Supporting Information

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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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S1: Hydrodistillation of Essential Oils

A total of 3.4-4.6 kg of shredded flower, leaf, and vine stem samples of *C. volubilis* were used for hydrodistillation. Each sample was separately placed in a 5-L flask, followed by the addition of 1.2 L of distilled water. Hydrodistillation was performed using a Clevenger-type apparatus designed according to specifications outlined by the Ministry of Health [1] to extract essential oils. The distillation process lasted for 4 hours under normal atmospheric pressure. The volatile oils were separated from the water and collected in clean, pre-weighed sample bottles. The collected oils were stored at 4 °C until further testing and analysis. Each distillation was conducted in triplicate.

S2: Analysis of Essential Oils

Essential oil analysis was conducted using Gas Chromatography/Mass Spectrometry with Flame Ionization Detection (GC/MS-FID). The GC/MS analysis employed an Agilent GC7890A system coupled with a Mass Selective Detector (Agilent 5975C). A HP-5MS fused silica capillary column (60 m × 0.25 mm i.d. × 0.25 μm film thickness) was used, with helium as the carrier gas at a flow rate of 1.0 mL/min. The inlet temperature was set to 250 °C, and the oven temperature program was as follows: an initial 60 °C, ramping to 240 °C at 4 °C/min. The split ratio was 100:1, with an injection volume of 1 μL. The MS interface temperature was maintained at 270 °C, with the MS mode set to Electron Ionization (E.I.), detector voltage at 1258V, and a mass range of 35–450 Da scanned at 1.0 scan/s. FID analysis was conducted under the same conditions, utilizing a HP-5MS fused silica capillary column (60 m × 0.25 mm i.d. × 0.25 μm film thickness), with the FID temperature at 270 °C. Essential oil constituents were identified by their relative retention indices, determined through co-injection of a homologous series of *n*-alkanes (C5–C30), and by comparing their mass spectral fragmentation patterns with those stored in the MS library NIST08, Wiley09, and HPCH1607 [2-3]. Data processing was performed using MassFinder4.0 software [4]. Each analysis was performed in triplicate, and component relative concentrations were calculated based on FID chromatographic peak areas without standardization.

S3: Assay for NO Inhibitory Effect using RAW264.7 Cells

RAW 264.7 macrophage cells (ATCC®-TIB-71TM) were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, 100 μg/mL streptomycin, and 0.25 μg/mL Gibco amphotericin B. The cells were seeded in 96-well plates at a density of 2×10^5 cells per well and incubated for 24 hours at 37 °C with 5% carbon dioxide (CO_2). After the initial incubation, the culture medium was replaced with DMEM without FBS, and the cells were incubated for an additional 3 hours. The cells were then pre-treated with essential oil samples and L-N^G-methyl arginine acetate (L-NMMA), a positive control, at concentrations of 128, 64, 32, 16, 8, and 4 μg/mL for 2 hours. This was followed by stimulation with 10 μg/mL lipopolysaccharides (LPS) for 24 hours. Nitric oxide (NO) production was assessed using the Griess Reagent System (Promega Corporation, WI, USA), with L-NMMA (Sigma) serving as the reference standard. The nitrite (NO_2^-) concentration in the medium was determined by measuring absorbance at 540 nm (A540) using a microplate reader. A standard curve was constructed based on $NaNO_2$ concentrations. Each experiment was performed in triplicate. The half-maximal inhibitory concentration (IC_{50}) for NO production inhibition was calculated using TableCurve 2Dv4. Cell viability was determined using the MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) [5, 6].

The formulas that was used to calculate the percentage of cell viability (1) and percentage of NO production inhibition (2) are as follows:

$$\text{Cell viability } (\%) = 100\% - \frac{OD_{control(+)} - OD_{test\ agent}}{OD_{control(+)} - OD_{control(-)}} \times 100\% \quad (1)$$

$$\text{NO production inhibition } (\%) = \frac{OD_{control(+)} - OD_{test\ agent}}{OD_{control(+)} - OD_{control(-)}} \times 100\% \quad (2)$$

Where:

OD: optical density; control (+): only cells in medium without cytotoxic agent; test agent: corresponds to a known concentration of cytotoxic agent; control (-): culture medium without cells.

The percentage of NO production inhibition will be calculated for the sample at concentrations where cell viability is greater than 80%, to avoid obtaining false positive results.

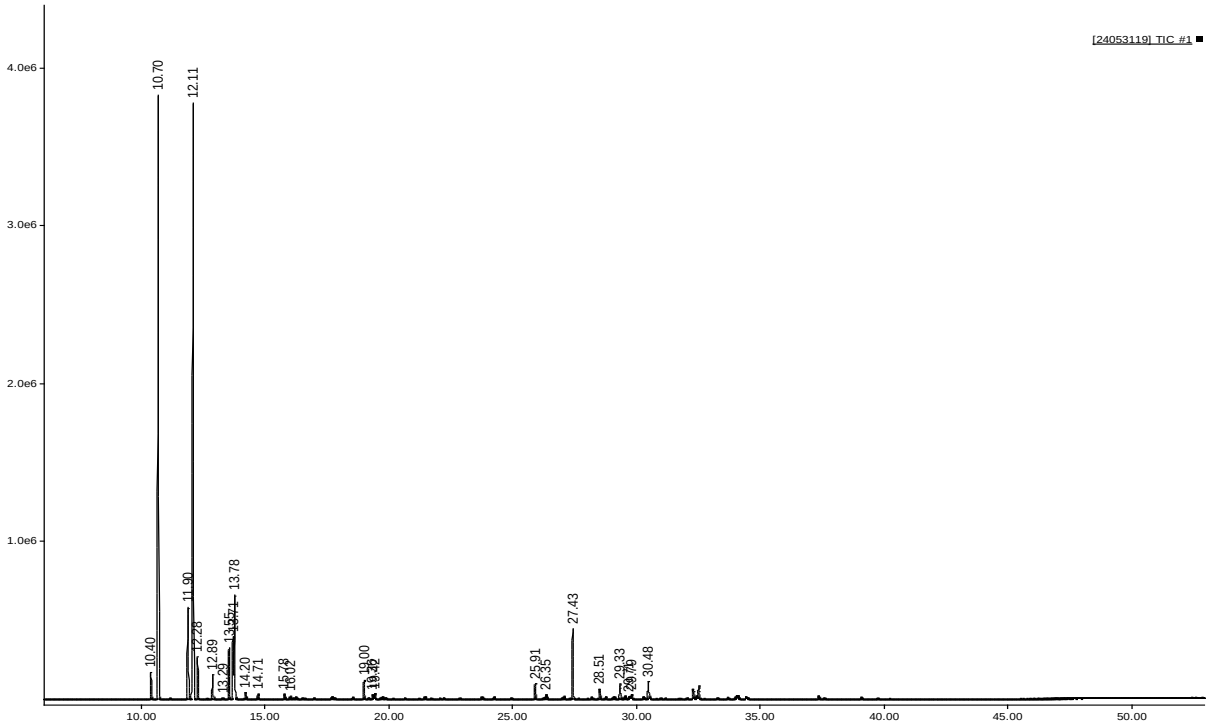

Figure S1 : GC chromatogram of the *cissampelopsis volubilis* flower essential oil

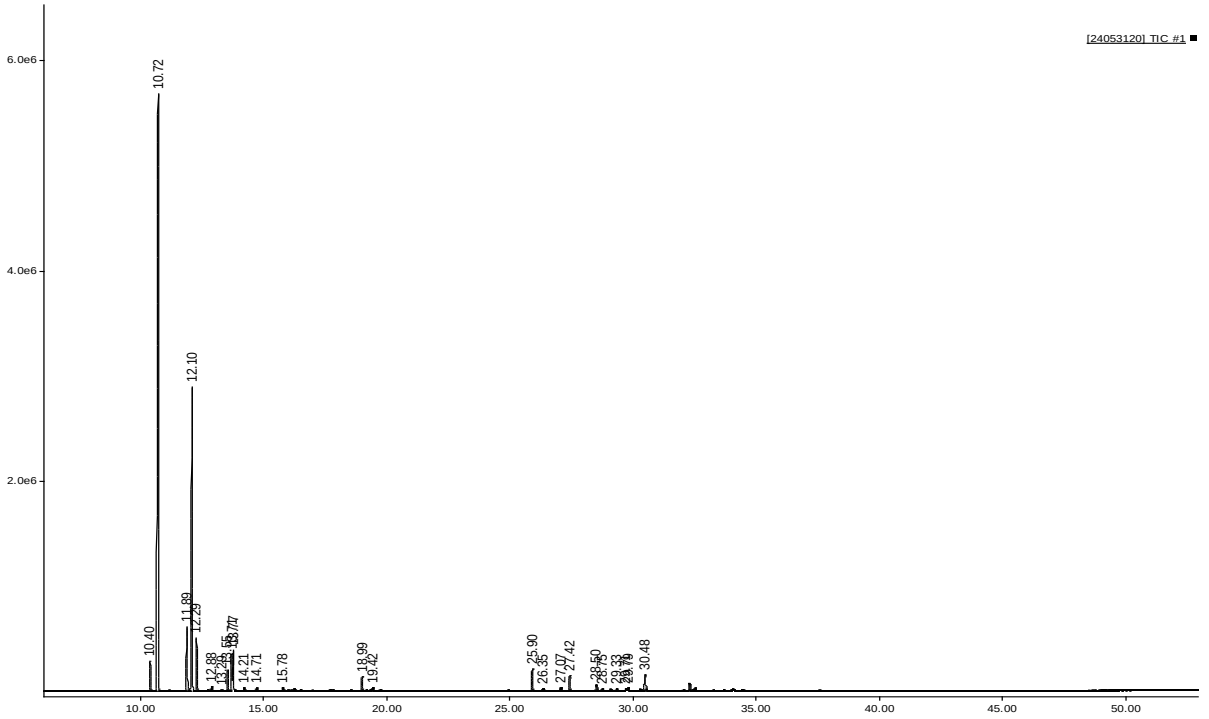

Figure S2 : GC chromatogram of the *cissampelopsis volubilis* leaf essential oil

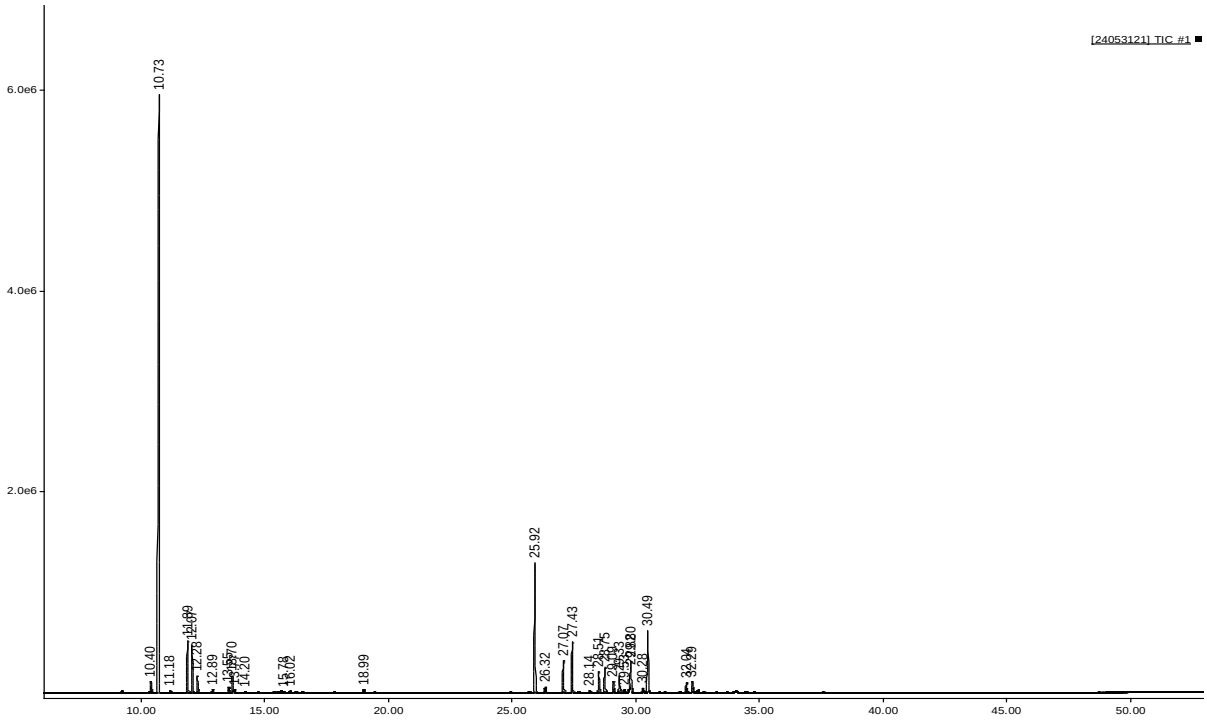

Figure S3 : GC chromatogram of the *cissampelopsis volubilis* vine stem essential oil

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