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Chemical Constituents, Biological Activities and Molecular Docking Studies of Root and Aerial Part Essential Oils from *Erigeron sublyratus* Roxb. ex DC. (Asteraceae)

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In this work, the volatile components of *Erigeron sublyratus* essential oils and their anti-inflammatory and cytotoxic activities were investigated for the first time. Gas chromatography-mass spectrometry (GC-MS) analysis identified 28 components in the root and aerial part essential oils. The main components included *cis*-lachnophyllum ester (53.4–64.2 %), germacrene D (5.6–8.6 %), *trans*-β-ocimene (2.6–7.5 %), β-caryophyllene (4.7–6.8 %), β-myrcene (2.0–6.3 %), and (*E*)-β-farnesene (4.8–5.0 %). The aerial part essential oil inhibited nitric oxide (NO) production on LPS-induced RAW 264.7 cells, with an IC_{50} value of 1.41 ± 0.10 μg/mL. In addition, both root and aerial part essential oils exhibited cytotoxic activity against MCF-7, SK-LU-1, and HepG2. Molecular docking simulation results revealed that (*E*)-β-farnesene strongly binds to the VEGFR-2 enzyme, while δ-cadinene has a high affinity to the COX-2 enzyme *via* hydrophobic interactions. These findings proposed that *E. sublyratus* essential oils can be exploited for their anti-inflammatory and anti-cytotoxicity potential.

Introduction

Asteraceae is one of the largest families of flowering plants in the world, with approximately 30000 species distributed throughout 1900 genera in 12 subfamilies.[1] Among them, the *Erigeron* L. (Asteraceae) comprises about 400 species, which is distributed mainly in Europe, mainland Asia, and North America, with a few species found in Africa and Oceania.[2,3] Traditional medicine has made extensive use of *Erigeron* species. *Erigeron* plants have been applied in folk medicine as a natural insecticide to repel fleas and treat headaches, rheumatism, gout, cystitis, nephritis, dysmenorrhea, and tooth discomfort.[4] This plant also showed antinociceptive and anti-inflammatory activities in rats and mice.[5]

Erigeron species are known for their essential oils with diverse biological activities. The essential oils of three Himalayan *Erigeron* species (*E. mucronatus*, *E. annuus* and *E. karwinskianus*) contain monoterpene hydrocarbons, sesquiterpene hydrocarbons, oxygenated sesquiterpene, and polyacetylenic esters compositions. These essential oils showed significant antifungal effects against the tested fungi with respective IC_{50} values ranging from 88.8 to 660.0 μg/mL.[6] The essential oil of *E. mucronatus*, including sesquiterpenoids (59.6 %), monoterpenoids (16.1 %), and polyacetylene esters (24.3 %), exhibited maximum anti-bacterial activity against both *Staphylococcus aureus* and *Escherichia coli* but moderate activity against *Pseudomonas aeruginosa*.[7] The essential oil of *E. acris* roots also showed antiproliferative activity against breast cancer MCF-7 cells with an IC_{50} value of 14.5 μg/mL.[8] The essential oil of *E. floribundus* aerial parts was dominated by spathulenol (12.2 %), caryophyllene oxide (12.4 %), and limonene (8.8 %) and exhibited strong cytotoxicity on HCT-116 colon carcinoma cells with an IC_{50} value of 14.89 μg/mL.[9] However, the anti-inflammatory activity of *Erigeron* species essential oils has not been as extensively studied as some bioactives.

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Erigeron sublyratus Roxb. ex DC. (Syn. *Aster benghalensis*, *Blumea sonbhadrensis*) is a species that grows up to 45 cm tall and is characterized as an annual erect aromatic plant with a terete stem and branches covered in whitish spreading hairs. Simple, alternating, pubescent leaves are present on both surfaces. Single inflorescence, either terminal or axillary cyme.[10] Up to now, the chemical compositions and bioactivities of the essential oils from this plant have not been reported. This study analyzed the volatile profile of the essential oils extracted from the *E. sublyratus* roots and aerial parts. Additionally, their potential anti-inflammatory and cytotoxic activities, as well as molecular docking simulations, were also evaluated.

Results and Discussion

Chemical Composition of the Essential Oils

The essential oil yields of the fresh roots and aerial parts of *E. sublyratus* were 0.09 and 0.12% (v/w, calculated based on the fresh weight of samples), respectively. The chemical constituents of these essential oils were identified using Gas chromatography-mass spectrometry (GC-MS) analysis (Figures 1 and 2).

A total of 28 volatile components (representing 98.6–99.1% of the total oil content) were characterized and shown in Table 1. The main components (≥5%) in the essential oil of *E. sublyratus* roots were *cis*-lachnophyllum ester (64.2%), germacrene D (5.6%), δ-cadinene (5.3%) and (*E*)-β-farnesene (5.0%), while the aerial part oil of *E. sublyratus* was found to contain *cis*-lachnophyllum ester (53.4%), followed by germacrene D (8.6%), *trans*-β-ocimene (7.5%), β-caryophyllene (6.8%) and β-myrcene (6.3%). Furthermore, several prominent compounds (≥2%) were also observed, including β-caryophyllene (4.7%), *trans*-β-ocimene (2.6%), β-elemene (2.6%) and β-myrcene (2.0%) in the root essential oil, while (*E*)-β-farnesene (4.8%), β-elemene (3.7%) and β-pinene (2.1%) were found in the aerial part essential oil. To the best of our knowledge, the chemical constituents of *E. sublyratus* have been reported here for the first time.

The chemical constituents of the essential oils of several *Erigeron* species (Asteraceae) were identified in the previous studies. For example, the compositions of the fresh aerial part essential oils of three Himalayan *Erigeron* species were described, with isomeric polyacetylenic esters viz., *trans*-2-*cis*-8-matricaria ester and *cis*-lachnophyllum ester (two major components), representing for 83.3% of *E. mucronatus*, 69.3% of *E. annuus* and 30.1% of *E. karwinskianus* oils.[6] In another study, germacrene D (47.2%) and *cis*-lachnophyllum ester (10.2%) were the major components of the flower oil of *E. annuus* from natural habitat in Poland. The main volatile compounds of the aerial parts of *E. multiradiatus* from Uttarakhand, India were analyzed and composed mainly of *trans*-2-*cis*-8-matricaria-ester (77.8%), *cis*-lachnophyllum ester (11.0%), and zingiberene (4.4%).[11] The essential oil of a mixture of *E. bonariensis* leaves and stems from Pakistan comprised *trans*-β-farnesene (10.2%), *cis*-lachnophyllum ester (24.9%), and matricaria ester (43.1%), whereas the main volatile components of *E. canadensis* were limonene (28.4%), *cis*-lachnophyllum ester (16.3%), and matricaria ester (31.7%).[12] By comparison of the present results with other previous studies, *cis*-lachnophyllum ester was found to predominate the high concentration in most

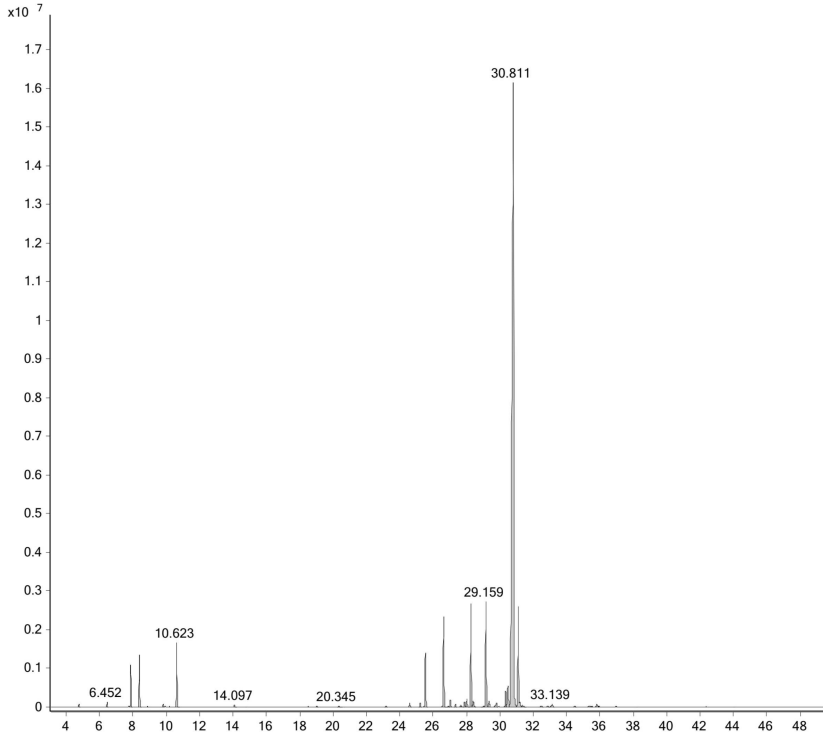

Figure 1. Gas chromatogram of the essential oil of *Erigeron sublyratus* roots.

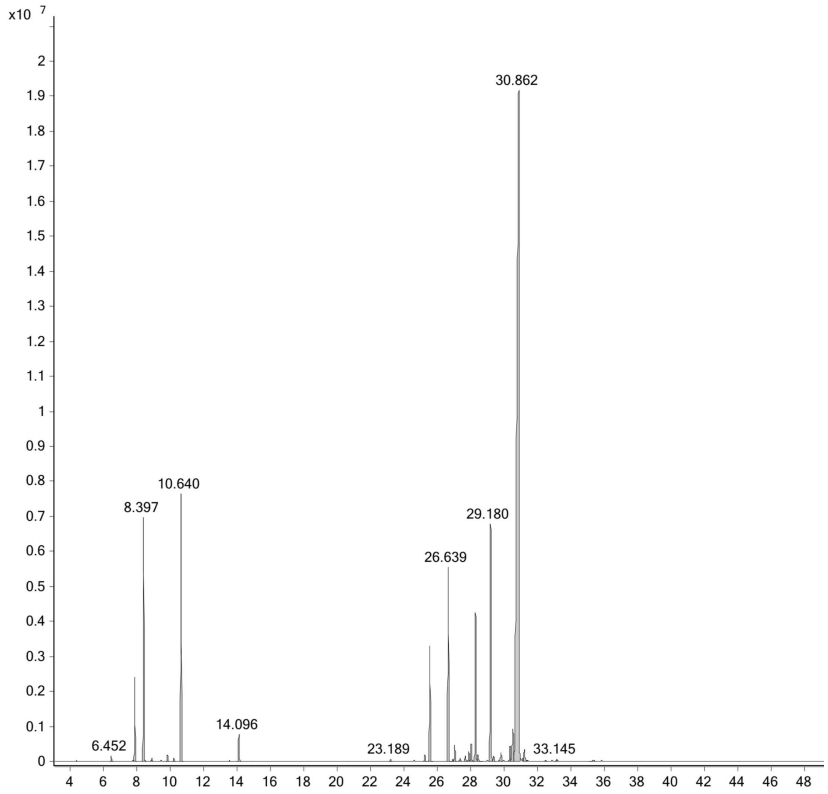

Figure 2. Gas chromatogram of the essential oil of *Erigeron sublyratus* aerial parts.

essential oils from the genus *Erigeron*. Perhaps, this component is the chemotaxonomic marker of *Erigeron* essential oils.

Biological Activities

Nitric oxide (NO) has a dual role in inflammation, acting as both pro-inflammatory and anti-inflammatory, depending on its level and where it is released. Normal levels of NO have anti-inflammatory effects, while high levels produced by iNOS during inflammation help protect blood vessels and reduce inflammation. NO decreases inflammation by stopping neutrophils and monocytes from sticking to blood vessel walls. Low NO levels from eNOS can promote inflammation, but high levels lower oxidative stress and inflammation. iNOS levels rise during inflammation, and drugs like glucocorticoids can help reduce this response. Additionally, NO triggers autophagy through the AMPK/mTOR pathway, which helps suppress inflammation.[13]

Researchers often measure the NO levels in LPS-activated RAW 264.7 macrophages to evaluate the anti-inflammatory potential of the samples. In the present work, we also investigated the effects of essential oils of *E. sublyratus* roots and aerial parts on NO production in LPS-stimulated RAW 264.7 macrophages. Our findings revealed that only the aerial part essential oil exhibited potent activity, demonstrating a significant inhibitory effect with an IC_{50} value of 1.41 ± 0.10 μg/mL, compared to the positive control dexamethasone, which had an IC_{50} value of 5.43 ± 0.54 μg/mL (Table 2). Furthermore, the aerial part essential oil showed no toxicity to macrophage cells, with 92.28% cell survival at a concentration of 4 μg/mL. In contrast, the anti-inflammatory activity of *E. sublyratus* root essential oil has not been determined due to its exhibited cytotoxic effects on macrophage cells, with only 66.07% cell survival at the same concentration. The anti-inflammatory effects of the essential oils may be related to their major compounds. It is reported that β-caryophyllene has exhibited anti-inflammatory properties by inhibiting the proliferation of inflammatory cells and modulating several intracellular pathways, including PI3 K/Akt, ERK/MAPK, and calcium homeostasis. It has reduced the expression of key proteins like Akt, MAPK, p38, and p44/42, while activating caspase-3 to induce apoptosis. Additionally, β-caryophyllene has decreased the activity of COX-1, COX-2, and NF-κB, effectively suppressing vascular inflammation and atherosclerosis. It has also reduced oxidative stress, enhanced the activity of antioxidant enzymes, and regulated nitric oxide (NO) levels, helping to prevent plaque formation and tissue damage.[14] On the other hand, β-myrcene has mitigated colon inflammation by inhibiting the MAP Kinase and NF-κB signaling pathways.[15] It has exhibited strong anti-inflammatory activity, particularly in protecting against gastric and duodenal ulcers by enhancing gastric mucosa defense factors.[16] At non-cytotoxic concentrations, β-myrcene has inhibited IL-1β-induced nitric oxide production and has also decreased IL-1β-induced activation of NF-κB, JNK, and p38, along with reducing the expression of inflammatory genes such as iNOS and catabolic genes like MMP-1 and MMP-13.[17]

The cytotoxic effects of the root and aerial part essential oils of *E. sublyratus* on MCF-7, SK-LU-1, and HepG2 cancer cell lines

Table 1. Chemical constituents of the essential oils from the roots and aerial parts of *Erigeron sublyratus* collected in Da Nang, Vietnam.

No.	RT	Components	Class	RI (Exp.)	RI (Lit.)	Percentage (%)	
						Roots	Aerial parts
1	6.452	α-Pinene	MH	937	937	0.2	0.1
2	7.865	β-Pinene	MH	979	979	1.6	2.1
3	8.386	β-Myrcene	MH	992	991	2.0	6.3
4	9.816	β-Terpinene	MH	1031	1028	0.1	0.2
5	10.211	*Cis*-β-ocimene	MH	1042	1038	-	0.1
6	10.623	*Trans*-β-ocimene	MH	1052	1049	2.6	7.5
7	14.096	Cosmene	MH	1132	1131	0.1	0.7
8	19.029	(*Z*)-Citral	OM	1243	1240	0.1	-
9	20.345	(*E*)-Citral	OM	1273	1270	0.1	-
10	23.183	δ-Elemene	SH	1338	1338	0.1	0.1
11	25.535	β-Elemene	SH	1392	1391	2.6	3.7
12	26.628	β-Caryophyllene	SH	1418	1419	4.7	6.8
13	27.028	Clovene	SH	1429	1425	0.2	0.5
14	27.343	α-Bergamotene	SH	1436	1435	0.2	0.1
15	27.658	Aromandendrene	SH	1442	1440	0.1	0.2
16	27.875	Isogermacrene D	SH	1450	1448	0.2	0.3
17	28.018	α-Humulene	SH	1453	1454	0.4	0.6
18	28.264	(*E*)-β-Farnesene	SH	1459	1457	5.0	4.8
19	29.159	Germacrene D	SH	1480	1481	5.6	8.6
20	29.346	β-Eudesmene	SH	1485	1486	0.3	0.2
21	29.798	Valencene	SH	1495	1492	0.2	0.6
22	30.336	α-Bisabolene	SH	1509	1504	0.8	0.5
23	30.479	β-Bisabolene	SH	1513	1509	1.4	1.6
24	30.811	*Cis*-lachnophyllum ester	PE	1522	1516–1527	64.2	53.4
25	31.097	δ-Cadinene	SH	1529	1524	5.3	–
26	33.139	Caryophyllene oxide	OS	1582	1581	0.2	0.1
27	35.337	Caryophylladienol II	OS	1640	1637	0.1	–
28	35.823	α-Cadinol	OS	1654	1653	0.2	–
		Total (%)				**98.6**	**99.1**

Note. RT: Retention time (min); RI (Exp.): Retention indices on HP-5MS UI column; RI (Lit.): Retention indices from the literature; MH: Monoterpene hydrocarbon; OM: Oxygenated monoterpene; SH: Sesquiterpene hydrocarbon; PE: Polyacetylenic ester; OS: Oxygenated sesquiterpene.

Table 2. NO production inhibitory activity of the essential oils from the roots and aerial parts of *Erigeron sublyratus.*

Conc. (μg/mL)	REO		APEO		Dexamethasone	
	% I	% CS	% I	% CS	% I	% CS
100	97.92 ± 1.02	5.35 ± 0.39	99.04 ± 1.74	5.74 ± 0.21	88.02 ± 2.34	93.53 ± 2.23
20	91.56 ± 2.40	14.43 ± 1.09	87.10 ± 3.30	37.54 ± 3.29	53.19 ± 1.02	100.31 ± 1.51
4	75.56 ± 2.91	66.07 ± 1.79	63.19 ± 1.23	92.28 ± 1.68	42.21 ± 0.91	–
0.8	32.00 ± 1.41	87.23 ± 2.40	37.06 ± 1.69	–	32.66 ± 0.82	–
0.16	–	–	5.06 ± 0.53	–	–	–
IC_{50}	**NA**		**1.41 ± 0.10**		**13.85 ± 1.39 (μM) = 5.43 ± 0.54 (μg/mL)**	

Note. Data are shown as mean ± standard deviation (n = 3); Conc.: Concentration; REO: Root essential oil; APEO: Aerial part essential oil; Dexamethasone: Positive control; % I: % inhibition; % CS: % cell survival; IC_{50}: Half-maximal inhibitory concentration; NA: Not available.

were evaluated. The results in Table 3 demonstrate that these essential oils exhibited strong activity with $IC_{50} < 2$ μg/mL on all evaluated cell lines. Specifically, the IC_{50} values of the root essential oil of *E. sublyratus* for MCF-7, SK-LU-1, and HepG2 cell lines were 1.32 ± 0.09, 1.70 ± 0.05, and 1.27 ± 0.04 μg/mL, respectively, while the figures for the aerial part essential oil were 1.18 ± 0.05, 1.25 ± 0.04, and 1.11 ± 0.04 μg/mL, respectively. The cytotoxic activity of both essential oils may be due to the presence of main components. Previous research has suggested that δ-cadinene inhibited the growth of ovarian cancer cells through caspase-dependent apoptosis and cell cycle arrest.[18] Similarly, β-caryophyllene inhibited the proliferation of various cancer cell lines, including colon (HT-29, HCT-116), pancreatic (PANC-1), and breast cancer (MCF-7). It enhanced the effects of other anticancer agents like isocaryophyllene and α-humulene by inducing apoptosis through ROS production, loss of mitochondrial membrane potential, increased Bax expression, and decreased Bcl-2 expression. It also showed synergistic activity with components from essential oils, stopping the cell cycle at G0/G1 or sub-G1 phases, and reducing precancerous risks in obese mouse models.[14] Additionally, β-myrcene strongly inhibited tumor necrosis factor-α (TNFα)-induced nuclear factor κB (NF-κB) activity. It suppressed TNFα-induced phosphorylation of inhibitor of κB kinase, NF-κB, and matrix metalloproteinase-9 (MMP-9) gene expression, while also inhibiting the invasion of MDA-MB-231 breast cancer cells induced by TNFα.[19]

To date, there is no research on the essential oil of *E. sublyratus*, but various species within the *Erigeron* genus have been explored for their potential anti-cancer properties. A previous study found that the aerial part essential oil of *E. canadensis* had cytotoxic effects on HaCaT cells, with an IC_{50} of 0.027 μg/mL.[20] Similarly, the essential oil of *E. acris* roots showed significant antiproliferative activity against the MCF-7 cell line, with an IC_{50} value of 14.5 μg/mL.[8] Additionally, the essential oil of *E. floribundus* aerial parts exhibited strong cytotoxicity against HCT-116 colon carcinoma cells, with an IC_{50} value of 14.89 μg/mL.[9] Likewise, the essential oil from the above-ground parts of *E. bonariensis* demonstrated potent cytotoxicity against HepG2 cells, with an IC_{50} of 25.6 μg/mL.[21] Moreover, there are indications that the essential oils from the upper region of *E. canadensis* may have the potential to inhibit the growth of cervical cancer cells.[22]

Table 3. Cytotoxic activity of the essential oils from the roots and aerial parts of *Erigeron sublyratus*.

Samples	Half-maximal inhibitory concentration (IC_{50}, μg/mL)		
	MCF-7	SK-LU-1	HepG2
REO	1.32 ± 0.09	1.70 ± 0.05	1.27 ± 0.04
APEO	1.18 ± 0.05	1.25 ± 0.04	1.11 ± 0.04
Ellipticine	0.40 ± 0.03	0.50 ± 0.03	0.33 ± 0.02

Note. Data are shown as mean ± standard deviation (n = 3); REO: Root essential oil; APEO: Aerial part essential oil; MCF-7: Human breast carcinoma; SK-LU-1: Human lung carcinoma; HepG2: Human hepatocellular carcinoma; Ellipticine: Positive control.

Molecular Docking Approach

In this section, under the assumption that the main components may play a pivotal role in the anti-inflammatory and anticancer activities of *E. sublyratus* essential oils, molecular docking studies were conducted to elucidate the molecular mechanisms of action as well as the binding modes of the identified compounds. VEGFR-2 is known as a receptor for vascular endothelial growth factor (VEGF), which is a major regulator of tumor angiogenesis and overexpression observed in various cancer cells, while COX-2 represents a therapeutic target for inflammation-related diseases.[23,24] Therefore, these target proteins were selected for docking simulation in the current research.

To validate the protocol before docking, co-crystallized ligands were extracted from the crystal structures of the VEGFR-2 and COX-2 enzymes. Subsequently, the docking protocol was validated by re-docking these co-crystallized ligands into the respective active sites of the corresponding proteins (Figure S1). The root mean square deviation (RMSD) between the crystallographic pose and the docked pose was calculated, resulting in 0.327895 Å and 0.902459 Å for co-crystallized ligands within the VEGFR-2 and COX-2 original complexes, respectively, all of which were less than 2 Å, indicating the good predictive capability of the docking protocol. Next, the selected main compounds were evaluated for their interaction ability and binding affinity on two targets, VEGFR-2 and COX-2 (Figures S2, S3 and S4). The co-crystallized compounds methyl (5-{4-[({[2-fluoro-5-(trifluoromethyl)phenyl]amino}carbonyl)amino]phenoxy}-1H-benzimidazol-2-yl)carbamate (GIG) and rofecoxib were considered as reference compounds to compare the interaction modes with the selected compounds. As described in Figure S2, all major components in the essential oils achieved negative binding affinities with both target enzymes, indicating potential favorable interaction capabilities. Among the 7 tested compounds, (*E*)-β-farnesene exhibited the best affinity to interact with the VEGFR-2 enzyme, achieving a docking score of −7.295 kcal/mol. Meanwhile, δ-cadinene showed the strongest binding affinity with a value of −8.047 kcal/mol for the COX-2 enzyme. Additionally, the compounds including β-myrcene, *trans*-β-ocimene, β-caryophyllene, germacrene D, and *cis*-lachnophyllum ester displayed significant binding affinities with both protein targets ranging from −5.081 to −6.974 kcal/mol. It can be observed that their biological effects stem from the collective contribution of these major compounds.

From the analysis of the interaction between receptors and observed ligands in Figures S3 and S4, the main interactions are hydrophobic interactions due to the inherently hydrophobic nature of the separated oil formed entirely from hydrocarbon compounds. The reference compounds demonstrate hydrophobic interactions at amino acid residues Val897, Leu887, Glu915, Phe1045, Val914, Leu1033, Leu838, Phe916, Lys918, Gly920, Ala864, Cys1043, Val846. Some of these amino acid residues of the VEGFR-2 enzyme also form with the studied compounds, indicating significant inhibition of the enzyme with contributions when interacting with this active region (Fig-

ure S3). Regarding the COX-2 enzyme, the reference compound rofecoxib was indicated to interact primarily at the amino acid residues Ala516, His90, Ile517, Gln192, Phe518, Leu352, Tyr387, Met522, Gly526, Ser530, Val349, Leu531, Ala527, Ser353, Val523, Tyr355, Ala516. Most of the studied compounds also interact with these amino acid residues except for the compound β-caryophyllene (Figure S4).

According to a previous report, β-myrcene has been demonstrated to alleviate colitis conditions by inhibiting mitogen-activated protein kinase (MAPK) and nuclear factor-kappa B (NF-κB) pathways.[25] Meanwhile, δ-cadinene, identified as a major compound from the essential oil of *Zingiber* species, is predicted to possess potent anti-inflammatory activity with increased binding affinity to COX-2 and favorable physicochemical properties.[26] This compound also demonstrates a binding energy of −5.92 kcal/mol to the serine/threonine-protein kinase (MST3) target associated with anti-proliferative activity.[27] Another configuration of (*E*)-β-farnesene, known as *cis*-β-farnesene (= (*Z*)-β-farnesene), has also been reported to show promising dual affinity when docked with two proteins, COX-2 and TNF-α, with binding energies of −9.4392 kcal/mol and −5.9222 kcal/mol, respectively.[28] These findings contribute to a better understanding of the potential effects of the major components of *E. sublyratus* essential oils and their interaction mechanisms with two protein targets related to anti-inflammatory and anticancer activities.

Conclusions

In conclusion, a total of 28 components were identified in the root and aerial part essential oils of *E. sublyratus*. Principal components, *cis*-lachnophyllum ester, followed by germacrene D, *trans*-β-ocimene, β-caryophyllene, β-myrcene, and (*E*)-β-farnesene were indicated. The essential oil from *E. sublyratus* aerial parts significantly inhibited NO production compared to the positive control, while the essential oils from both roots and aerial parts of *E. sublyratus* exhibited cytotoxic activity against three cancer cell lines MCF-7, SK-LU-1, and HepG2. (*E*)-β-Farnesene exhibited the strongest binding energy among the studied compounds with the VEGFR-2 enzyme, while δ-cadinene demonstrated the strongest affinity towards the COX-2 enzyme obtained from molecular docking results. This is the first report on the essential oils of *E. sublyratus* roots and aerial parts. These new findings provide additional insights and guidance for developing future anti-cancer and anti-inflammatory drug products.

Experimental Section

Materials

Fresh roots and aerial parts of *Erigeron sublyratus* Roxb. ex DC. were collected in the morning from Da Nang, Vietnam (15°58′8.22″ N, 108°13′59.15″ E) on 20th March 2023, and identified by Assoc. Prof. Dr. Nguyen Hoang Tuan (a botanist from Hanoi University of Pharmacy). A voucher specimen (No. TTH.ES 20.03.23) has been deposited at the Department of Pharmacy, Da Nang University of Medical Technology and Pharmacy, Vietnam.

Extraction of the Essential Oils

The fresh roots and aerial parts of *E. sublyratus* were cleaned, cut into small pieces, and subjected to hydrodistillation for 3.5 h using Clevenger-type apparatus (at 100 °C using an electric stove) as previously described with slight modifications.[29–31] The extracted essential oils were dried over anhydrous sodium sulfate to remove any trace of water, collected in 1.5 mL vials, and stored under refrigeration until analysis. All experiments were conducted in triplicate.

Gas Chromatography–Mass Spectrometry (GC-MS) Analysis

The analysis of volatile constituents of *E. sublyratus* essential oils was done using an Agilent Technologies 7890B GC System fitted with an HP-5MS UI column (30 m ×0.25 mm, 0.25 μm film thickness), and coupled with an Agilent 5977B MSD model. In brief, one μL of each essential oil solution (diluted with dichloromethane, Merck) was injected with a split ratio of 25:1. The carrier gas was helium with a flow rate of 1.0 mL/min (pressure 8.23 psi). The temperature of Inlet-F, Aux-2, MS Source, and MS Quad, Inlet-F was set at 300 °C, 250 °C, 230 °C, and 150 °C, respectively. The oven temperature was programmed from 60 °C (hold 3 min) to 180 °C at a rate of 3 °C/min, then increased at a rate of 5 °C/min to 240 °C (hold 5 min). The GC-MS data was captured with an ionization voltage of 70 eV, mass range of 50–550 amu at 2.0 scan/s. A homologous series of *n*-hydrocarbons (C_7-C_{30}, Merck) was used to calculate retention indices (RIs) under identical conditions. The chemical compositions of the *E. sublyratus* essential oils were confirmed based on comparing their RIs and mass spectra with those of standard components available from the literature.[11,12,32] The relative peak area percentage was used for quantification.

Measurement of Nitric Oxide (NO) Production

The inhibitory effect of *E. sublyratus* essential oils on NO production in lipopolysaccharide (LPS)-stimulated RAW 264.7 cells was evaluated as previously described.[33,34] Briefly, cells were seeded in 96-well plates, incubated for 24 hours, then treated with various sample concentrations for 2 hours before LPS stimulation for another 24 hours. Nitrite levels were measured using the NO kit (Griess Reagent System, Promega, WI, USA). The experiment was conducted in triplicate, and half-maximum inhibitory concentration (IC_{50}) values were calculated using Table Curve Version 4.0 (Systat Software Inc., San Jose, CA, USA).

MTT Cell Viability Assay

The essential oil samples were added to a 96-well plate containing RAW 264.7 cells at the same concentrations as in the NO experiment. After 24 hours, MTT solution (5 mg/mL) was added and incubated for another 24 hours. Formazan crystals were dissolved in 50 μL DMSO, and absorbance was measured at 540 nm. Cell viability was calculated relative to the blank control.[33,34]

Cytotoxicity Assay

The cytotoxic effects of *E. sublyratus* essential oils on MCF-7 (human breast carcinoma), SK-LU-1 (human lung carcinoma), and HepG2 (human hepatocellular carcinoma) cell lines were evaluated using the Sulforhodamine B (SRB) assay following the same reported

method.[35] In summary, cells were cultured in 96-well plates, treated with test samples in 10% dimethyl sulfoxide (DMSO), and incubated. After fixation with trichloroacetic acid and staining with SRB, optical density (OD) was measured at 540 nm, with ellipticine used as the positive control. Cell growth inhibition was calculated using the formula: (%) inhibition = 100% − [(OD_{sample} − $OD_{day\ 0}$)/($OD_{blank\ control}$ − $OD_{day\ 0}$)] ×100. Experiments were conducted in triplicate, and data analysis for IC_{50} calculation was performed using TableCurve 2Dv4 software.

Statistical Analysis

Experiments were performed in triplicate. Data are presented as the mean ± standard deviation (Microsoft Excel, Microsoft, 2018). The IC_{50} was calculated using TableCurve 2Dv4 software.

Molecular Docking Study

The AutoDock Vina v1.2.3 program is one of the docking programs known for its rapid computation and high reliability, widely used in current research endeavors.[36,37] The chemical structures of the main compounds β-myrcene, *trans*-β-ocimene, β-caryophyllene, (*E*)-β-farnesene, germacrene D, *cis*-lachnophyllum ester, and δ-cadinene were drawn using ChemSketch software and energy-minimized using the MMFF94s force field.[38] Crystal structures of Vascular endothelial growth factor receptor-2 (VEGFR-2) and cyclooxygenase-2 (COX-2) were retrieved from the RCSB Protein Data Bank with corresponding PDB IDs 2OH4 and 5KIR, respectively.[39,40] All target proteins, main compounds in the essential oil, and co-crystallized ligands were prepared as per prior studies. Docking simulation parameters, including grid center coordinates based on the co-crystallized ligands within the active site regions of VEGFR-2) (x = 3.172 Å, y = 33.766 Å, and z = 17.175 Å) and COX-2 (x = 23.3 Å, y = 0.4 Å, and z = 34.4 Å) with a grid size of 24×24×24 Å, were set to ensure full coverage. The exhaustiveness value was adjusted to 400, while other parameters remained unchanged. Upon completion of the docking simulation, the top-ranked docked poses were identified for each ligand. LigPlot+ v2.2 software was utilized to visualize the interactions between the best-posed ligands and amino acid residues within the active sites of VEGFR-2 and COX-2 enzymes.[41]

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Author Contributions

N.T.G.A., L.D.G. conceived and designed the experiments, H.T.T., D.X.D. performed the experiments, N.T.T., N.T.T.H. analyzed the data, D.K.N. prepared the original draft, N.X.H. calculated docking, V.S.V. collected the plant sample, L.D.G. edited the manuscript. All authors have read and approved the finalized manuscript.

Conflict of Interests

The authors declared no conflict 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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