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Insight into Chemical Composition and Anti-inflammatory Activities of Essential Oil from Flowers, Leaves and Vine Stems of *Cissampelopsis volubilis* Miq.

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Abstract: Fresh flower, leaf, and vine stem materials of *Cissampelopsis volubilis* Miq., collected from the wild in Lao Cai province of Vietnam, were hydrodistilled to obtain essential oils. The oil yields were 0.49%, 0.82% and 0.28% (v/w) for flowers, leaves, and vine stems, respectively, calculated on a dry weight basis. The major components of these essential oils, that were analyzed using gas chromatography-flame ionization detector (GC-FID) and gas chromatography-mass spectrometry (GC-MS), included: *α*-pinene (32.4%) and *β*-pinene (39.6%) in flower oil; *α*-pinene (55.8%) and *β*-pinene (23.2%) in leaf oil; *α*-pinene (65.7%) and α-copaene (5.9%) in vine stem oil. The essential oil from leaves exhibited strong anti-inflammatory effect, with IC_{50} of 22.07 ± 2.01 µg/mL in inhibiting nitric oxide (NO) production in RAW 264.7 (ATCC®-TIB-71TM) cells. This finding paves the way for further research into the potential use of this plant's leaves in treating inflammation-related diseases. To the best of our knowledge, this is the first report on the chemical composition and anti-inflammatory activity of essential oils of *C. volubilis* flowers, leaves, and vine stems.

Keywords: Anti-inflammatory activities; *α*-pinene*;* *β*-pinene; *Cissampelopsis volubilis*; essential oil composition.

1. Plant Source

In research programs focused on plant species with medicinal properties for treating skin diseases among ethnic minorities in Vietnam, we investigated composition and anti-inflammatory activity of essential oils extracted from flowers, leaves, and vine stems of *Cissampelopsis volubilis* Miq. (Asteraceae). Fresh materials of *C. volubilis* were collected from Lao Cai province of Vietnam in January 2024 (flower sample) and May 2024 (leaf and vine stem samples) and were identified by Dr. Thanh Van Bui. A voucher specimen (No. BX19) was deposited at the Herbarium of the Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology (code: HN000076377).

2. Previous Studies

Many species in the Asteraceae family hold significant value and potential for human health protection [1]. *Cissampelopsis* is a perennial scandent genus in the Asteraceae family, comprising 10

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species and 2 varieties. Its members are typically climbing shrubs or small trees found at the margins of mixed deciduous and evergreen forests at altitudes of (700-) 1000-2500 m [2]. *Cissampelopsis volubilis* is one of three species in the genus *Cissampelopsis* from the Asteraceae family recorded in Vietnam. It is a large, sprawling shrub or herbaceous plant, reaching heights of 3 m or more [3]. This species is also distributed in India, Myanmar, China, Thailand, Indonesia, Malaysia [3,4]. Based on our project's research into the traditional practices of the Dao ethnic minority in Lao Cai, all parts of this species are used for bathing postpartum women to help them recover quickly; as well as in boiled water solution to wash skin pimples and soak feet to treat arthritis. Studies on *C. volubilis* are scarce, and to the best of our knowledge, this is the first time data on its essential oils have been reported.

3. Present Study

A total of 3.4-4.6 kg of shredded fresh flowers, leaves, and vine stems of *C. volubilis* were subjected to hydrodistillation for 4 hours using a Clevenger-type distillation unit [5]. The obtained essential oils were colorless, less dense than water, with respective yields of 0.49 ± 0.01%, 0.82 ± 0.02, and 0.28 ± 0.01 (v/w) calculated on a dry weight basis.

The identification of compounds in the *C. volubilis* essential oils collected from Lao Cai province, Vietnam, was conducted using mass spectral (MS) and retention index (RI) data. Table 1 lists the identified compounds in the order of their elution on the HP-5MS column used for the GC-MS analysis. A total of 25, 25 and 30 compounds, accounting for 99.8%, 99.7% and 99.1% of the compositions, was identified in *C. volubilis* floral, leaf, and vine stem essential oils, respectively. These three essential oil samples were all dominated by monoterpene hydrocarbons (90.4%, 92.9% and 76.5%). The next group was sesquiterpene hydrocarbons with respective contents at 5.5%, 3.8% and 21.7%. Only one aromatic compound, *p*-cymene (2.7%, 2.1% and 0.1%), was detected in all three essential oils. Oxygenated compounds were present in very low amounts in these essential oils.

The compound with the highest content in the leaf and vine stem oils was *α*-pinene, while in the flower oil it was *β*-pinene. The *α*-pinene concentrations in the flower, leaf, and vine stem essential oils were 32.4%, 55.8% and 65.7%, respectively, while the *β*-pinene concentrations were 39.6%, 23.2% and 3.3%. The next most abundant compound in the studied oils was sabinene, with concentrations ranging from 3.3% to 4.6%. The compounds like myrcene, *α*-phellandrene, *β*-phellandrene and *α*-copaene were detected at concentrations ranging from 0.2% to 5.9% across these three essential oils. The remaining compounds accounted for 0.1% to 3.0% of the essential oil concentration (Table 1).

Table 1. The composition (%) of *Cissampelopsis volubilis* flower, leaf, and vine stem essential oils

Compounds[a]	**RI**[b]	**RI**[c]	**RI**[d]	**Flower oil**[e]	**Leaf oil**[e]	**Vine stem oil**[e]
α-Thujene	924	924	909-937	1.1	1.6	0.7
α-Pinene	934	932	912-944	32.4	55.8	65.7
Camphene	949	946	930-969	-	-	0.1
Sabinene	972	969	945-980	4.6	3.9	3.3
β-Pinene	978	974	958-997	39.6	23.2	3.3
Myrcene	985	988	955-998	2.2	3.7	1.4
α-Phellandrene	1003	1002	966-1010	3.5	0.8	0.5
α-Terpinene	1014	1014	1007-1057	0.3	0.2	-
p-Cymene	1022	1022	1015-1039	2.7	2.1	0.1
Limonene	1027	1024	995-1037	2.1	1.3	1.1
β-Phellandrene	1028	1025	995-1036	3.2	1.3	0.2
(*E*)-*β*-Ocimene	1041	1044	1015-1056	0.5	0.3	0.1

Table 1 continued…

γ-Terpinene	1056	1054	1023-1067	0.5	0.4	-
Terpinolene	1087	1086	1052-1094	0.4	0.4	0.1
Linalool	1094	1095	1062-1125	0.1	-	0.1
Terpinen-4-ol	1174	1174	1140-1207	0.8	0.7	0.2
Cryptone	1185	1183	1172-1192	0.1	-	-
α-Terpineol	1187	1186	1153-1224	0.2	0.2	-
α-Copaene	1376	1374	1335-1419	0.4	0.8	5.9
β-Cubebene	1388	1387	1350-1409	-	-	0.3
trans-*β*-Elemene	1389	1389	1366-1407	0.2	0.1	-
α-Gurjunene	1412	1409	1383-1417	-	0.2	1.6
(*E*)-β-Caryophyllene	1424	1417	1395-1462	2.7	0.8	2.9
(*Z*)-*β*-Farnesene	1446	1440	1426-1476	-	-	0.2
α-Humulene	1458	1452	1418-1495	0.4	0.3	1.2
9-*epi*-(*E*)-Caryophyllene	1466	1464	1465-1474	-	0.1	1.4
γ-Muurolene	1477	1478	1457-1540	-	-	0.6
Germacrene D	1484	1484	1436-1521	0.8	0.2	1.1
β-Selinene	1491	1489	1475-1496	-	-	0.2
trans-Muurola-4(14),5-diene	1497	1493	1491	0.2	0.3	-
α-Muurolene	1500	1500	1475-1540	0.3	0.3	1.5
Bicyclogermacrene	1501	1500	1453-1534	-	-	1.5
γ-Cadinene	1516	1513	1470-1553	-	-	0.3
δ-Cadinene	1523	1522	1480-1562	0.5	0.7	3.0
Palustrol	1570	1567	1550-1571	-	-	0.2
Spathulenol	1578	1577	1543-1624	-	-	0.3
Total identified				99.8	99.7	99.1
Monoterpene hydrocarbons				90.4	92.9	76.5
Oxygenated monoterpenes				1.1	0.9	0.3
Sesquiterpene hydrocarbons				5.5	3.8	21.7
Oxygenated sesquiterpenes				0.0	0.0	0.5
Benzenoids				2.7	2.1	0.1
Others				0.1	-	-

Note: [a]Elution order on HP-5MS column; [b]Retention indices on HP-5MS column; [c,d]Literature retention indices: [c][6]; [d][7] on HP-5MS column; [e]Standard deviation were insignificant and excluded from the Table to avoid congestion; (-) Not identified.

Different parts of the plant accumulate organic compounds in essential oils with varying compositions and concentrations. In the flower oil, one compound (cryptone, 0.1%) was not detected in the leaf and stem oils. In contrast, two compounds, *α*-gurjunene (0.2% and 1.6%) and 9-*epi*-(*E*)-caryophyllene (0.1% and 1.4%) were detected in the leaf and stem oils but not in the flower oil. The stem oil contained 9 compounds that were not found in the flower and leaf oils, though these compounds were present in low amounts. Conversely, the flower and leaf oils contained five compounds not detected in the stem oil, but their concentrations were also low. Additionally, linalool (0.1% in both) was detected in the flower and stem oils but not in the leaf oil.

Some species in the Asteraceae family produced essential oils dominated by monoterpenes [8]. Previous studies showed that monoterpene hydrocarbons are the most abundant compounds in the essential oil from *Chrysothamnus nauseosus* var. *nauseosus* aerial part, with *β*-pinene (19.8%) being one of the two major compounds [9]. This aligns with the chemical characteristics of the *C. volubilis* flower and leaf essential oils in the present study (Table 1).

Many species in the Asteraceae family have essential oil compositions that significantly differ from those of *C. volubilis* in the present study [10]. However, some Asteraceae species contain essential oil components that show similarities to those found in our samples. Specifically, essential oils from *Pulicaria undudata*, *Achillea wilhelmsii*, *Ormenis multicaulis*, *Aldama arenaria* [11-14], and *Santolina rosmarinifolia* [15], contain high amounts of *α*-pinene or *β*-pinene. These two compounds were shown to exhibit anti-inflammatory effects through various mechanisms. Specifically, *α*-pinene was reported to inhibit cerulein-induced acinar cell death and the production of inflammatory mediators [16]. Additionally, both *α*-pinene and *β*-pinene were shown to inhibit NO production induced by lipopolysaccharide (LPS), with respective IC_{50} values of 30 ± 4 and 46 ± 9 µg/mL [17].

Inflammation is a protective response of the host microcirculation against various injuries caused by external abiotic and biotic factors. However, chronic and persistent inflammation can lead to various diseases, including cancer [18]. Macrophages play a key role in chronic inflammation by producing various inflammatory mediators. Among these, nitric oxide (NO) is produced in excessive amount by one of the inflammatory enzymes, inducible nitric oxide synthase (iNOS), contributing to tissue damage and leading to various serious diseases such as rheumatoid arthritis, cancer, and neurodegenerative diseases [19-21]. Thus, discovering novel inhibitors of NO production is of significant interest. Among them, essential oils are increasingly being evaluated as potential sources of new therapeutic agents due to their biological properties.

Anti-inflammatory activity of essential oils from *C. volubilis* flowers, leaves and vine stems was assessed using the NO assay, and their effects on the viability of RAW 264.7 (ATCC®-TIB-71TM) cells was evaluated by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) assay [22]. The cells were pre-treated with essential oils at the concentrations of 128, 64, 32, 16, 8, 4 µg/mL (corresponding to 2^7, 2^6, 2^5, 2^4, 2^3, 2^2 µg/mL). The results revealed that as the concentration of essential oils increased, cell viability decreased. Specifically, the floral, leaf and vine stem oils of *C. volubilis* at concentrations of 16, 64, and 32 mg/mL or higher resulted in cell viability below 80%. When the cell death rate exceeded 20%, the NO production inhibition value could not be calculated, to avoid obtaining false positive results. The highest NO production inhibition values (22%, 68% and 44%) were observed for the flower, leaf, and vine stem oils at concentrations of 8, 32 and 16 µg/mL, respectively (Figure 1).

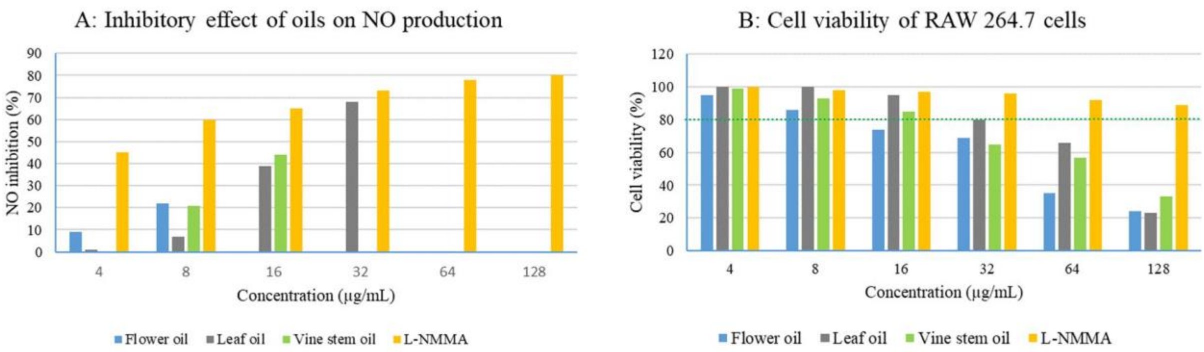

Figure 1. Inhibitory effect of *Cissampelopsis volubilis* flower, leaf, and vine stem essential oils on NO production in LPS-mediated macrophage RAW 264.7 cells (A) and cell viability of RAW 264.7 cells (B) (The dashed green line represents 80% of cell viability)

Table 2. Anti-inflammatory activity of *Cissampelopsis volubilis* flower, leaf, and vine stem essential oils

Inhibition of NO production	Flower oil	Leaf oil	Vine stem oil	L-NMMA[a]
IC_{50} (µg/mL)	UD	22.07±2.01	UD	4.38±0.5

Note: [a]N^G-Methyl-L-arginine acetate (reference material); UD = undetermined.

The leaf oil of *C. volubilis* demonstrated highly potent NO inhibitory activity against LPS-induced NO release, with an IC_{50} value of 22.07±2.01 µg/mL, indicating significant anti-inflammatory activity. In comparison, the IC_{50} value for the reference compound N^G-Methyl-L-arginine acetate (L-NMMA) was 4.38±0.5 µg/mL, while the IC_{50} values for the other two oils were could not be determined within the tested concentration range due to increased cytotoxic effects at higher oil concentrations (Table 2).

Previous study showed that the essential oil of *Santolina rosmarinifolia* (Asteraceae), which contained β-pinene (29.6%), borneol (16.9%), and myrcene (15.4%) as its main compositions, exhibited lower anti-inflammatory activity (IC_{50} = 580 µg/mL) [15] compared to the leaf oil of *C. volubilis* in the present study. The observed anti-inflammatory activity in the essential oils of different parts of *C. volubilis* may be attributed to the two main compounds α-pinene and β-pinene, or possibly due to the synergistic effects of other components present in smaller quantities. For instance, sabinene was reported to significantly inhibit the production of pro-inflammatory cytokines, chemokines, and NO in LPS and interferon gamma (IFNγ)-stimulated macrophages [23]. In addition, myrcene was also noted for its anti-inflammatory properties, such as inhibiting the production of pro-inflammatory cytokines, NO, interleukin 4 (IL-4), and IFNγ in LPS induced inflammation in rats [24], as well as preventing pro-inflammatory cytokines induced production of NO, nuclear factor-kappa B (NF-κB) and activation of p38, and reducing iNOS expression and catabolic (MMP1 and MMP13) genes [25].

Thus, the essential oils from the flowers, leaves, and vine stems of *C. volubilis* collected in Lao Cai, Vietnam, contain *α*-pinene (32.4-65.7%) and *β*-pinene (3.3-39.6%) as their main compounds, with two different chemotypes observed. The essential oils from flowers and leaves were rich in *α*-pinene (32.4 and 55.8%, respectively) and *β*-pinene (39.6 and 23.2%, respectively). In contrast, the essential oil from vine stems was rich in *α*-pinene (65.7%), followed by *α*-copaene (5.9%). The anti-inflammatory activity of the leaf oil, with an IC_{50} value of 22.07±2.01 µg/mL, highlights its potential for further exploration in treating inflammation-related diseases. This study, for the first time, provides data on the chemical composition and anti-inflammatory activity of essential oils from different parts of *C. volubilis*, paving the way for further research into their therapeutic potential.

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Supporting Information

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