidal properties [32,33]. Notably, *Ocimum americanum* essential oil has demonstrated potent larvicidal and repellent effects against *Anopheles gambiae*, the primary malaria vector, showing mortality and repellence rates comparable to commercial insecticides, suggesting synergistic activity among

its volatile constituents [33]. Given the urgent need for eco-friendly alternatives to synthetic insecticides amid rising insecticide resistance and vector adaptation, *Ocimum americanum* represents a promising botanical source for developing novel malaria vector control agents and adjunct therapeutics that align with integrated malaria management strategies.

Phytochemical analysis revealed a chemically diverse profile dominated by flavonoids (mosloflavone and apigenin), pentacyclic triterpenoids (ursolic acid, and betulinic acid), phenolic derivatives (rosmarinic acid and eugenol), and terpenoids (camphor, camphene, and humulene), accompanied by the phytosterol *β*-sitosterol. The presence of mosloflavone, a polymethoxylated flavone, is particularly relevant because methoxylation enhances lipophilicity and metabolic stability, properties often associated with improved biological efficacy of flavonoids [34]. Apigenin is a well-established bioactive flavone with documented antioxidant and anti-inflammatory activities mediated through modulation of NF-κB and MAPK signaling pathways [35]. Rosmarinic acid, a characteristic phenolic ester of the Lamiaceae family, is recognized as a potent radical scavenger and inhibitor of inflammatory mediators, providing strong mechanistic support for the antioxidant and anti-inflammatory activities frequently reported for *Ocimum* species [36]. In addition, ursolic acid and betulinic acid represent two major pentacyclic triterpenoids known for broad pharmacological effects, including anti-inflammatory, metabolic disease, and neurological disorders, and other diseases of the brain, largely mediated through regulation of oxidative stress, mitochondrial function, and apoptosis-related pathways [37]. The volatile terpenoids camphor, camphene, and humulene contribute to the essential oil fraction and are associated with antimicrobial and anti-inflammatory properties, with humulene in particular reported to suppress pro-inflammatory cytokine production [38]. Eugenol further reinforces this activity spectrum through its well-documented antioxidant and membrane-active effects, while *β*-sitosterol is known to modulate immune and inflammatory responses [39,40]. Collectively, this combination of polar phenolics and non-polar terpenoids and sterols supports a multi-target mode of action and provides a strong phytochemical rationale for the antioxidant and anti-inflammatory potential of *Ocimum americanum* , justifying its further evaluation using network pharmacology and *in silico* approaches.

Flavonoids serve as potent antioxidants and anti-inflammatory agents through multiple mechanisms, including free radical scavenging, metal chelation, enzyme induction, and modulation of inflammatory pathways. Their consumption is linked to various health benefits, particularly in reducing the risk of chronic diseases associated with oxidative stress and inflammation [41]. Flavonoids are prominent bioactive compounds in *Ocimum americanum* , contributing to its medicinal, antioxidant, and antimicrobial properties. Nevadensin is the most important flavonoid found in *Ocimum americanum* , and it is abundant in chemotypes across Africa and Asia [21]. Ocimum species show significant infraspecific differences in flavonoid profiles, with luteolin 5-O-glucoside being a key characteristic of the genus [42].

A total of forty targets were identified as the central or core targets, as illustrated in the Venn diagram (Figure 1). These targets represent the key intersection points among the biological pathways associated with *Ocimum americanum* compound and the three major pathophysiological processes investigated: malaria, oxidative stress, and inflammation. Specifically, 13 targets were shared between *Ocimum americanum* and malaria, suggesting the plant's potential to influence parasitic or host-related mechanisms relevant to antimalarial activity (CYP2C19, CCR5, G6PD, ACP1, NOS2, CD81, CAPN2, CAPN1, IDO1, MET, TLR4, ADRB2, and HMOX1). Sixteen targets were found in common between *Ocimum americanum* and oxidation-related pathways, indicating strong antioxidant potential through modulation of redox-associated genes or enzymes (ESR1, PPARG, FABP3, PPARD, CYP3A4, XDH, GSK3B, FABP2, IL6, GNPAT, PLA2G7, KDM1A, ADH1B, ADH1C, ADH1A, and DNMT3A). Four targets overlapped among *Ocimum americanum* ,

inflammation, and oxidation, implying a dual modulatory effect on inflammatory and oxidative stress cascades (PLA2G4A, PTPN22, PARP1, and GCG).

Additionally, nine targets were shared between oxidation and inflammation, highlighting their interconnected biological roles in cellular damage and immune regulation (MUTYH, GBA1, HMCN1, CFHR3, CFHR1, ADIPOR1, PXDN, NFE2L2, and CTLA4). Two targets were identified as common to inflammation, oxidation, and malaria, reflecting critical molecular nodes where these three processes converge (FCGR2A and FCGR2B). Another two targets were shared across *Ocimum americanum* , inflammation, oxidation, and malaria—representing the most integrated points of interaction within the network (TNF and IL10). Finally, three targets were common to inflammation and malaria, emphasizing the immunopathological link between inflammatory responses and malarial infection (ACKR1, CD55, and CR1). Collectively, these overlaps suggest that *Ocimum americanum* exerts multifaceted pharmacological actions by simultaneously modulating oxidative, inflammatory, and parasitic pathways.

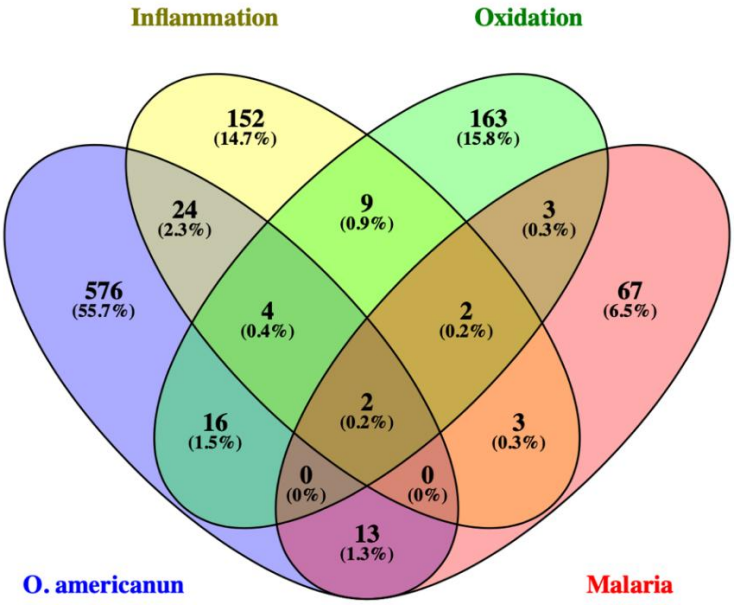


Figure 1: Overlapping of oxidant target, inflammation target, and *Ocimum americanum* target

The network topology analysis identified twenty-nine core targets that represent the intersection between *Ocimum americanum* target compound and key BP related to malaria, inflammation, and oxidative stress. Among these, TNF and IL-6 emerged as the most influential hub nodes, exhibiting the highest degree and betweenness centrality values (Figure 2). These findings highlight their central role in coordinating pro-inflammatory and immune signaling during *Plasmodium* infection. Elevated TNF and IL-6 levels are well-documented hallmarks of malaria pathogenesis, as they mediate fever, endothelial activation, and severe inflammatory responses leading to complications such as cerebral malaria and anemia [43]. Their high closeness centrality further indicates that these cytokines communicate efficiently across the network, amplifying immune responses to infection while also increasing the risk of immunopathology when dysregulated. Supporting this, a recent systematic review and meta-analysis reported that inflammatory biomarkers, C-reactive protein (CRP), IL-6, and TNF-α, were significantly elevated in patients with complicated malaria compared to those with uncomplicated disease. The pooled results demonstrated markedly higher levels of CRP (SMD = 0.90 mg/L), IL-6 (standard mean difference or SMD = 0.89 pg/mL), and TNF-α (SMD = 1.18 pg/mL) in severe cases, confirming their strong association with malaria severity [44]. However,

the study also concluded that no single biomarker alone provided sufficient diagnostic specificity; rather, a combined assessment of these markers offered a more accurate reflection of disease progression. This aligns with the network findings in which TNF and IL-6 function as central regulators within the inflammatory cluster, interacting with multiple targets to propagate downstream responses.

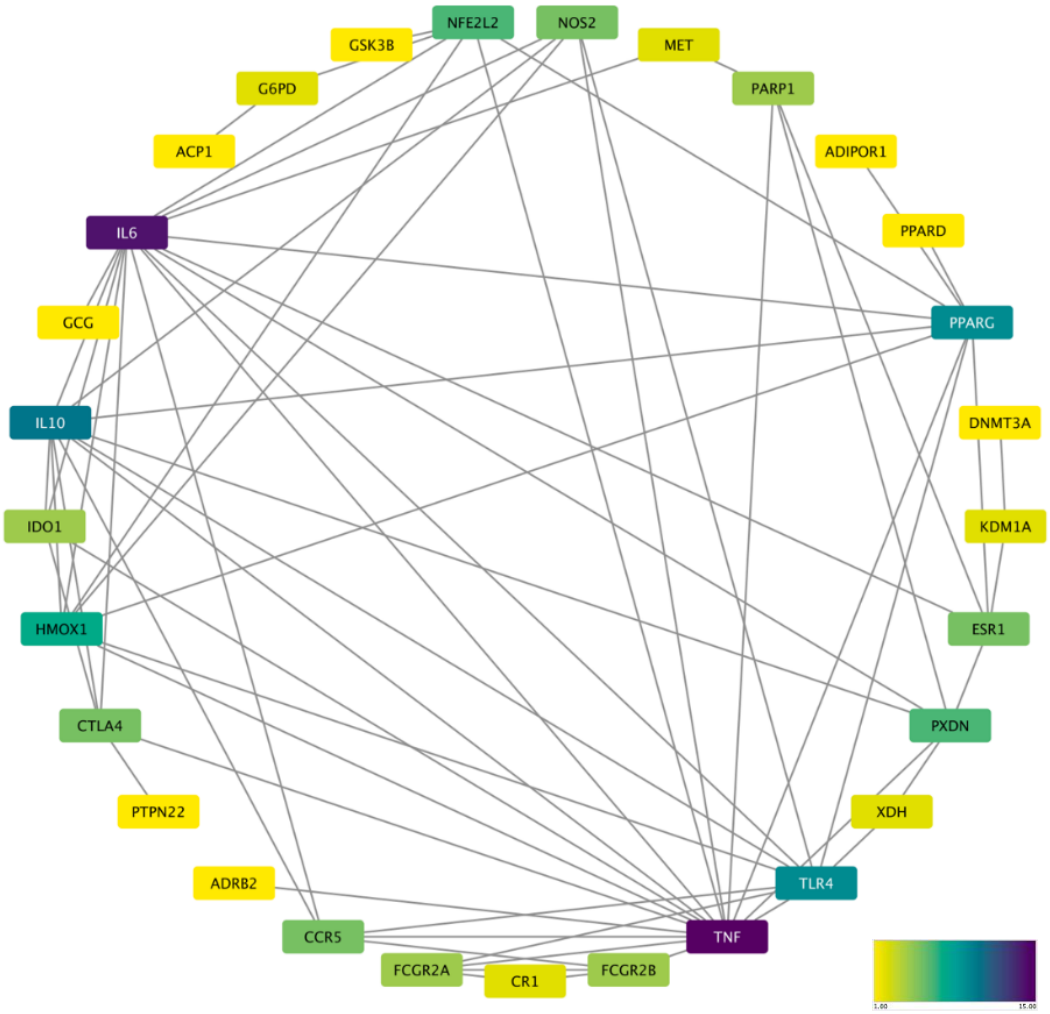


Figure 2: Network analysis related to malaria-inflammation-oxidation targeted with *Ocimum americanum*

Network pharmacology and systems biology approaches provide insights into the key genes regulating complex BP, including inflammation and malaria. This study examines the centrality measures of key genes to determine their importance in a biological network [45]. Network analysis was conducted to evaluate the connectivity and influence of each gene using three key centrality measures: (1) Degree centrality: represents the number of direct connections a node has, indicating its immediate influence. (2) Betweenness centrality measures the extent to which a node acts as a bridge in the network, influencing communication between other nodes, and (3) Closeness centrality represents how efficiently a node can reach all other nodes in the network, reflecting its accessibility and influence [46]. The analyzed genes include TNF, IL-6, Interleukin-10 (IL-10), Peroxisome Proliferator-Activated Receptor Gamma (PPARG), Toll-like receptor 4 (TLR4), Heme Oxygenase 1 (HMOX1), Peroxidasin Homolog (PXDN), Nuclear Factor Erythroid 2-Related Factor 2 (NFE2L2), C-C Chemokine Receptor Type 5 (CCR5), Cytotoxic T-Lymphocyte Protein 4 (CTLA4), Estrogen Receptor (ESR1), and

Nitric Oxide Synthase (NOS2), each of which plays a significant role in malaria, inflammatory responses, and disease mechanisms (Table 1).

TNF and IL-6 are the most central nodes in this network, indicating their pivotal roles in inflammation and immune responses. As a pro-inflammatory cytokine, IL-6 is involved in various inflammatory diseases, autoimmune conditions, and cancer progression [47]. Its high betweenness suggests that IL-6 is a key regulatory hub, mediating interactions between other genes. TNF plays a crucial role in the inflammatory response and apoptosis [48]. It is a key regulator of immune signaling pathways and is implicated in chronic inflammatory diseases, including rheumatoid arthritis and inflammatory bowel disease. Its high degree and betweenness centrality indicate significant network involvement.

Table 1: Summary of gene related triad of malaria-inflammation-oxidation activity of *Ocimum americanum* compounds

Gene symbol	Description	Degree	Betweenness centrality	Closeness centrality	Clustering coefficient
TNF	TNF	15	0.317	0.623	0.295
IL6	IL-6	14	0.278	0.623	0.318
IL10	Interleukin-10	10	0.034	0.518	0.534
PPARG	Peroxisome proliferator-Activated receptor gamma	9	0.185	0.549	0.389
TLR4	Toll-like receptor 4	9	0.069	0.509	0.500
HMOX1	Heme oxygenase 1	7	0.012	0.509	0.809
PXDN	Peroxidasin homolog	6	0.041	0.474	0.400
NFE2L2	Nuclear factor erythroid 2-related factor 2	6	0.204	0.500	0.400
CCR5	C-C chemokine receptor type 5	5	0.0112	0.459	0.700
CTLA4	Cytotoxic T-lymphocyte protein 4	5	0.072	0.451	0.600
ESR1	Estrogen receptor	5	0.145	0.459	0.300
NOS2	Nitric oxide synthase	5	0	0.451	1

The enrichment of the GO study was conducted utilizing the FDR. This metric indicates the degree of enrichment significance. The GO enrichment analysis revealed key BP, MF, and CC associated with inflammation targets [25]. *P*-values adjusted for multiple testing within each category are presented, utilizing the Benjamini-Hochberg procedure. In Figure 3, the signal on the x-axis of BP is defined as a weighted harmonic mean of the observed/expected ratio and -log(FDR). FDR prioritizes larger terms for their capacity to yield lower *p*-values, whereas the observed/expected ratio underscores smaller terms, which possess a high foreground-to-background ratio but fail to attain low FDR values due to their size [26]. The strongest enrichment of BP was observed in the regulation of the immune response, suggesting that the bioactive compounds in *Ocimum americanum* may play a pivotal role in modulating mediators of inflammation. This aligns with the observed anti-inflammatory activity of the extract, potentially through the regulation of pro-inflammatory cytokines such as IL-6, which was identified as a key central node in the network. The regulation of acute inflammation is critical in controlling immune responses and preventing chronic inflammatory diseases [49].

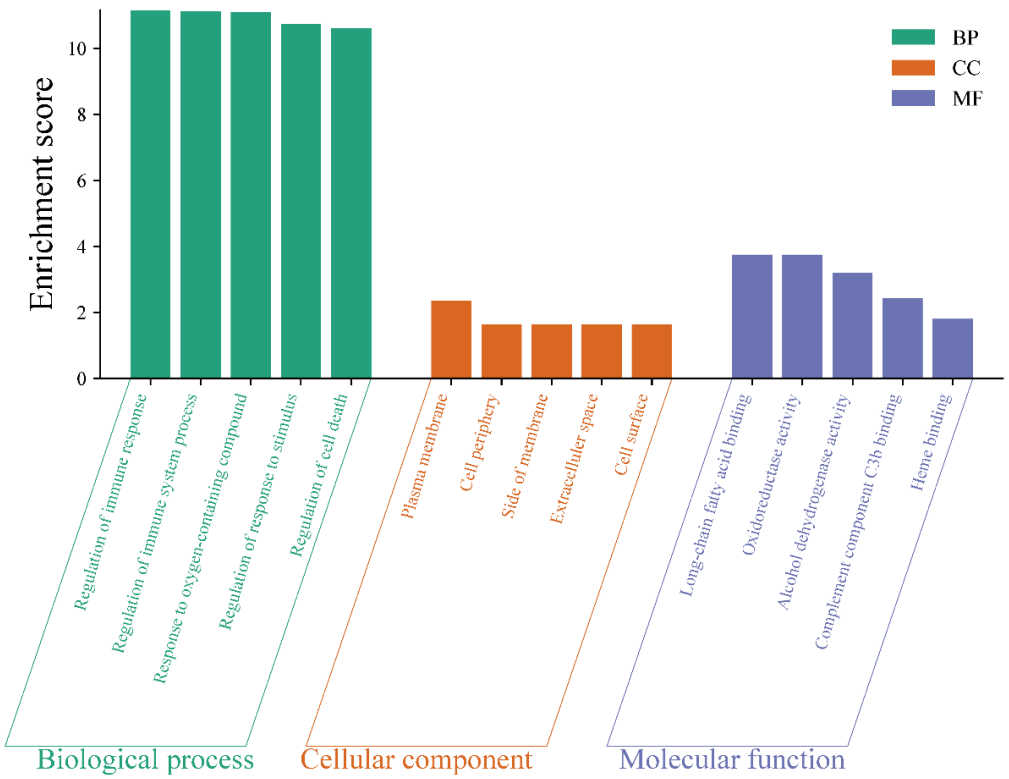


Figure 3: Enrichment analysis of BP, CC, and MF from GO. Enrichment scores were calculated from FDR value

The highest enrichment in CC was observed at the plasma membrane, suggesting that the key molecular targets are membrane-associated proteins. This is significant as many receptors, transporters, and signaling proteins involved in inflammation and oxidative stress are membrane-bound. The presence of bioactive compounds targeting these proteins implies that *Ocimum americanum* may exert its effects by modulating cell surface receptors, such as cytokine receptors, thereby influencing signal transduction pathways related to inflammation and immune responses. The most enriched MF was long-chain fatty acid binding with the highest gene count, indicating that the bioactive compounds likely interact with various proteins involved in inflammation and oxidative stress pathways. In GO term detail, binding can be defined as the selective, non-covalent, often stoichiometric interaction of a molecule with one or more specific sites on another molecule [50]. This suggests a broad mechanism of action, including enzyme inhibition, receptor modulation, and interaction with inflammatory mediators. Such interactions may contribute to the observed pharmacological effects of *Ocimum americanum* , particularly in antioxidant defense and immune modulation. The GO analysis provides mechanistic insights into the therapeutic potential of *Ocimum americanum* . The extract's bioactive compounds appear to influence acute inflammation regulation, protein binding interactions, and membrane-associated signaling, which may underlie its observed antioxidant and anti-inflammatory activities. These findings support the potential application of *Ocimum americanum* in developing natural anti-inflammatory agents and warrant further investigation into its molecular mechanisms.

The molecular docking analysis revealed differential binding affinities of the identified phytocompounds toward the pro-inflammatory cytokines IL-6 and TNF, indicating variability in their predicted anti-inflammatory potential (Table 2). Overall, most compounds exhibited stronger binding to TNF than to IL-6, suggesting a higher likelihood of modulating TNF-mediated inflammatory signaling. Binding free energy values ranged from −5.3 to −7.3 kcal/mol for IL-6 and from −5.7 to −9.4 kcal/mol for TNF, reflecting moderate to strong ligand–protein interactions across the dataset. Among the tested compounds, pentacyclic triterpenoids demonstrated the most

favorable binding profiles. Ursolic acid showed strong affinity toward both IL-6 (−7.3 kcal/mol) and TNF (−8.9 kcal/mol), while betulinic acid exhibited the lowest binding free energy against TNF (−9.4 kcal/mol), indicating particularly stable complex formation. The relatively high binding affinity of ursolic acid to IL-6 further supports its potential as a dual modulator of TNF and IL-6, which are critical in the exaggerated pro-inflammatory responses associated with severe malaria [8]. The high binding affinity of ursolic acid is particularly noteworthy, as it aligns with previous studies indicating its anti-inflammatory properties through decreased inflammatory cytokine levels, elevated antioxidant enzyme levels, and reduced oxidative stress levels [51]. These findings are consistent with the known anti-inflammatory properties of triterpenoids, which are often attributed to their bulky hydrophobic skeleton combined with polar functional groups that facilitate hydrogen bonding and hydrophobic interactions within cytokine binding pockets. Similarly, rosmarinic acid, a phenolic ester rich in hydroxyl groups, displayed strong binding to both targets (−7.0 kcal/mol for IL-6 and −8.4 kcal/mol for TNF), highlighting the importance of multiple hydrogen bond donors and acceptors in stabilizing cytokine–ligand interactions.

Table 2: Binding free-energy value of metabolite compounds from *Ocimum americanum* with two target receptors, namely (a) TNF and (b) IL-6

No.	Name	Molecular formula	Class of compounds	Binding Free Energy (kcal/mol)	
				IL-6	TNF
1	Mosloflavone	$C_{17}H_{14}O_5$	Polymethoxylated flavone	-6.2	-7.6
2	Ursolic acid	$C_{30}H_{48}O_3$	Pentacyclic triterpenoid	-7.3	-8.9
3	Apigenin	$C_{15}H_{10}O_5$	Flavone	-6.5	-7.6
4	Camphor	$C_{10}H_{16}O$	Monoterpene ketone	-5.4	-5.9
5	Camphene	$C_{10}H_{16}$	Monoterpene hydrocarbon	-5.6	-5.7
6	Betulinic acid	$C_{30}H_{48}O_3$	Pentacyclic triterpenoid	-6.8	-9.4
7	Rosmarinic acid	$C_{18}H_{16}O_8$	Phenolic ester	-7	-8.4
8	Humulene	$C_{15}H_{24}$	Sesquiterpene hydrocarbon	-5.8	-7.1
9	Eugenol	$C_{10}H_{12}O_2$	Phenylpropanoid	-5.3	-5.9
10	β-sitosterol	$C_{29}H_{50}O$	Phytosterol	-7.1	-8.5

Flavonoids such as mosloflavone and apigenin showed moderate but consistent binding affinities toward both cytokines (approximately −6.2 to −6.5 kcal/mol for IL-6 and −7.6 kcal/mol for TNF). Notably, mosloflavone, a polymethoxylated flavone, exhibited comparable binding to TNF (−7.6 kcal/mol) despite fewer hydroxyl groups, suggesting that methoxylation may enhance lipophilicity and improve accommodation within hydrophobic regions of the binding site. In contrast, smaller and more volatile terpenoids, including camphor, camphene, and humulene, displayed weaker binding energies, particularly toward IL-6, which can be attributed to their limited functional groups and reduced capacity for specific intermolecular interactions. The moderate binding affinities of flavonoids like mosloflavone and apigenin are consistent with their reported roles in modulating inflammatory pathways [34,35]. These results indicate that compounds with larger molecular frameworks and a balanced distribution of hydrophobic and polar functionalities such as triterpenoids, phenolic esters, and phytosterols that exhibit superior binding affinities toward IL-6 and TNF.
This result supports their potential role as key contributors to the anti-inflammatory activity of the extract. The stronger and more consistent

interactions observed with TNF further suggest that TNF-mediated pathways may represent a primary molecular target, providing a mechanistic basis for the anti-inflammatory effects observed in subsequent *in vitro* or *in vivo* evaluations.

Molecular docking analysis showed that three major phytochemicals, namely ursolic acid, rosmarinic acid, and *β*-sitosterol, exhibited distinct and partially overlapping interaction modes in the active sites of their predicted target proteins (Figure 4). Their binding regions consisted of functionally important residues, including His15, Tyr119, Leu120, Gly121, Gly122, Leu57, Ile58, Tyr59, Ser60, Gln61, Gln149, Tyr151, and Ile155, which together exhibited the typical aromatic-polar-hydrophobic of the ligand recognition pocket in TNF-α. Ursolic acid exhibited the most stable and deepest-penetrating binding behavior, interacting with Tyr97(A), Asn63(A), Glu93(A), Leu64(A), and Pro65(A). The presence of Tyr97, located adjacent to the hydrophobic-aromatic region around Tyr59 and Tyr119, suggests that ursolic acid is oriented toward one of the key anchorage points. The combination of polar contacts (Asn63 and Glu93) and hydrophobic stabilization (Leu64 and Pro65) favors robust insertion into the pocket, consistent with published evidence showing that ursolic acid frequently interacts with aromatic-binding regions on TNF-α [52].

MDs were performed to evaluate the structural stability of IL-6 in complex with four ligands namely, ursolic acid, rosmarinic acid, *β*-sitosterol, and aspirin, over a 10,000 ps trajectory. The conformational snapshots of each ns (Figure 5i, panel a) demonstrate that all ligand–protein complexes remained intact throughout the simulation period, with no evidence of ligand dissociation or major structural collapse. Although minor positional adjustments were observed in loop regions, the overall folding of IL-6 was preserved, indicating that each ligand forms a stable interaction within the binding cavity. Notably, the complexes containing ursolic acid and rosmarinic acid appeared more compact across the 1–10,000 ps time points, whereas the IL-6–aspirin complex displayed slightly greater conformational movement, suggesting weaker stabilization. These qualitative observations were supported by RMSD analysis (Figure 5i, panel b), which revealed that all systems reached equilibrium within the first 1–2 ns, indicating good convergence of the trajectories. Average RMSD values ranged between approximately 1.5 and 2.5 Å, consistent with a stable protein–ligand system for MD simulations. Among the ligands, the IL-6–ursolic acid complex exhibited the smallest fluctuations after equilibration, followed closely by rosmarinic acid, suggesting that these phytochemicals impart higher structural rigidity to the IL-6 backbone. *β*-Sitosterol showed moderate stability, whereas the IL-6–aspirin complex demonstrated slightly elevated RMSD variation, reinforcing the qualitative impression of reduced binding stability for the control compound.

Residue-level fluctuations assessed through RMSF analysis (Figure 5i, panel c) provided further insights into local flexibility changes upon ligand binding. All complexes showed low RMSF values for the majority of residues (<1 Å), reflecting a stable core structure. Peaks were consistently observed at residues approximately 30–40, 100–110, and 150–160, which correspond to loop or flexible surface-exposed regions of IL-6. Importantly, the amplitudes of these fluctuations differed among ligands; ursolic acid and apigenin produced the lowest RMSF intensities across all major peaks, indicating greater dampening of local mobility. *β*-Sitosterol induced moderate fluctuations, while aspirin resulted in the highest flexibility in loop segments, consistent with a weaker stabilizing interaction. Throughout the simulation, all complexes retained the ligand within the TNF binding region, and the global fold of TNF remained recognizable. Representative conformations captured at 1 ns intervals for each complex are displayed in Figure 5ii, panel a. Variations in loop positioning and side-chain orientations appear across frames, but no major deviations or unfolding events are observed in any of the systems.

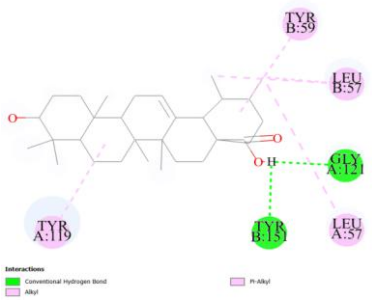
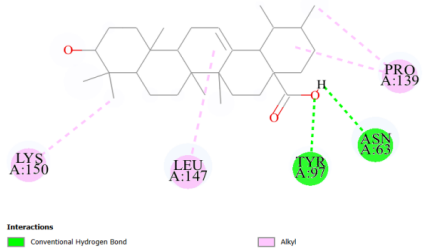
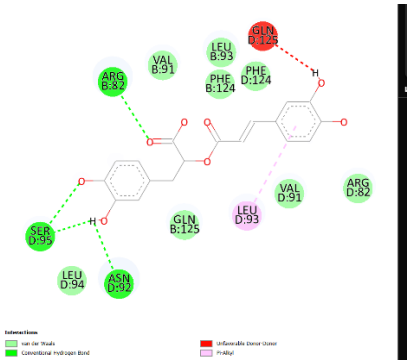
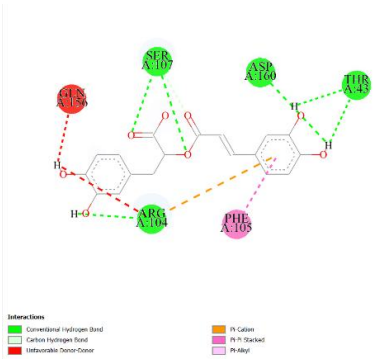
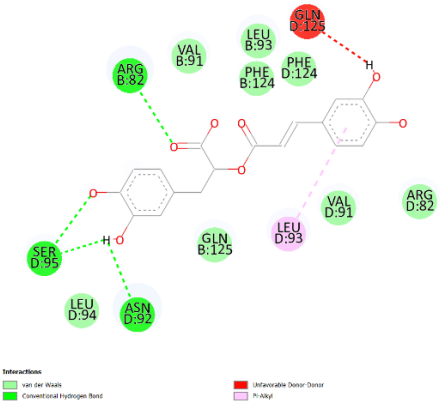
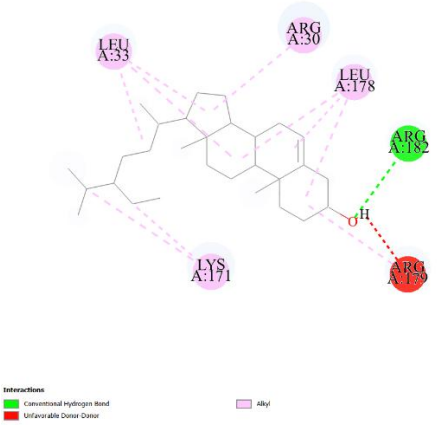
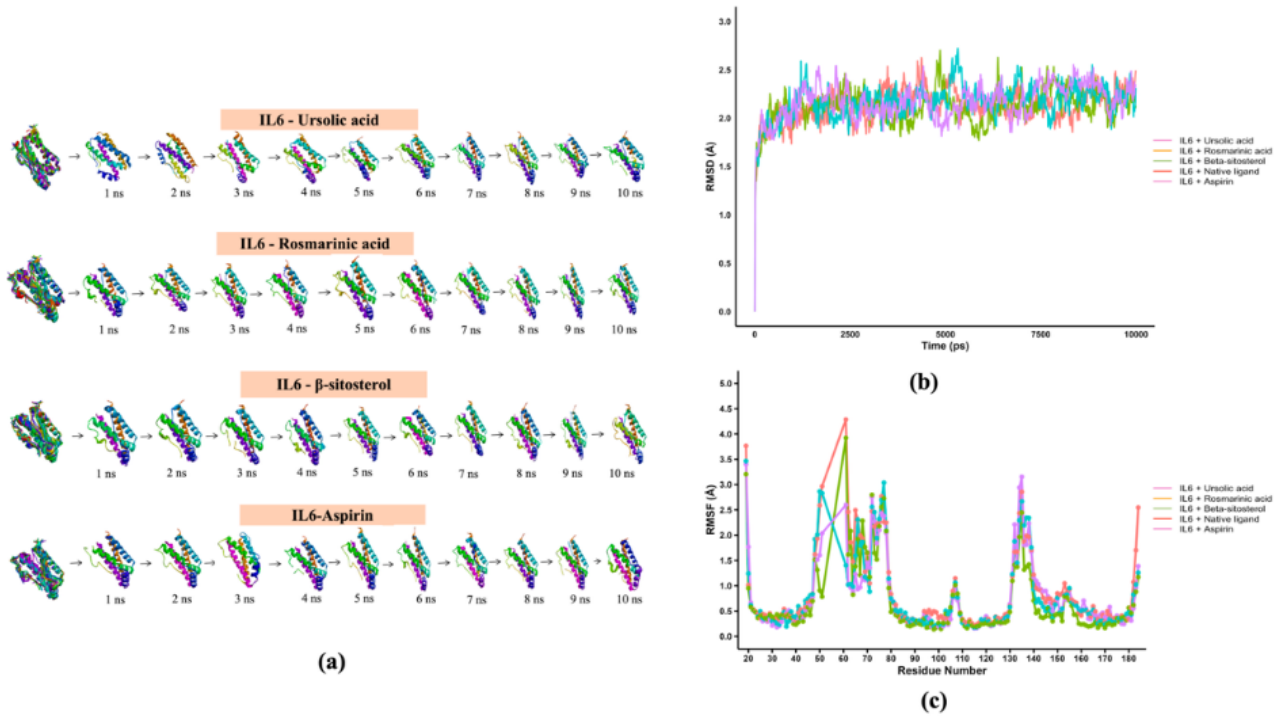
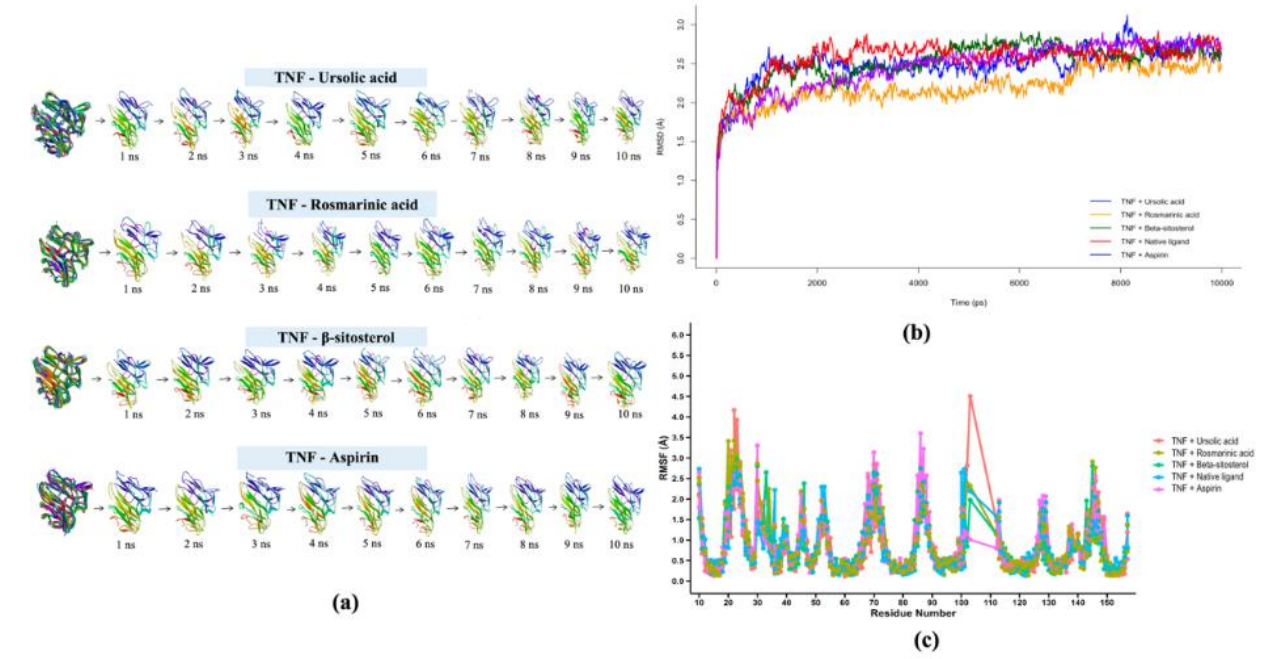
TNF	**IL-6**
	
Ursolic acid	
	
rosmarinic acid–TNF	
	
***β*-sitosterol–IL-6**	
	
Aspirin	

Figure 4: The 2D visualisation of selective ligand with IL-6 and TNF receptors

The RMSD profiles for the four TNF–ligand complexes, along with the native TNF reference, are presented in Figure 5ii, panel b. All trajectories exhibit a rapid rise in RMSD during the initial portion of the simulation, after which the plots level off, indicating entry into a more stable phase. For the remainder of the simulation, RMSD values fluctuate within a range of approximately 1.5–2.7 Å. Although the curves for the different systems are not identical, they follow broadly similar trends over the full 10,000 ps. Finally, residue fluctuations are illustrated by the RMSF results in Figure 5ii, panel c.



(i) IL-6



(ii) TNF

Figure 5: The molecular dynamic simulation of IL-6 and TNF in complex with ursolic acid, rosmarinic acid, β-sitosterol, and aspirin (a) Panel, (b) RMSD, and (c) RMSF values of the complexes

The RMSF curves show a repeating pattern of low- and high-mobility regions along the TNF sequence. Most residues exhibit values below 1 Å, while several clearly defined peaks occur at specific residue positions in all systems. The overall location of these peaks is consistent among the ligand-bound complexes and the native TNF reference line. Differences between ligands appear primarily in the height of individual peaks rather than in the general distribution of fluctuating segments.

The molecular dynamic simulations of IL-6 and TNF in complex with ursolic acid, rosmarinic acid, *β*-sitosterol, and aspirin provide insight into how each ligand influences the local flexibility and dynamic stability of the cytokine during simulation. Overall, the fluctuations across all systems remain within the expected range for globular cytokines such as IL-6, whose loop regions and terminal segments are characteristically more flexible than the core α-helical bundle. Across the four systems, ursolic acid produced the lowest RMSF values over most residues, indicating superior stabilization of the IL-6 backbone. The marked reduction in fluctuation, particularly in flexible loops around residues 18–30, 50–80, and 150–170, suggests stronger or more persistent interactions between ursolic acid and key microdomains of IL-6. Ursolic acid is known for its polycyclic hydrophobic framework and multiple hydrogen bond donors and acceptors, which support deep and stable occupation of protein pockets; similar stabilizing behavior has been reported in MD studies of ursolic acid interacting with inflammatory mediators.

Table 3 presents the predicted drug-like and ADME-related properties of three representative secondary metabolites from *Ocimum americanum* with the best docking scores, namely ursolic acid, rosmarinic acid, and *β*-sitosterol. Although all three compounds originate from the same plant source, they display distictly different physicochemical characteristics that may influence their pharmacological behavior and translational potential. Ursolic acid, a pentacyclic triterpenoid, exhibited the highest overall bioavailability score (0.85) among the evaluated compounds, despite its relatively high molecular weight (456.70 g/mol) and lipophilicity (Log P = 5.93). These properties are consistent with its hydrophobic scaffold, which is often associated with a strong affinity toward protein binding pockets involved in inflammatory and metabolic regulation. The limited number of hydrogen bond donors and acceptors suggests a balanced polarity that may favor stable ligand–target interactions. Nevertheless, the predicted low gastrointestinal absorption indicates that oral delivery of ursolic acid may be suboptimal without formulation enhancement, a limitation commonly reported for triterpenoid-based compounds.

Table 3: Druglike properties of selective compound from *Ocimum americanum*

No.	Parameter	Ursolic Acid	Rosmarinic acid	*β*-sitosterol
1	Molecular formula	$C_{30}H_{48}O_3$	$C_{18}H_{16}O_8$	$C_{29}H_{50}O$
2	Molecular weight (g/mol)	456.70	360.31	414.71
3	Number H-bond acceptors	3	8	1
4	Number H-bond donors	2	5	1
5	Log $P_{o/w}$	5.93	1.58	7.24
6	Gastrointestinal absorption	Low	Low	Low
7	Blood brain barrier permeant	No	No	No
8	P-glycoprotein substrate	No	No	No
9	Bioavailability score	0.85	0.56	0.55
10	Meet lipinski rules	Yes	Yes	Yes

Other compounds, rosmarinic acid displayed a markedly different profile. With a lower molecular weight (360.31 g/mol) and a substantially reduced Log P value (1.58), rosmarinic acid is considerably more polar than the other two compounds. Its high number of hydrogen bond donors and acceptors reflects the presence of multiple phenolic and carboxyl functional groups, which can facilitate strong hydrogen-bonding interactions with biological targets. While rosmarinic acid complies with Lipinski's rule of five and demonstrates moderate bioavailability (0.56), its predicted low gastrointestinal absorption suggests that excessive polarity may hinder passive membrane permeation, potentially limiting oral uptake. *β*-Sitosterol showed the highest lipophilicity among the tested compounds (Log P = 7.24), consistent with its phytosterol backbone. Although its molecular weight remains within acceptable limits and it satisfies Lipinski's criteria, the combination of extreme hydrophobicity and minimal hydrogen-bonding capacity likely contributes to its low predicted gastrointestinal absorption and moderate bioavailability score (0.55). These features suggest that *β*-sitosterol may preferentially interact with lipid-rich environments or membrane-associated targets rather than aqueous compartments.

Notably, none of the evaluated compounds were predicted to cross the blood–brain barrier or act as substrates of P-glycoprotein. This profile is advantageous for applications targeting peripheral tissues, as it reduces the likelihood of central nervous system–related side effects and efflux-mediated clearance. The results indicate that all three compounds possess acceptable drug-like characteristics, with ursolic acid emerging as the most promising candidate in terms of predicted bioavailability. Meanwhile, rosmarinic acid and *β*-sitosterol present complementary pharmacokinetic profiles that may be further optimized through formulation strategies or chemical modifications to enhance their therapeutic potential.

The *Ocimum americanum* has been studied for its potential anti-inflammatory effects, particularly through its essential oil and various extracts. The essential oil of *Ocimum americanum* contains various bioactive compounds that contribute to its anti-inflammatory properties attributed to the oil's ability to modulate inflammatory mediators. For instance, it has been observed to suppress the production of pro-inflammatory cytokines and reduce paw edema in experimental models. This suggests a protective effect against joint damage and inflammation [53]. Research has consistently highlighted the pharmacological relevance of *Ocimum americanum* . The ethanolic extract has shown promising results in reducing markers of oxidative stress and inflammation in rat models [54]. These findings suggest that *Ocimum americanum* may be a valuable natural remedy for various health conditions.

Studies on related Ocimum species, including *O. basilicum* and *O. sanctum*, have revealed similar pharmacological activities. The ethanolic extracts of *O. basilicum* have exhibited strong antioxidant and anti-inflammatory properties, attributed to the presence of rosmarinic acid and eugenol [55]. Similarly, *O. sanctum* has demonstrated anti-skin aging effects [56]. The essential oils derived from the leaves of *O. basilicum* and *Ocimum americanum* showed reasonable antioxidant, antibacterial, and mosquito larvicidal properties, and can be employed as an alternative medication for human health and mosquito control [32]. These comparative analyses highlight the potential of *Ocimum americanum* as a source of therapeutic compounds for oxidative stress and inflammatory disorders.

Collectively, these data affirm *Ocimum americanum*'s therapeutic promise for inflammatory disorders, including severe malaria where heightened cytokines such as TNF and IL-6 play a central role in clinical deterioration. Moreover, the integration of phytochemical profiling with MD-based stability assessment strengthens the justification for further investigation into *Ocimum americanum* as a candidate therapeutic resource. Future studies should prioritize structured clinical evaluations to confirm efficacy and safety, while also isolating and characterizing active constituents, especially those such as ursolic acid, rosmarinic acid, and *β*-sitosterol. Complementary metabolomic network

analysis and formulation optimization may also clarify synergistic interactions within the extract and support development of standardized preparations.

Conclusion

This study highlights *Ocimum americanum* as a promising source of phytochemicals with the potential to modulate inflammatory and oxidative stress–related pathways implicated in malaria-associated pathology. Using an integrated network pharmacology, molecular docking, and molecular dynamics framework, the analysis identified a set of core molecular targets involved in cytokine-mediated signaling, with IL-6 and TNF emerging as central regulatory nodes within the interaction network. Among the evaluated phytochemicals, ursolic acid exhibited the most favorable and consistent binding profiles toward key inflammatory mediators, supporting its potential role as a lead compound in modulating cytokine-driven inflammatory processes. The systems-level interactions observed in this study underscore the relevance of multi-target mechanisms in addressing complex inflammatory conditions such as malaria-associated hyperinflammation. Overall, these findings provide mechanistic *in silico* evidence supporting the pharmacological relevance of *Ocimum americanum*–derived phytochemicals and establish a rational framework for future experimental validation and therapeutic exploration. Further studies incorporating biochemical, cellular, and *in vivo* approaches will be necessary to confirm these predictions and to assess their translational potential.

Ethical Approval

Not applicable.

Declaration of Competing Interest

The authors declared that they had no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data Availability

The data are available on request from the author.

ORCID

Nur Balqis Maulydia
https://orcid.org/0009-0000-6497-5825
Syahputra Wibowo
https://orcid.org/0000-0003-3979-4277
Josephine Elizabeth Siregar
https://orcid.org/0000-0001-9894-3207
Rinaldi Idroes
https://orcid.org/0000-0003-2264-6358
Rony Abdi Syahputra
https://orcid.org/0000-0003-2016-0151
Putri Cahaya Situmorang
https://orcid.org/0000-0002-9895-1853

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