

# Essential Oils of the Leaf and Stem of *Polyalthia viridis* Craib and Their Biological Activities

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## Abstract

Plants of the genus *Polyalthia* can be seen as a rich resource of essential oils type terpenoids. In this study, the essential oils obtained by hydro-distillation from the leaf and stem of *Polyalthia viridis* were analysed by gas chromatography/mass spectrometry–flame ionization detection. Thirty-nine constituents (95.3%) were identified in the leaf oils and 42 constituents (90.9%) in the stem oils. Sesquiterpene hydrocarbons and oxygenated sesquiterpenes were the main constituents of both oils, in which 2 sesquiterpene hydrocarbons, germacrene D (45.1%–47.4%) and bicyclogermacrene (8.2%–17.1%), were the 2 major compounds. The stem oils inhibited the growth of 3 cancer cell lines, HepG2, MCF7, and A549, with half inhibitory concentration (IC<sub>50</sub>) values of 56.7 to 68.4 µg/mL. The stem oils also successfully suppressed the growth of the filamentous fungus *Aspergillus niger* and the yeast *Candida albicans* with minimum inhibitory concentration values of 50 µg/mL. *P. viridis* oils suppressed NO production in lipopolysaccharide-stimulated BV2 microglial cells, with IC<sub>50</sub> values of 57.6 to 76.7 µg/mL.

## Keywords

*Polyalthia viridis*, leaf, stem, essential oils, cytotoxicity, antimicrobial, anti-inflammatory

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## Introduction

*Polyalthia* is a genus of flowering plants in the family Annonaceae. There are >70 species, distributed in Africa, Madagascar, and Asia.<sup>1</sup> Because of the high percentages of alkaloid and diterpenoid derivatives, *Polyalthia* plants have been used as folk medicines in each country.<sup>2</sup> One of the most important plants in this genus is *Polyalthia longifolia*, because it has been used to treat headache, fever, infection, skin disease, and to reduce blood pressure.<sup>2</sup>

*Polyalthia* plants are also of interest due to their essential oils (EOs), with oxygenated monoterpenes and oxygenated sesquiterpenes as major components.<sup>3–8</sup> The EOs of Nigerian *P. longifolia* contained 57 constituents (86.1%), with the main components  $\alpha$ -zingiberene (9.4%),  $\alpha$ -humulene (8.5%), and  $\alpha$ -selinene (2.6%).<sup>9</sup>  $\beta$ -Caryophyllene reached the highest percentage of 16.0% in the stem bark EO of Cameroonian *Polyalthia suaveolens*.<sup>10</sup> Monoterpene hydrocarbons were rich in the leaf EO of Indian *Polyalthia korintii*, in which 2 main isomeric monoterpene hydrocarbons,  $\alpha$ -pinene and  $\beta$ -pinene, accounted for 43.2% and 25.5%, respectively.<sup>11</sup>

The EOs of *Polyalthia* species have displayed valuable pharmacological activities. The oil of Nigerian *P. longifolia* seed showed potential for antioxidative, anti-inflammatory, antiparasitic and cytotoxic activities,<sup>12</sup> whereas the seed oil of this plant

collecting from India showed an MBC/MFC value of 39.0 µg/mL against 2 bacterial species, *Streptococcus mutans* and *Staphylococcus aureus*.<sup>13</sup> In the DPPH radical scavenging assay, the bark EO of *P. suaveolens* had an SC<sub>50</sub> value of 0.71 µg/mL, which was better than that of the bark EO of *Enantia chlorantha* (SC<sub>50</sub> 8.0 µg/mL).<sup>10</sup>

According to The Plant List, *Polyalthia viridis* Craib is the accepted name of this species, with a high confidence level.<sup>14</sup> Several clerodane derivatives have been isolated from this plant,<sup>15–18</sup> but little pharmacology has been documented. Ichino et al<sup>18</sup> reported that the 80% EtOH extract of *P. viridis*

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and its major component, 16-hydroxycyclohexa-3,13(14)Z-dien-15,16-olide, showed antimalarial activity against *Plasmodium falciparum*, with half inhibitory concentration (IC<sub>50</sub>) values of 10.0 and 3.6 µg/mL, respectively. Here, we report for the first time the chemical composition of the EOs of *P. viridis* leaf and stem, collected in Vietnam. These oils were also subjected to cytotoxic, antimicrobial, and anti-inflammatory assays.

## Materials and Methods

### Materials

The fresh leaves and stems of *P. viridis* were collected from Camlo, Quangtri, Vietnam in November, 2020, around 16°46'59.2"N and 106°51'11.5"E. The plant materials were identified by Professor Vu Tien Chinh, Vietnam National Museum of Nature, Vietnam Academy of Science and Technology (VAST). The voucher specimens (PVL-88 [leaf] and PVS-88 [stem]) were deposited at the Department of Applied Biotechnology, Institute of Chemistry, VAST.

Three cancer cell lines HepG2 (hepatocellular carcinoma), MCF-7 (human breast adenocarcinoma), and A-549 (human lung adenocarcinoma epithelial) were supplied from American Type Culture Collection (ATCC). They were maintained at 37 °C in 5% CO<sub>2</sub> in suitable media (RPMI 1640, MEM, DMEM; Merck KGaA) containing 10% fetal bovine serum (FBS), penicillin (100 UI/mL), streptomycin (100 mg/mL), and L-glutamine (2 mM).

Eight microorganisms, comprising 2 Gram (+) bacteria, *Bacillus subtilis* ATCC 27212 and *S. aureus* ATCC 12222, 2 Gram (-) bacteria, *Escherichia coli* ATCC 8739 and *Pseudomonas aeruginosa* ATCC 25923, 2 filamentous fungi, *Aspergillus niger* ATCC 9763 and *Fusarium oxysporum* ATCC 48112, and 2 yeasts, *Candida albicans* ATCC 10231 and *Saccharomyces cerevisiae* ATCC9763, were used for antimicrobial assays. They were purchased from the ATCC.

The materials for the anti-inflammatory assay, comprising BV2 microglial cells, lipopolysaccharide (LPS), Griess reagent, butein, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), were also purchased from ATCC.

### Extraction of Volatile Compounds

The fresh leaves (4.0 kg) were cleaned, cut into pieces, and immediately subjected to a Clevenger-type apparatus for 3 h to produce a yellowish and pungent-smelling oil in 0.04% yield (w/w), which was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> before analysis. The same procedure was applied to the fresh stems (1.5 kg) to afford a yellowish and pungent oil in 0.05% (v/v) yield.

### Gas Chromatography–Mass Spectrometry Procedure

The EOs of the leaf and stem of *P. viridis* were analysed by gas chromatography/mass spectrometry–flame ionization

detection (GC/MS-FID). The GC–MS analysis was carried out using an Agilent Technologies 7890A GC coupled with a mass spectrum detector (MSD) (Agilent Technologies 5975C) and HP-5 MS column. The column dimensions were 60 m × 0.25 mm, and film thickness was 0.25 µm. The injector was maintained at 250 °C. The temperature program was 60 °C, then a ramp of 4 °C/min up to 240 °C. Helium was used as the carrier gas with a flow rate of 1 mL/min. The split ratio was 100:1 and 1 mL of oil was injected. The MSD was based on full scan modes under an electron impact ionization voltage of 70 eV, emission current 40 mA, and an acquisitions scan mass range of 35 to 450 amu. The GC–FID analytical procedure was performed under the same conditions as those for the GC–MS method. Compound identification of the EOs was performed by comparing their retention index (RI) and MS data with those from HPCH1607 and W09N08 libraries, and the NIST standard database. The relative percentage amount of each volatile component was calculated based on the GC–FID peak area, without any correction factor.

### Cytotoxic Assay

Cytotoxic examination of the 2 samples was carried out using the MTT assay.<sup>19</sup> Briefly, the cells were diluted in a 96-well microplate (5 × 10<sup>4</sup> cells per well of 200 µL mixture). The samples (1–100 µg/mL) and the positive control, doxorubicin (0.05–1.56 µg/mL), were added to the cells and incubated at 37 °C for 48 h with 5% CO<sub>2</sub>. MTT (20 µL) was added to the wells and incubation was continued at 37 °C for 4 h. Absorbance was recorded at 540/720 nm using a Spark multi-mode reader (Tecan). Each experiment was repeated in triplicate.

Inhibitory percentage (%) =  $([1 - \text{OD}_{\text{sampl}}/\text{OD}_{\text{con}}]) \times 100\%$ , where OD<sub>sampl</sub> and OD<sub>con</sub> stand for the optical densities of the samples and the control, respectively.

### Antimicrobial Activity

The antimicrobial activity test has been described in previous publications.<sup>20–23</sup> The Gram (+) and Gram (-) bacteria were cultured in tryptic soy broth (Merck KGaA) while the fungi were grown in Saboraud-2% dextrose broth (Merck) to a final inoculum size of about 150 × 10<sup>6</sup> colony-forming units per mL (or 0.5 McFarland standard at λ of 550 nm). Two samples at various concentrations ranging from 12.5 to 200 µg/mL were loaded into 96-well microplates containing fresh cultures and were incubated at 37 °C for 24 h. The minimum inhibitory concentration (MIC) value is determined as the lowest sample concentration which inhibited the visible microorganism growth after 24 h treatment. Streptomycin and tetracyclin were used as the positive controls for the Gram (+) and Gram (-) bacteria, respectively, whereas nystatin was used for fungi and yeasts. The negative control was 5% DMSO only. Each experiment was repeated in triplicate.

### Anti-inflammatory Activity

The antiinflammatory activity of *P. viridis* EOs was determined by their inhibitory effects on NO production in the LPS-stimulated BV2 cell line.<sup>24</sup> Briefly, BV2 microglial cells were cultured in DMEM (5% FBS, and 1% penicillin–streptomycin). The cells ( $4 \times 10^4$  cells/each well) were loaded into a 96-well microplate and incubated at 37 °C in 5% CO<sub>2</sub> and 95% air. After a full day of incubation, the cells were treated either with or without the EOs (0–100 µg/mL), then stimulated with LPS (100 ng/mL) for 30 min. After an additional day of incubation, the cell culture medium (50 µL) was mixed with Griess reagent (50 µL) for 10 min. Absorbance was measured at 540 nm on a Spark multimode reader. The rate of accumulated nitrite in the medium was determined from a standard curve in which the absorption coefficient was calibrated from a NaNO<sub>2</sub> solution standard; butein was used as the positive control. Cell viability was measured by the MTT-based colorimetric assay.<sup>24</sup> Each experiment was repeated in triplicate.

### Results and Discussion

The leaf EOs of *P. viridis* were yellowish and pungent. As shown in Table 1, 39 constituents (95.3%) were determined. The characteristic features of the oil were the presence of monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, and oxygenated sesquiterpenes. Sesquiterpene hydrocarbons formed 89.8%, while 4.52%, 0.77%, and 0.19% were assigned to oxygenated sesquiterpenes, monoterpene hydrocarbons, and oxygenated monoterpenes, respectively. The major components were 2 sesquiterpene derivatives, germacrene D (45.1%) and bicyclgermacrene (17.1%). Nine components reached amounts of >1.0%, including 8 sesquiterpenes,  $\delta$ -elemene (5.0%), (*E*)-caryophyllene (3.8%),  $\delta$ -cadinene (3.4%),  $\beta$ -selinene (3.1%),  $\beta$ -elemene (1.9%),  $\gamma$ -muurolene (1.8%),  $\alpha$ -humulene (1.2%), and  $\gamma$ -amorphene (1.0%), and an oxygenated sesquiterpene, muurola-4,10(14)-dien-1-ol (1.3%).

Forty-two constituents were identified in the stem EOs of *P. viridis*, which accounted for 90.8%. Sesquiterpene hydrocarbons in the stem EOs were lower in amount than in the leaf EOs, but monoterpene hydrocarbons, oxygenated monoterpenes, and oxygenated sesquiterpenes were in higher amounts. The major sesquiterpene hydrocarbon in the stem EOs, germacrene D, was in greater amount (2.3%) than in the leaf EOs but bicyclgermacrene was in lower amount than that in the leaf EOs (8.9%) (Table 1). Similarly, the percentages of several components in the stem EOs, such as the sesquiterpene hydrocarbons  $\delta$ -elemene,  $\beta$ -elemene, and (*E*)-caryophyllene, were decreased. In contrast to the low percentages in the leaf EOs, the oxygenated sesquiterpenes  $\alpha$ -*epi*-cadinol and  $\alpha$ -cadinol in the stem EOs achieved percentages of 1.6% and 3.6%, respectively.

Three sesquiterpene hydrocarbons,  $\beta$ -bourbonene (0.3%),  $\alpha$ -gurjunene (0.1%), and *trans*-cadin-1(6),4-diene (0.2%), and 1 oxygenated sesquiterpene muurola-4,10(14)-dien-1-ol

**Table 1.** Chemical Composition of the Leaf and Stem EOs from *Polyalthia viridis*.

No	<sup>a</sup> RI <sub>E</sub>	<sup>b</sup> RI <sub>L</sub>	Constituents	Leaf (%)	Stem (%)
1	938	939	$\alpha$ -Pinene	0.11	0.19
2	954	954	Camphene	0.26	0.45
3	991	991	Myrcene	0.12	0.22
4	1048	1050	( <i>E</i> )- $\beta$ -ocimene	0.28	0.31
5	1100	1097	Linalool	0.19	0.66
6	1196	1189	$\alpha$ -Terpineol	—	0.12
7	1203	1196	Methyl chavicol	—	0.11
8	1237	1239	Isobornyl formate	—	0.19
9	1244	1238	Neral	—	0.15
10	1273	1267	Geranial	—	0.27
11	1347	1338	$\delta$ -Elemene	5.02	2.91
12	1359	1351	$\alpha$ -Cubebene	0.18	0.24
13	1383	1375	$\alpha$ -Ylangene	0.30	0.17
14	1388	1377	$\alpha$ -Copaene	0.69	0.39
15	1398	1388	$\beta$ -Bourbonene	0.32	—
16	1402	1391	$\beta$ -Elemene	1.94	1.54
17	1424	1410	$\alpha$ -Gurjunene	0.14	—
18	1434	1432	$\beta$ -Copaene	0.38	0.38
19	1436	1419	( <i>E</i> )-Caryophyllene	3.83	2.38
20	1444	1434	$\beta$ -Gurjunene	0.84	0.75
21	1455	1441	Aromadendrene	0.57	0.38
22	1459	1450	<i>Cis</i> -Muurola-3,5-diene	0.13	0.11
23	1470	1455	$\alpha$ -Humulene	1.23	1.05
24	1477	1466	9- <i>Epi</i> -( <i>E</i> )-caryophyllene	—	0.71
25	1478	1477	<i>Trans</i> -cadin-1(6),4-diene	0.18	—
26	1491	1480	$\gamma$ -Muurolene	1.83	2.02
27	1500	1485	Germacrene D	45.12	47.41
28	1504	1490	$\beta$ -Selinene	3.11	0.57
29	1509	1496	$\gamma$ -Amorphene	1.01	1.54
30	1514	1500	Bicyclgermacrene	17.10	8.22
31	1520	1512	$\delta$ -Amorphene	0.53	0.53
32	1528	1514	$\gamma$ -Cadinene	0.77	0.98
33	1536	1523	$\delta$ -Cadinene	3.36	3.35
34	1546	1535	<i>Trans</i> -cadin-1,4-diene	0.13	0.25
35	1551	1539	$\alpha$ -Cadinene	0.13	0.28
36	1558	1546	$\alpha$ -Calacorene	0.26	0.12
37	1576	1561	Germacrene B	0.70	0.61
38	1589	1572	Caryophyllenyl alcohol	0.28	0.65
39	1595	1578	Spathulenol	0.76	0.87
40	1643	1615	Muurola-4,10(14)-dien-1-ol	1.34	—
41	1644	1629	1- <i>Epi</i> -cubenol	—	1.37
42	1656	1640	$\alpha$ - <i>Epi</i> -cadinol	0.40	1.64
43	1657	1642	$\alpha$ - <i>Epi</i> -Muurolol	0.40	1.63
44	1661	1646	$\alpha$ -Muurolol	0.22	1.00
45	1670	1654	$\alpha$ -Cadinol	0.62	3.62
46	1674	1660	<i>Neo</i> -intermedeol	0.50	0.50
			Total	95.26	90.83
			Monoterpene hydrocarbons	0.77	1.17
			Oxygenated monoterpenes	0.19	1.5
			Sesquiterpene hydrocarbons	89.78	76.88
			Oxygenated sesquiterpenes	4.52	11.28

Abbreviations: EO, essential oil; RI, retention indices; MS, mass spectrometry; NIST, National Institute of Standards and Technology.

<sup>a</sup>RIs relative to *n*-alkanes (C<sub>7</sub>–C<sub>30</sub>) on the HP-5 MS column.

<sup>b</sup>RIs from NIST standard database.

(1.3%), were only found in the leaf EOs, whereas 5 oxygenated monoterpenes  $\alpha$ -terpineol (0.1%), methyl chavicol (0.1%), isobornyl formate (0.2%), neral (0.1%), and geranial (0.3%), 1 sesquiterpene hydrocarbon, 9-*epi*-(*E*)-caryophyllene (0.7%), and 1 oxygenated sesquiterpene 1-*epi*-cubenol (1.4%), were only detected in the stem EOs.

Our results are in agreement with previous publications on Vietnamese *Polyalthia* oils. As an example, Dai et al.<sup>5</sup> reported that the EOs from either the leaf or stem bark of *Polyalthia barmandii*, *Polyalthia jucunda*, and *Polyalthia thorelii* contained 2 major sesquiterpene hydrocarbons, germacrene D (4.4%-0.1%) and bicyclgermacrene (4.2%-27.9%). The fresh leaf EO of Malaysian *Polyalthia rumphii* had germacrene D (33.1  $\pm$  0.2%) as its main component.<sup>2</sup>

The EOs of leaf and stem were further subjected to cytotoxic, antimicrobial, and anti-inflammatory assays, and the results are outlined in Tables 2 to 4. Regarding cytotoxicity, the stem EO showed activity with IC<sub>50</sub> values of 65.3  $\pm$  0.2, 56.7  $\pm$  0.3, and 68.4  $\pm$  0.3  $\mu$ g/mL against the 3 cancer cell lines HepG2, MCF7, and A549, respectively. Especially, this sample, at the concentration of 100  $\mu$ g/mL, controlled >77% of the growth of the 3 cancer cell lines (Figure 1). The leaf EO failed to do so (IC<sub>50</sub> value > 100  $\mu$ g/mL) since, at the concentration of 100  $\mu$ g/mL, it induced an inhibitory percentage <20% (Figure 2). To the best of our knowledge, this is the first time that the EO of a Vietnamese *Polyalthia* species has been explored for anticancer activity.

Taking the antimicrobial results into consideration, as shown in Table 3, both samples showed MIC values of 200  $\mu$ g/mL against the Gram (–) bacterium *E. coli*, but the stem EO only inhibited the growth of the Gram (–) bacterium *P. aeruginosa*. Both samples were inactive against the Gram (+) bacteria, the

fungus *F. oxysporum*, and the yeast *S. cerevisiae*. The stem EO, once again, showed remarkable activity, with a MIC value of 50  $\mu$ g/mL against the growth of the fungus *A. niger*, as compared to that of the leaf EO (MIC value of 200  $\mu$ g/mL). Finally, the stem EO showed a low MIC value of 50  $\mu$ g/mL against *C. albicans*, in contrast to the leaf EO, which was inactive.

Plants in the family Annonaceae seem to be a rich resource of EOs for drug development against multidrug resistant pathogenic bacterial strains. Regarding the genus *Polyalthia*, antimicrobial examinations have only been focused on *P. longifolia*.<sup>12,13</sup> The EOs of this plant inhibited the growth of 2 micro-organisms, *S. aureus* ATCC-29213 and *C. albicans* ATCC-10231, with MIC values of 78  $\mu$ g/mL.<sup>13</sup> This is the first report of antimicrobial activity of a Vietnamese *Polyalthia* species.

Annonaceae plants are also a good resource for anti-inflammatory compounds. For instance, a survey conducted by Oyemitan et al.<sup>25</sup> reported that *Dennettia tripetala* EOs significantly revealed an anti-inflammatory effect in the carrageenan-induced paw edema model. *P. viridis* EOs were evaluated for anti-inflammatory activity by controlling NO production in LPS stimulated BV2 cells (Table 4). At the concentration of 100  $\mu$ g/mL, *P. viridis* EOs induced an inhibitory percentage of >80.0%. The leaf and stem EOs reached IC<sub>50</sub> values of 76.7  $\pm$  1.3 and 57.6  $\pm$  2.0  $\mu$ g/mL, respectively, as compared with that of the positive control butein (IC<sub>50</sub>: 16.1  $\pm$  1.0  $\mu$ g/mL). Finally, it can be assumed that the differences in the biological results between the leaf and stem EOs of *P. viridis* may be due to the percentage of each compound, especially in terms of the major components, sesquiterpene hydrocarbons and oxygenated sesquiterpenes.

**Table 2.** Cytotoxicity of Tested Samples (Half Inhibitory Concentration [IC<sub>50</sub>] in  $\mu$ g/mL).

No	HepG2	MCF7	A549
Leaf oils	>100	>100	>100
Stem oils	65.3 $\pm$ 0.2	56.7 $\pm$ 0.3	68.4 $\pm$ 0.3
Doxorubicin	0.78 $\pm$ 0.11	0.19 $\pm$ 0.07	0.96 $\pm$ 0.12

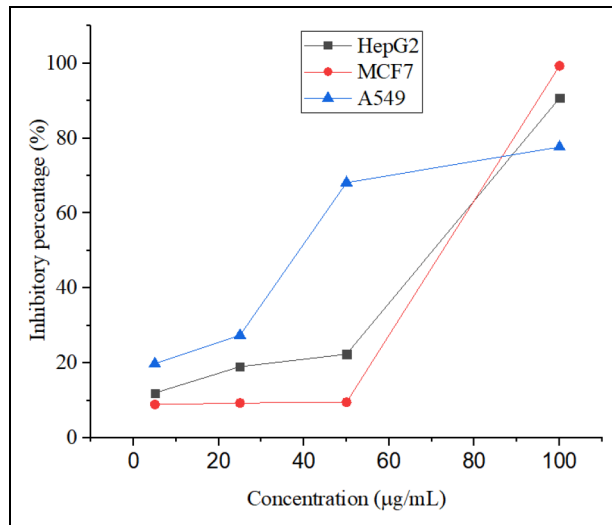
**Table 3.** Antimicrobial Activity of Test Samples.

Microbial strains	MIC ( $\mu$ g/mL)				
	Leaf	Stem	Streptomycin	Tetracyclin	Nystatin
Gram (+)					
<i>Bacillus subtilis</i>	(–)	(–)	7.2		
<i>Staphylococcus aureus</i>	(–)	(–)	14.4		
Gram (–)					
<i>Escherichia coli</i>	200	200		5.5	
<i>Pseudomonas aeruginosa</i>	(–)	200		11.0	
Fungi					
<i>Aspergillus niger</i>	200	50			23.1
<i>Fusarium oxysporum</i>	(–)	(–)			11.6
Yeasts					
<i>Candida albicans</i>	(–)	50			11.6
<i>Saccharomyces cerevisiae</i>	(–)	(–)			5.8

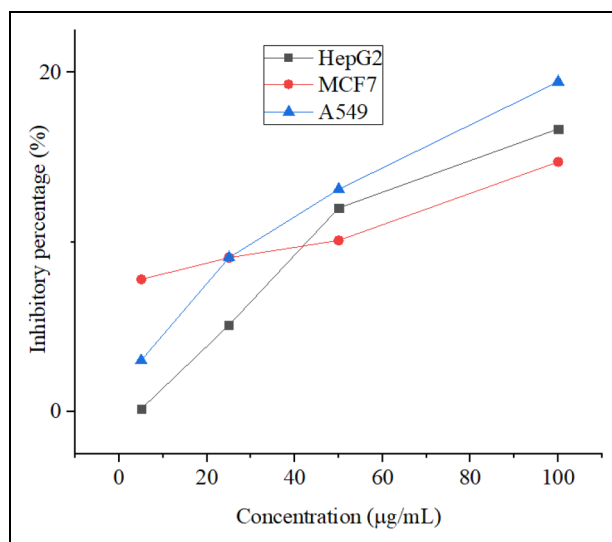
Abbreviations: MIC, minimum inhibitory concentration; (–), inactive.

**Table 4.** Anti-inflammation Activity of Test Samples.

No	Inhibitory percentage	Half inhibitory concentration (IC <sub>50</sub> ) ( $\mu$ g/mL)
Leaf oils	80.8	76.7 $\pm$ 1.3
Stem oils	87.2	57.6 $\pm$ 2.0
Butein	91.8	16.1 $\pm$ 1.0



**Figure 1.** The inhibitory effect of the stem oils on 3 cancer cell lines (HepG2, MCF7, and A549) at the tested concentrations of 5 to 100 µg/mL.



**Figure 2.** The inhibitory effect of the leaf oils on 3 cancer cell lines (HepG2, MCF7, and A549) at the tested concentrations of 5 to 100 µg/mL.

## Conclusion

For the first time, phytochemical investigation based on GC/MS-FID analysis was applied to the EOs of *P. viridis* leaf and stem. Sesquiterpene hydrocarbons and oxygenated sesquiterpenes were the main classes of compounds detected. Germacrene D was the major component, forming >45.0% of both oil samples. The stem oils showed cytotoxic activity against the 3 cancer cell lines, HepG2, MCF7, and A549, but the leaf oils failed to do so. The stem oils also showed antimicrobial activity against the fungus *A. niger* and the yeast *C. albicans*.

Finally, both oil samples indicated anti-inflammatory activity against NO production in LPS stimulated BV2 microglial cells.

## Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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