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RESEARCH ARTICLE

Essential Oil of *Polyalthia obliqua* Leaves: GC-MS Composition, Antioxidant, Anti-Inflammatory, Antimicrobial Effects, and Molecular Docking Analysis

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ABSTRACT

Polyalthia obliqua Hook.f. & Thomson is a noteworthy species of the genus *Polyalthia* with promising medicinal value. The leaf essential oil was analyzed for its chemical composition and biological activities. Gas chromatography-mass spectrometry identified 59 constituents (85.98% of total oil), predominantly oxygenated sesquiterpenoids (73.20%) with spathulenol (28.72%) as the major compound. The oil exhibited antioxidant activity in the 2,2-diphenyl-1-picrylhydrazyl assay (IC_{50} = 157.74 ± 3.93 μg/mL); the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) assay (IC_{50} = 170.00 ± 0.88 μg/mL), and notable inhibition of nitric oxide production (IC_{50} = 54.85 μg/mL). Selective antibacterial effects were observed against *Staphylococcus aureus* (MIC = 64 μg/mL). Molecular docking suggested that khusinol contributes to anti-inflammatory activity via inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), while both khusinol and 14-oxy-α-muurolene may contribute to antibacterial activity via dihydrofolate reductase (DHFR) and UDP-N-acetylglucosamine enolpyruvyl transferase (MurB). These findings underscore the chemotaxonomic and bioactive potential of *P. obliqua* essential oil.

1 | Introduction

The genus *Polyalthia*, belonging to the Annonaceae family, comprises approximately 120 species distributed worldwide [1]. The genus *Polyalthia* is widely distributed in the palaeotropical area, especially in Southeast Asia, extending from Southern India and Sri Lanka, through continental Southeast Asia to Northern Australia and Melanesia, even with some occurrences in the lowland humid regions of East Africa and Madagascar [2]. Many species within this genus hold significant medicinal value and have been traditionally used in folk medicine to treat ailments such as stomachache, intestinal parasites, dysmenorrhea, pharyngeal neurosis, fever, skin diseases, diabetes, and hypertension [3–5]. Due to their rich content of bioactive compounds, numerous species of *Polyalthia* have become valuable sources of medicinal agents in modern medical research. Previous studies on plants of the *Polyalthia* genus have identified a wide range of bioactive compounds, including alkaloids, diterpenoids, triterpenoids, sesquiterpenoids, flavonoids, styryllactones, furans, polyacetylenes, acetogenins, and essential oils [5–8]. Pharmacological research has demonstrated that both the plant extracts and their isolated compounds exhibit diverse biological activities, such as cytotoxic, antiplasmodial, anti-HIV, antineoplastic, antibacterial, antioxidant, and antitrypanosomal effects [8–12].

Trang Huyen Xuan Hoang and Tuan Quoc Doan contributed equally to this study.

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FIGURE 1 | *Polyalthia obliqua* Hook.f. & Thomson (Annonaceae).

Polyalthia obliqua Hook.f. & Thomson (Figure 1) is one of the notable species within the genus *Polyalthia*, exhibiting remarkable medicinal potential. Studies have identified ten compounds from the roots of *P. obliqua*, including suberosol, marcanine A, and several others [13]. Additionally, two lanostane-type triterpenoids have been isolated from its branches and leaves. Notably, a newly discovered alkaloid, consanguine B, along with ten previously known alkaloids, has been identified from the ethanol extract of the branches and leaves of *P. obliqua* collected in Hainan Province, China. These compounds have demonstrated strong bioactive properties, particularly in anticancer activities [14].

However, the essential oil composition of *P. obliqua* remains largely unexplored. Therefore, this study was conducted to elucidate the essential oil composition from the leaves of *P. obliqua* and evaluate some of its biological activities, thereby contributing to a better understanding of its potential applications in medicine and pharmaceuticals.

2 | Results and Discussion

2.1 | Chemical Composition of Essential Oils Isolated From Leaves of *P. obliqua*

The chemical composition of the essential oil is summarized in Table 1. The extraction yield was 0.09 ± 0.02% (w/w) based on fresh plant material. The essential oil was lighter than water, exhibiting a pale-yellow color and a characteristic aromatic odor. A total of 59 compounds were identified, accounting for 85.98% of the total chromatographic peak area, indicating a comprehensive characterization of the oil composition (Figure S1).

The chemical profile was dominated by oxygenated sesquiterpenoids (73.20%), while sesquiterpene hydrocarbons (11.16%) and oxygenated monoterpenes (0.24%) were present in much lower proportions, suggesting a metabolic tendency toward highly oxidized terpenoid structures. Among the identified constituents, spathulenol (28.72%) was the most abundant compound, followed by 14-oxy-α-muurolene (4.58%), globulol (3.99%), khusinol (3.07%), humulene epoxide II (2.91%), and α-cadinol (2.80%) (Figure S2). These compounds mainly belong to sesquiterpene alcohols and epoxides, reflecting structural diversity within the oil, including alcohol, ketone, and epoxide functionalities. The exceptionally high proportion of spathulenol is particularly noteworthy, as this compound is typically reported at moderate levels in many plant species, suggesting its potential role as a chemophenetic marker of *P. obliqua*.

Another notable feature is the richness of oxygenated sesquiterpenoids belonging to various structural classes, such as muurolane, cadinane, and eudesmane types. This diversity indicates a highly active terpenoid biosynthetic system capable of extensive oxidative transformations. In contrast, the negligible amounts of monoterpenes (<0.5%) highlight a metabolic preference against monoterpene biosynthesis, further emphasizing the chemical uniqueness of this species.

When compared to other species of the same genus, *P. obliqua* shares the general dominance of sesquiterpenoids but differs by its higher proportion of oxygenated derivatives rather than hydrocarbon sesquiterpenes such as germacrene D, which is typical of *P. rumphii* [2] or *P. stenopetalla* [2]. Interestingly, *P. obliqua* oil was almost devoid of monoterpene hydrocarbons, in contrast to *P. korintii* [15], *P. suberosa* [16], and *P. jucunda* [17], which are rich in β-pinene.

From a biological perspective, oxygenated sesquiterpenoids have been reported to exhibit antimicrobial, anti-inflammatory, and antioxidant activities [18, 19]. Therefore, the abundance and structural diversity of this chemical class in *P. obliqua* essential oil not only underline its distinctive phytochemical profile but also provide a strong foundation for evaluating its pharmacological potential, particularly in the search for new bioactive natural compounds. This rationale forms the scientific basis for the subsequent investigation of its antioxidant, anti-inflammatory, and antimicrobial properties in this study.

2.2 | Antioxidant Activity

The antioxidant potential of *P. obliqua* leaf essential oil was evaluated using both DPPH and ABTS radical scavenging assays. The results indicated that the oil exhibited moderate antioxidant activity, with IC_{50} values of 157.74 ± 3.93 μg/mL (DPPH) and 170.00 ± 0.88 μg/mL (ABTS), which were substantially higher than those of the standard ascorbic acid (IC_{50} = 2.69 ± 0.09 and 1.27 ± 0.08 μg/mL, respectively). Although the activity was weaker than the reference compound, the essential oil demonstrated a distinct radical scavenging capability.

Notably, the predominance of spathulenol (28.72%) along with other oxygenated sesquiterpenes such as 14-oxy-α-muurolene (4.58%), globulol (3.99%), and humulene epoxide II (2.91%) in the essential oil composition may account for its antioxidant effects. These sesquiterpenoids have previously been reported to display significant free radical scavenging activities in various aromatic species. For instance, spathulenol has been identified as a key contributor to the antioxidant capacity of essential oils from the Myrtaceae and Lamiaceae families due to its ability to stabilize reactive oxygen species [20, 21]. Globulol is the main antioxidant component in the essential oil of *Schinus areira*, and this compound exhibits DPPH free radical scavenging activity with an IC_{50} value of 5.6 ± 0.91 μg/mL [22]. Humulene epoxide derivatives also contribute to the ability to inhibit lipid oxidation [23].

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TABLE 1 | Chemical composition of the essential oil from the leaves of *P. obliqua*.

No	RT	Compound[a]	RI[b]	RI[c]	CAS ID	Concentration (%)
1	13.58	Linalool*	1095	1099	78-70-6	0.24
2	24.15	*δ*-Elemene	1335	1338	20307-84-0	0.21
3	24.69	*α*-Cubebene	1348	1350	17699-14-8	0.25
4	25.39	Cyclosativene	1369	1366	22469-52-9	0.42
5	25.87	*α*-Ylangene	1373	1377	14912-44-8	1.46
6	26.23	*β*-Bourbonene	1387	1386	5208-59-3	0.23
7	26.45	*β*-Cubebene	1387	1391	13744-15-5	0.32
8	26.55	*β*-Elemene	1389	1393	515-13-9	0.88
9	27.10	*α*-Funebrene	1402	1406	50894-66-1	0.26
10	27.69	*β*-Caryophyllene*	1417	1420	87-44-5	0.43
11	28.03	(*E*)-*α*-Ionone	1428	1429	127-41-3	0.14
12	28.09	*β*-Copaene	1430	1430	18252-44-3	0.17
13	28.51	Aromadendrene	1439	1440	489-39-4	0.43
14	29.03	Geranyl acetone	1453	1453	3796-70-1	0.23
15	29.11	*α*-Humulene	1452	1455	6753-98-6	0.22
16	30.08	*trans*-Cadina-1(6),4-diene	1475	1479	20085-11-4	0.92
17	30.47	*β*-Selinene	1489	1488	17066-67-0	0.38
18	30.69	*cis*-*β*-Guaiene	1492	1493	88-84-6	0.17
19	30.83	*γ*-Amorphene	1495	1497	6980-46-7	0.97
20	31.03	*α*-Muurolene	1500	1502	31983-22-9	0.39
21	31.61	*γ*-Cadinene	1513	1517	39029-41-9	0.91
22	31.98	*δ*-Cadinene	1522	1526	483-76-1	1.21
23	32.52	*α*-Cadinene	1537	1540	82468-90-4	0.14
24	32.75	*α*-Calacorene	1544	1546	21391-99-1	0.58
25	34.53	**Spathulenol**	1577	1591	6750-60-3	**28.72**
26	34.62	**Globulol**	1590	1593	51371-47-2	**3.99**
27	34.89	Salvial-4(14)-en-1-one	1594	1600	73809-82-2	0.73
28	34.89	Fokienol	1596	1600	33440-00-5	0.73
29	35.31	Guaiol	1600	1611	489-86-1	0.58
30	35.49	**Humulene epoxide II**	1608	1616	19888-34-7	**2.91**
31	35.59	Isolongifolan-7-*α*-ol	1618	1619	57566-26-4	2.11
32	35.79	Junenol	1618	1624	472-07-1	0.32
33	36.15	*α*-Acorenol	1632	1634	28296-85-7	1.88
34	36.31	1,7-diepi-*α*-Cedrenal	1639	1638	288249-27-4	0.51
35	36.59	*allo*-Aromadendrene epoxide	1639	1646	85760-81-2	0.69
36	36.70	*epi*-*α*-Muurolol	1640	1649	19912-62-0	1.35
37	36.91	3-*iso*-Thujopsanone	1641	1654	25966-81-8	1.21
38	37.05	Khusilal	1647	1658	2221-68-3	0.78
39	37.22	**α-Cadinol**	1652	1663	481-34-5	**2.80**
40	37.49	Intermedeol	1665	1670	6168-59-8	0.91
41	37.76	Bulnesol	1670	1677	22451-73-6	0.85
42	37.88	Cadalene	1675	1680	483-78-3	1.86
43	38.03	(*Z*)-*α*-Santalol	1675	1684	115-71-9	0.89

(Continues)

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TABLE 1 | (Continued)

No	RT	Compound[a]	RI[b]	RI[c]	CAS ID	Concentration (%)
44	38.15	**Khusinol**	1679	1687	24268-34-6	**3.07**
45	38.40	Eudesma-4(15),7-dien-1β-ol (impure)	1687	1694	119120-23-9	2.23
46	38.53	Eudesm-7(11)-en-4-ol	1700	1698	473-04-1	0.99
47	38.80	Caryophyllene acetate	1701	1705	57082-24-3	0.25
48	38.98	*cis*-Thujopsenal	1708	1710	470-41-7	0.47
49	39.08	14-hydroxy-α-Humulene	1713	1713	000-00-0	0.86
50	39.86	Isobicyclogermacrenal	1733	1735	73256-82-3	0.12
51	40.06	Eremophilone	1734	1741	562-23-2	0.54
52	40.59	7,14-anhydro-Amorpha-4,9-diene	1755	1756	394251-67-3	0.07
53	40.96	Cyclocolorenone	1759	1766	489-45-2	2.11
54	41.25	**14-oxy-α-Muurolene**	1767	1774	135118-53-5	**4.58**
55	41.46	α-Costol	1773	1780	65018-15-7	1.46
56	41.57	14-hydroxy-α-Muurolene	1779	1783	135118-51-3	1.18
57	42.27	(*E*)-Isovalencenol	1793	1803	22387-74-2	1.17
58	42.39	14-hydroxy-δ-Cadinene	1803	1807	135118-52-4	0.55
59	46.22	(5*E*,9*E*)-Farnesyl acetone	1913	1921	3953-35-3	0.99
Oxygenated monoterpene						**0.24**
Sesquiterpene hydrocarbon						**11.16**
Oxygenated sesquiterpene						**73.20**
Others						**1.39**
Total						**85.98**

Note: All components that were found in the essential oils at greater than 2.0% were highlighted with bold letters.

Abbreviation: RT, Retention time.

[a]Elution order on Equity-5 column.

[b]Retention indices on Equity-5 column.

[c]Literature retention indices.

*Co-injection with an authentic sample.

Furthermore, the presence of α-cadinol (2.80%) and khusinol (3.07%) likely enhances the overall bioactivity [24]. This suggests that the synergistic contribution of these oxygenated sesquiterpenoids plays a crucial role in the overall antioxidant potential of the studied essential oil. The collective action of these constituents, rather than a single compound, often defines the efficacy of natural volatile mixtures in neutralizing oxidative stress.

2.3 | Inhibitory Activity on No Production

The essential oil of *P. obliqua* significantly reduced the production of NO in LPS-stimulated RAW 264.7 cells in a dose-dependent manner, achieving 87.31% inhibition at 100 μg/mL, comparable to dexamethasone (84.26%). Although the IC_{50} value (54.85 μg/mL) was higher than that of the reference drug (14.41 μg/mL), the results still demonstrated a remarkable anti-inflammatory potential. In addition, the oil exhibited no marked cytotoxicity, as the cell viability remained at 99.11 ± 1.49% even at the highest tested concentration (100 μg/mL) (Table S1).

This anti-inflammatory effect could be attributed to the high proportion of oxygenated sesquiterpenoids (73.20%), among which spathulenol, globulol, humulene epoxide II, α-cadinol, khusinol, and 14-oxy-α-muurolene are the principal constituents. These compounds have previously been reported to modulate iNOS expression, suppress NO production, and attenuate inflammatory responses [25].

In comparison with other species of the genus *Polyalthia*, *P. obliqua* exhibited a distinct chemical profile and stronger bioactivity. For instance, the essential oils of *P. longifolia* and *P. rumphii*, which are rich in germacrene D and other sesquiterpene hydrocarbons, were found to display only moderate anti-inflammatory activities. Meanwhile, the leaf and stem oils of *P. viridis* showed significant inhibition of NO production, with IC_{50} values of 57.6 ± 2.0 μg/mL and 76.7 ± 1.3 μg/mL, respectively, compared with the positive control butein (IC_{50} = 16.1 ± 1.0 μg/mL) [26].

The anti-inflammatory activity of the essential oil of *P. obliqua* may be partly attributed to the high abundance of spathulenol (28.72%), the dominant constituent in the oil. Previous studies have demonstrated the important role of spathulenol in modulating inflammatory responses. Specifically, spathulenol significantly inhibited carrageenan-induced paw edema and reduced leukocyte migration in a pleurisy model, indicating its

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effects on both the acute and cellular phases of inflammation [20]. In addition, a previous study elucidated the underlying mechanisms, showing that spathulenol is capable of regulating pro-inflammatory cytokines, thereby contributing to the attenuation of inflammatory responses and nociception. This activity may be associated with the inhibition of inflammatory mediators such as TNF-α and IL-1β, as well as signaling pathways related to oxidative stress [27].

Therefore, given the predominance of spathulenol, the essential oil of *P. obliqua* is likely to exhibit significant anti-inflammatory activity. This effect may be further enhanced through synergistic interactions with other oxygenated sesquiterpenoids present in the oil, such as α-cadinol [28], globulol, and humulene epoxide II [29].

These findings suggest that the unique phytochemical composition of *P. obliqua* contributes to its notable anti-inflammatory activity. Although its efficacy does not yet match that of dexamethasone, the oil possesses distinct advantages owing to its natural origin, diverse spectrum of bioactive constituents, and potential concurrent antioxidant and antimicrobial properties. Collectively, these results not only highlight the pharmacological value of *P. obliqua* but also support its potential as a promising natural source for the development of plant-derived anti-inflammatory agents.

2.4 | Antimicrobial Activity

The essential oil of *P. obliqua* exhibited a selective antibacterial spectrum, showing strong inhibitory effects against Gram-positive bacteria, particularly *Staphylococcus aureus* (IC_{50} = 40.00 μg/mL; MIC = 64 μg/mL), followed by *Bacillus subtilis* (IC_{50} = 160 μg/mL; MIC = 256 μg/mL). In contrast, the oil displayed negligible activity against Gram-negative bacteria (*Escherichia coli*, *Salmonella enterica*, *Pseudomonas aeruginosa*) and *Lactobacillus fermentum* (Table S2). This activity may be associated with the lipophilic nature of oxygenated sesquiterpenoids, which facilitates their interaction with bacterial cell membranes, leading to increased permeability and disruption of cellular integrity in Gram-positive bacteria. In addition, molecular docking results suggest potential interactions with key bacterial enzymes, which may further contribute to the observed antibacterial effects.

When compared with other *Polyalthia* species, the antimicrobial profile of *P. obliqua* appears distinct. For example, *P. suberosa* essential oil demonstrates pronounced activity against Gram-negative bacteria, where the stem oil strongly inhibited *P. aeruginosa* (MIC = 50 μg/mL), and the leaf oil showed comparable inhibition against *E. coli* at the same concentration [16]. Similarly, essential oils obtained from the leaves and stems of *P. viridis* inhibited *P. aeruginosa* and *E. coli* with MIC values of approximately 200 μg/mL [26]. In contrast, the selective inhibition of *S. aureus* by *P. obliqua* highlights a species-specific antibacterial spectrum within the genus. The pronounced selectivity of *P. obliqua* toward Gram-positive bacteria, especially *S. aureus*, therefore underscores its unique phytochemical signature and may have chemotaxonomic implications for the genus.

The research results showed that *P. obliqua* leaf essential oil possesses three outstanding properties: moderate but significant antioxidant activity; high NO inhibition and cell safety, confirming its anti-inflammatory potential; selective and potent effects on Gram-positive bacteria, especially *S. aureus*. The predominance of spathulenol and rare oxygenated sesquiterpenoids is the basis for these properties, and also contributes to affirming the pharmacological and chemotaxonomic value of the species. Although the efficacy cannot be compared with standard drugs, *P. obliqua* essential oil opens up the prospect of developing into a new natural source of raw materials for anti-inflammatory and antibacterial products of plant origin.

2.5 | Molecular Docking Analysis

To clarify the possible biological mechanisms of the main compounds in *P. obliqua* essential oil, molecular docking simulations were carried out on four key enzymes related to inflammation and bacterial biosynthesis. The inflammatory targets iNOS and COX-2 regulate nitric oxide and prostaglandin production, whereas the antibacterial targets DHFR and MurB are essential for nucleotide and cell-wall biosynthesis. Inhibiting these enzymes could therefore contribute to the anti-inflammatory and antibacterial potential of the essential oil. The docking results (Figure S5) showed that the sesquiterpenoids present in *P. obliqua* essential oil exhibited different binding affinities toward the four target enzymes.

For iNOS, the docking results revealed that the major sesquiterpenoids, including spathulenol, 14-oxy-α-muurolene, and khusinol, interact with the iNOS active site predominantly through hydrophobic contacts. Khusinol showed the most negative binding energies (−8.9 kcal/mol), followed by 14-oxy-α-muurolene and spathulenol (−8.1 and −8.0 kcal/mol, respectively). These results suggest that these molecules may have favorable interactions with the iNOS active site and could potentially contribute to the inhibition of nitric oxide production, providing a possible molecular explanation for the observed anti-inflammatory activity.

In terms of molecular interactions, spathulenol and khusinol relied mainly on hydrophobic interactions. Spathulenol was stabilized mainly by alkyl and π–alkyl interactions with Trp194, Phe369, Ala197, and Cys200, while Tyr489, Val352, Asn370, and Gly371 contributed additional van der Waals contacts. For khusinol, the complex was stabilized mainly by hydrophobic interactions, including alkyl and π–alkyl contacts with Trp194, Cys200, Phe369, Ile244, Leu209, and Tyr489, while Ser242, Gly371, and Ala197 contributed additional van der Waals interactions (Figure 2A).

In contrast, 14-oxy-α-muurolene displayed a more favorable interaction pattern, characterized by a conventional hydrogen bond with Asn370, which likely serves as an important anchoring interaction and enhances binding specificity. In addition, this ligand formed a π–sigma interaction with Trp194 and multiple alkyl/π–alkyl contacts with Cys200, Phe369, Tyr489, and Ala197, whereas Ile244, Leu209, Ser242, Ala243, Gly371, Pro350, and Val352 contributed further van der Waals interactions (Figure S6).

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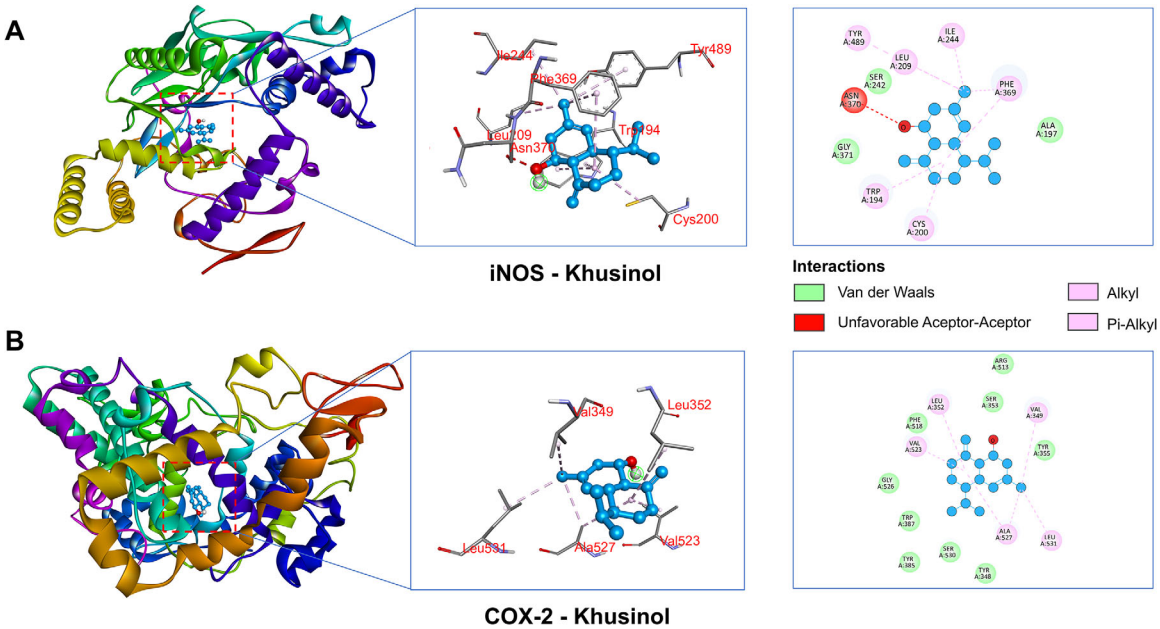

FIGURE 2 | Predicted binding modes of khusinol with the anti-inflammatory targets iNOS (A) and COX-2 (B).

For COX-2, most compounds showed moderate binding affinities (−6.2 to −8.1 kcal/mol), with khusinol again showing the strongest binding (−8.1 kcal/mol). The interaction profile showed that khusinol is predominantly stabilized through hydrophobic interactions, including alkyl contacts with Leu352, Val349, Val523, Ala527, and Leu531 (Figure 2B). In addition, several residues such as Phe518, Tyr355, Tyr348, Ser353, Ser530, Gly526, and Trp387 contributed van der Waals interactions, further enhancing the stability of the complex. No hydrogen bonds were detected, indicating that the ligand is mainly stabilized by non-polar contacts within the cyclooxygenase pocket, which is consistent with its moderate binding affinity. These findings indicate that iNOS may represent a more favorable target than COX-2 for the anti-inflammatory potential of *P. obliqua* essential oil.

For the antibacterial targets, khusinol (−8.3 kcal/mol), α-cadinol (−8.3 kcal/mol), and globulol (−8.3 kcal/mol) exhibited the strongest binding affinities toward DHFR. This suggests a plausible antibacterial mechanism of the essential oil, since DHFR plays a vital role in the folate pathway and nucleotide biosynthesis of bacteria.

Specifically, khusinol, α-cadinol, and globulol (−8.3 kcal/mol) formed hydrophobic interactions with Ile50 and Leu20 (Figure 3A). Notably, Leu20 and Ile50 have been identified as critical active-site residues that play a pivotal role in the enzyme inhibition mechanism. Previous studies have reported several key amino acids near the active site of bacterial DHFR, including Ile5, Val6, Ala7, Asn18, Leu20, Asp27, Leu28, Ile31, Ile50, Leu54, Phe92, and Tyr98 [30]. The strong interaction of ligands with these residues is therefore likely to contribute significantly to the inhibition of DHFR activity [31].

For the antibacterial enzyme MurB, 14-oxy-α-muurolene showed the strongest binding affinity (−9.0 kcal/mol), followed by khusinol (−8.9 kcal/mol). Within the MurB active pocket, 14-oxy-α-muurolene formed a hydrogen bond with Tyr77, a π–σ interaction with Tyr149, and an alkyl contact with Ile84 (Figure 3B). By comparison, khusinol established several alkyl contacts with Ile84 and Tyr149 (Figure S9).

The docking analysis indicates that khusinol may be a key contributor to the biological activities of *P. obliqua* essential oil, as it exhibited strong binding affinities toward both anti-inflammatory targets (iNOS and COX-2) and antibacterial targets (DHFR and MurB). In addition, spathulenol, the major constituent of the oil, also demonstrated favorable interactions with these target proteins, suggesting its significant role in the overall bioactivity. The combined effects of khusinol and spathulenol, together with the complementary contributions of other oxygenated sesquiterpenoids, likely account for the biological activities observed experimentally. These findings provide molecular-level insights into the possible mechanisms underlying the observed bioactivities and suggest that *P. obliqua* may represent a potential natural source for the development of plant-derived anti-inflammatory and antibacterial agents. However, further experimental validation is required to confirm these findings.

3 | Conclusions

The essential oil from the leaves of *P. obliqua* is characterized by a predominance of oxygenated sesquiterpenoids, particularly spathulenol, which defines its unique chemical fingerprint within the genus. The oil exhibited moderate antioxidant activity, strong inhibition of nitric oxide production with cell safety, and selective antibacterial activity against Gram-positive bacteria. Molecular docking further revealed that khusinol possesses strong affinities toward both anti-inflammatory (iNOS, COX-2) and antibacterial (DHFR, MurB) targets, supporting their pivotal roles in the observed biological effects. These results provide molecular insights into the mechanisms underlying the pharmacological potential of *P. obliqua* essential oil and suggest

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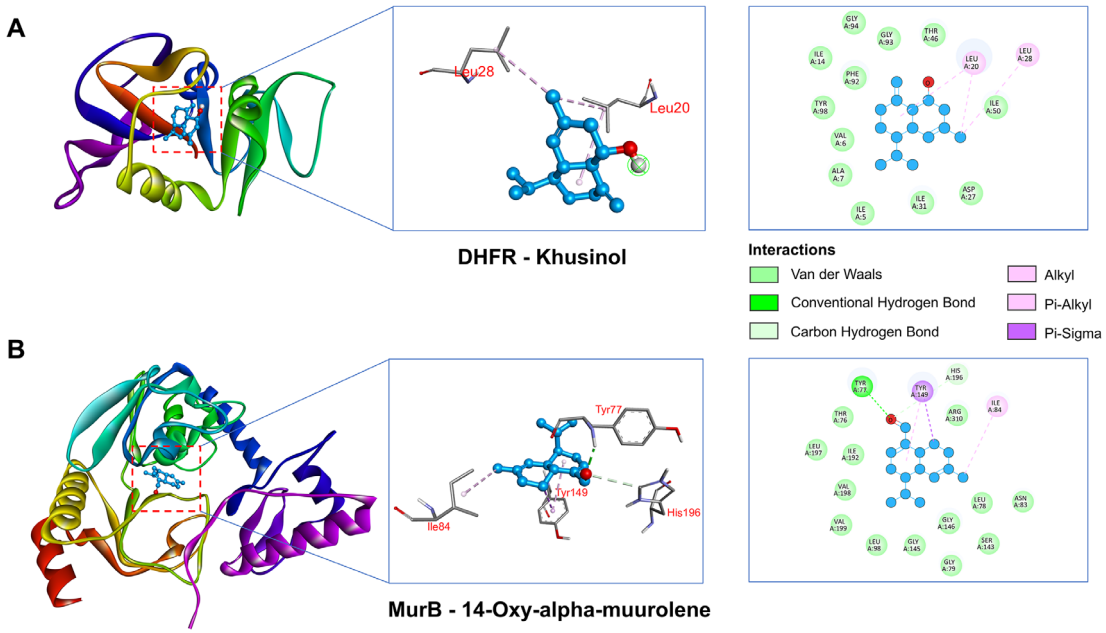

FIGURE 3 | Predicted binding modes of khusinol with dihydrofolate reductase (DHFR) (A) and 14-oxy-α-muurolene with MurB (B).

that this species could serve as a valuable natural resource for the development of plant-based anti-inflammatory and antibacterial products. To the best of our knowledge, this is the first report on the chemical composition and the biological activities, including antioxidant, nitric oxide inhibitory, and antibacterial effects of the leaf essential oil of *P. obliqua*. Future studies focusing on bioassay-guided isolation and in vivo evaluations are warranted to further substantiate these findings.

4 | Experimental Section

4.1 | Plant Materials

The fresh leaves of *P. obliqua* were collected from A Luoi district, Thua Thien Hue province, in November 2024 (16°08′34.6″N 107°23′52.0″E), and identified by Dr. Le Tuan Anh, Viet Nam National Museum of Nature. A voucher specimen (PO-T235) has been deposited in the Faculty of Pharmacy at Hue University of Medicine and Pharmacy, Hue University, Vietnam.

4.2 | Materials

Lipopolysaccharides (LPS) from *Escherichia coli*, sodium nitrite, sulfanilamide, N-1-napthylethylenediamine dihydrochloride, and dimethyl sulphoxide (DMSO) from Sigma Chemical Co. (St. Louis, MO, USA) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Dulbecco's Modified Eagle's Medium (DMEM) and fetal bovine serum (FBS) were from Life Technologies, Inc. (Gaithersburg, MD, USA).

4.3 | Cell Source and Culture

RAW 264.7 (RRID: CVCL_0493) murine macrophage cell line was kindly provided by Prof. Dr. Domenico Delfino (University of Perugia, Perugia, Italy). Stock cultures were cultured in DMEM medium supplemented with 2 mM L-glutamine, 10 mM HEPES, 1.0 mM sodium pyruvate, and 10% fetal bovine serum (FBS, GIBCO). The cells were subcultured every 3–5 days at a 1:3 ratio and maintained in a CO_2 incubator under controlled conditions of 37°C and 5% CO_2.

4.4 | Extraction of the Essential Oil

Following methods established in previous work [32], prior to the hydrodistillation process, the fresh leaves of *M. subssesilis* were shredded. Samples were then subjected to hydrodistillation using a Clevenger apparatus for three hours at normal pressure, according to the Vietnamese Pharmacopoeia V (2019) [33]. The essential oils were then collected, the water was removed by using sodium sulfate (Na_2SO_4), and they were stored at 4°C in a refrigerator. The experiments were performed in triplicate.

4.5 | Analysis of Essential Oil

The chemical composition of the essential oil was determined using a gas chromatography mass spectrometry (GC–MS) system (GCMS-QP2010 Plus, Shimadzu, Kyoto, Japan), following analytical conditions similar to those described in our previous studies [34–36]. The system was equipped with an Equity-5 fused silica capillary column (30 m × 0.25 mm in diameter, film thickness 0.25 μm, Supelco, USA). The essential oil sample was diluted in n-hexane (1:100, v/v), and 1 μL of the diluted solution was injected in split mode with a split ratio of 1:50. Helium was used as the carrier gas at a constant flow rate of 1.5 mL/min. The injector and interface temperatures were maintained at 280°C. The oven temperature program began at 60°C (held for 2 min), increased to 240°C at a rate of 3°C/min (held for 10 min), and then ramped to 280°C at 5°C/min with a final hold of 40 min. Mass spectra were acquired in the electron impact mode at

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70 eV over an *m/z* range of 45–500 with a scan rate of 1.0 scan/s. Compound identification was performed by comparing the obtained mass spectra with those in the NIST 2020 and WILEY 7 libraries, as well as with published data. Retention indices (RI) were calculated using a homologous series of *n*-alkanes (C_{8}–C_{32}) analyzed under identical conditions. Where available, co-injection with authentic standards was conducted to confirm compound identities. Quantification of constituents was based on the relative percentage of the GC peak areas.

4.6 | Antioxidant Activities

4.6.1 | DPPH• Radical Scavenging Activity

The free radical scavenging activity of the essential oil was evaluated using the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH•) radical [37]. A 0.135 mM DPPH solution in methanol was mixed with test samples at various concentrations (2 mL + 2 mL). After incubation for 30 min in the dark at room temperature, absorbance was measured at 517 nm using methanol as a blank and ascorbic acid as a positive control. The radical scavenging capacity was expressed as percentage inhibition (%*I*) calculated by the following equation:

$$\%I = (A_{\text{control}} - A_{\text{sample}})/A_{\text{control}} \times 100$$

where A_{control} is the absorbance of DPPH + methanol, and A_{sample} is that of the DPPH + sample. The IC_{50} value, defined as the concentration required to inhibit 50% of DPPH radicals, was determined from the dose–response curve. All measurements were carried out in triplicate.

4.6.2 | ABTS•$^{+}$ Radical Scavenging Activity

The ABTS•$^{+}$ radical cation was prepared by mixing equal volumes of 7 mM ABTS and 2.4 mM potassium persulfate in distilled water and allowing the mixture to stand in the dark for 14 h at room temperature [38, 39]. The resulting stock solution was diluted with methanol to an absorbance of 0.83 ± 0.01 at 734 nm. For the assay, 2.0 mL of the diluted ABTS•$^{+}$ solution was mixed with 2.0 mL of the test samples at various concentrations and incubated for 7 min in the dark. Absorbance was then measured at 734 nm using methanol as a blank and ascorbic acid as the positive control.

The radical scavenging activity was expressed as percentage inhibition (%*I*) using the following formula:

$$\%I = (A_{\text{control}} - A_{\text{sample}})/A_{\text{control}} \times 100$$

where A_{control} is the absorbance of ABTS•$^{+}$ with methanol, and A_{sample} is the absorbance of ABTS•$^{+}$ with the test sample. The IC_{50} value, defined as the concentration required to scavenge 50% of ABTS•$^{+}$ radicals, was calculated from the dose–response curves. All assays were performed in triplicate, and results are expressed as mean ± SD.

4.6.3 | NO Inhibition Assay

RAW 264.7 macrophages were seeded in 96-well plates at a density of 2 × 10^{5} cells/well and maintained at 37°C in 5% CO_2 for 24 h. After initial incubation, the culture medium was replaced with serum-free DMEM, and cells were subsequently treated with various concentrations of the test samples in the presence of lipopolysaccharide (LPS, 1 μg/mL) for another 24 h. Dexamethasone was applied as a positive control, whereas 1% DMSO served as the vehicle control.

Nitrite accumulation, indicative of nitric oxide (NO) production, was quantified using the Griess reagent system (Promega, WI, USA). For this purpose, 100 μL of culture supernatant was mixed with an equal volume of Griess reagent, prepared from 1% sulfanilamide in 5% phosphoric acid and 0.1% N-1-naphthylethylenediamine dihydrochloride. After 10 min incubation at room temperature, absorbance was measured at 540 nm using a microplate reader. The inhibitory effect of the essential oil was tested at concentrations of 100, 20, 4, and 0.8 μg/mL, and IC_{50} values were calculated by nonlinear regression analysis using Table Curve 4.0 (Systat Software Inc., San Jose, CA, USA) [40].

4.6.4 | MTT Cell Viability Assay

The cell culture plate used for the NO expression assay, after collecting the supernatant to determine NO levels, was supplemented with 90 μL of cell culture medium and 10 μL of MTT (final concentration: 5 mg/mL) per well. After 4 h, the medium was removed, and the formazan crystals were dissolved using 50 μL of 100% DMSO.

The OD values were measured at a wavelength of 540 nm using a BioTek Elx800 microplate reader. The cell viability in the presence of the tested substances was determined using the following formula:

$$\text{Inhibition}\,(\%) = \left(\text{OD}_{\text{sample}} - \text{OD}_{\text{blank}}\right) / \left(\text{OD}_{\text{DMSO}} - \text{OD}_{\text{blank}}\right)$$

4.6.5 | Antimicrobial Activity

The antimicrobial potential of the tested samples was assessed using the broth microdilution technique, which enables the determination of growth inhibition against selected microorganisms [41, 42]. The panel of test strains included *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 13709), *Lactobacillus fermentum* (N4), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 15442), and *Salmonella enterica*. Suitable culture media were employed depending on the microbial type: Mueller–Hinton Broth (MHB) and Mueller–Hinton Agar (MHA) for general bacteria, Tryptic Soy Broth (TSB) and Tryptic Soy Agar (TSA) for aerobic species.

Stock solutions of the samples were prepared in 100% DMSO and diluted with sterile distilled water to achieve serial concentrations (4–10 levels). Microbial suspensions were standardized to 5 × 10^{5} CFU/mL before inoculation. For the assay, 10 μL of each

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diluted sample was pipetted into wells of a 96-well microplate, followed by 190 µL of microbial suspension. Plates were incubated at 37°C for 16–24 h, and growth was monitored to determine the minimum inhibitory concentration (MIC), defined as the lowest sample concentration that completely suppressed visible microbial growth. In addition, the half-maximal inhibitory concentration (IC_{50}) was estimated from growth inhibition percentages across the tested range. Ampicillin and Cefotaxime served as positive controls for Gram-positive and Gram-negative bacteria, respectively.

4.6.6 | Molecular Docking Analysis

The molecular docking analysis was performed with AutoDock Vina 1.1.2 [43] to predict the binding modes of the major compounds identified from the essential oil of *P. obliqua* (Figure S3), such as spathulenol, khusinol, 14-oxy-α-muurolene, α-cadinol, humulene epoxide II, and globulol. The crystallographic structures of four target proteins, inducible nitric oxide synthase (iNOS, PDB ID: 3E7G) [44], cyclooxygenase-2 (COX-2, PDB ID: 5KIR) [45], *S. aureus* dihydrofolate reductase (DHFR, PDB ID: 2W9S) [46], and UDP-N-acetylenolpyruvylglucosamine reductase (MurB, PDB ID: 1HSK) [47], were obtained from the Protein Data Bank. Protein preparation was carried out in AutoDock Tools 1.5.7 [48] by removing crystallographic water molecules and co-crystallized ligands, followed by the addition of Kollman charges.

Ligand structures were drawn in ChemDraw 16.0, converted into 3D conformations, and subjected to energy minimization with Open Babel 3.1.1 [49] to ensure geometrical stability. After docking, the resulting complexes were examined using Discovery Studio 24.1 to identify key binding interactions, including hydrogen bonds and hydrophobic contacts, thereby providing molecular-level evidence for the observed binding affinities.

4.6.7 | Statistical Analysis

Means with standard deviation were reported for each measurement in three replicates. The Student's *t*-test with a significance level of $p < 0.05$ was used to statistically compare the data.

Author Contributions

Trang Huyen Xuan Hoanga: conceptualization, methodology, software, writing, reviewing, editing, and data analysis. **Tuan Quoc Doana**: conceptualization, methodology, software, writing, reviewing, editing, and data analysis. **Hoai Thi Nguyena**: conceptualization, methodology, software, writing, reviewing, editing, and data analysis. **The-Huan Trana**: conceptualization, methodology, software, writing, reviewing, editing, and data analysis. **Thuan Hai Hoanga**: conceptualization, methodology, software, writing, reviewing, editing, and data analysis. **Linh Thuy Thi Tran**: conceptualization, methodology, software, writing, reviewing, editing, and data analysis. **Dien Dinhc**: plant material collecting. **Trang Huyen Xuan Hoanga**: supervision, writing, reviewing, and editing. **Tuan Quoc Doana**: supervision, writing, reviewing, and editing. **Linh Thuy Thi Tran**: supervision, writing, reviewing, and editing.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Supporting File 1: cbdv71360-sup-0001-SuppMat.docx.

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