Supplementary Information

GC-MS profiling and biological activities of *Conamomum vietnamense* leaf extracts from Vietnam

Materials and Methods

Chemicals and reagents: n-Hexane (Chemsol, Vietnam), ethanol (OPC, Vietnam), sodium bicarbonate and acetic acid (China), MEME (Minimum Essential Medium with Eagle salts), DMEM (Dulbecco's Modified Eagle Medium), L-glutamine, penicillin G, streptomycin, TCA (trichloroacetic acid), SRB (Sulforhodamine B), Tris-base buffer, PBS (phosphate-buffered saline), MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), FBS (10% Fetal Bovine Serum), sodium pyruvate ($C_3H_3NaO_3$), Trypsin-EDTA (0.05%), ellipticine (Sigma, USA), DMSO (dimethyl sulfoxide) (Merck, Germany), DPPH (Sigma). Other analytical-grade chemical reagents were used in this work.

Cell lines: The cell lines were provided by Prof. JM Pezzuto (Long Island University, USA) and Prof. Jeanette Maier (University of Milan, Italy) and stored at the Institute of Biotechnology, Vietnam Academy of Science and Technology.

Total phenolic content (TPC) and Total flavonoid content (TFC)

TPC: Total phenolic compounds in the leaf extract were evaluated with Folin-Ciocalteu reagent according to previous reports in which gallic acid was used as a standard compound (1, 2). In brief, 1.0 mL of sample (200 μg/mL) was mixed with 5.0 mL of 10% Folin-Ciocalteu reagent. After 10 min incubation, 4.0 mL of 10% Na_2CO_3 was added to the above mixture and further incubated for 2 h at room temperature, after which the UV absorbance was recorded at 744 nm (UVD-2960, Labomed Inc., USA). The data are expressed as mg gallic acid (GAE) equivalent/g crude extract. The standard sample was performed using the same procedure as the extract.

TFC: The total flavonoid content was determined based on previous publications with some modifications (1, 2). The dried extract was dissolved with methanol to obtain a working solution of 400 μg/mL, which was further applied to perform a calibration curve. In detail, 1.0 mL of sample solution was added to a test tube containing 10% $NaNO_2$ (0.3 mL), followed by incubation for 10 min. Afterward, 0.3 ml of 10% $AlCl_3$ and 1.0 mL of 1.0 M NaOH were respectively added to the reaction mixture, and the total volume was adjusted to 10 mL with distilled water. After 30 min incubation at room temperature, the absorbance was recorded at 510 nm using a spectrophotometer (UDV-2960, Labbomed Inc., USA). The total flavonoid content was calculated as the rutin equivalent (RE) in mg/g crude extract. The standard sample was achieved by using the same protocol as the test sample and a standard line of rutin was established based on the standard substance.

DPPH free radical scavenging assay

The free radical scavenging activity of *C. vietnamense* extract was determined according to the previously published with some improvements (2, 3). Briefly, 1.0 mL of different concentrations (from 25 to 1000 μg/mL) of sample in methanol were combined with 6.0 mL of methanol, followed by adding 1.0 ml of 0.6 mM DPPH solution, and the mixture was incubated for 30 min in the dark. The absorbance was measured at 517 nm using a spectrophotometer (UDV-2960, Labbomed Inc., USA). The sample containing only methanol was used as a blank, while the DPPH solution alone in methanol was used as a control. Ascorbic acid was used as a positive control. The results were expressed as the percentage inhibition and calculated by the below formula:

$$\text{DPPH radical scavenging capacity (\%)} = ((Abs_{Sc} - Abs_{St})/Abs_{Sc}) \times 100\% \quad \text{(Eqn. 1)}$$

In which: Ab_{Sc} and Ab_{St} present the absorbance values of the control and test samples, respectively. The IC_{50} value was obtained *via* linear regression analysis of dose-response curve plotting between percentage inhibition and concentration.

In vitro cytotoxic activity assay

Cytotoxic Activity on MCF-7, SK-LU-1, Hela, and MKN-7 Cell Lines: The cytotoxic effects of the *n*-hexane fractionated extract from *C. vietnamense* leaves were evaluated following the protocol of (4) and (5). The MCF-7, SK-LU-1, Hela, and MKN-7 cell lines were cultured in MEME medium enriched with 2.0 mL of L-glutamine, 1.0 mM sodium pyruvate, penicillin G (100 IU/mL), streptomycin (100 µg/mL), and 10% fetal bovine serum (FBS). The cells were incubated at 37°C in 5% CO_2. Subsequently, the cells were trypsinized to detach them, and their numbers were determined using a hemocytometer. The stock solution of the n-hexane extract was prepared by diluting it in 100% DMSO to a final concentration of 0.020 µg/mL. This stock was then further diluted using cell culture medium without FBS to achieve concentrations of 500, 100, 20.0, 4.0, and 0.8 µg/mL. In a 96-well plate, 10 µL of each sample was combined with 190 µL of cell suspension and incubated in a warm incubator for 72 hours. Following the incubation, the cells were fixed using 20% TCA and stained with 0.2% SRB for 30 minutes at 37 °C. After staining, the cells were washed three times with acetic acid and allowed to dry at room temperature. SRB dye was dissolved using a 10 mM Tris-base buffer, and the mixture was gently agitated for 10 minutes. The optical density (OD) was then measured at 540 nm with an ELISA Plate Reader (Biotek, USA). Blank wells, containing 190 µL of cancer cells and 10 µL of 1% DMSO, were prepared in a similar manner. After 1 hour, the blank wells were fixed using 20% TCA. Ellipticine, used as a positive control, was prepared at concentrations of 10, 2.0, 0.4, and 0.08 µg/mL.
The inhibition rate of cancer cells was determined using the formula:

$$\% I = [1 - ((OD_{Sp} - OD_{Bl}) / (OD_{DMSO} - OD_{Bl}))] \times 100\% \quad \text{(Eqn. 2)}$$

Where I represent the inhibition rate of cancer cells, OD_{Sp} is the mean optical density of the test sample, OD_{Bl} is the mean optical density of the blank sample, and OD_{DMSO} is the mean optical density of the DMSO control.

Cytotoxic Activity on HL60 Cell Lines: The cytotoxic effects of the *n*-hexane fractionated extract from *C. vietnamense* leaves on HL60 cell lines were assessed using a modified version of the procedure established by (5) and (6). The HL-60 cell line was cultured in DMEM following the above protocol. After 72 hours of incubation, 10 µL of MTT solution with the final concentration of 500 µg/mL was added to each well. Following an additional 4-hour incubation, the medium was discarded, and the resulting formazan crystals were dissolved in 50 µL of pure DMSO. The OD was then recorded at 540 nm using a BioTek spectrophotometer (USA).
The cancer cell inhibition rate was determined using the formula:

$$\% I = [1 - ((OD_{Sp} - OD_{Bl}) / (OD_{DMSO} - OD_{Bl}))] \times 100\% \quad \text{(Eqn. 3)}$$

where: I represent the inhibition rate of cancer cells, OD_{Sp} is the average OD of the test sample, OD_{Bl} is the average OD of the blank control, and OD_{DMSO} is the average OD of the DMSO control.

References

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Table S1. Phytochemical screening of *C. vietnamense* leaf extract.

Constituents	**Tests**	**Inference**
Lipids	Stain test	-
Carbohydrate	Fehling's test, Molisch's test	+
Carotenoids	H_2SO_4 test	+
Essential oil	Scent test	+
Triterpenoids	Salkowski test	+
Alkaloids	Dragendoff's test, Wagner's test	+
Amino acids	Na_2CO_3 test	+
Steroid/Cardiac glycosides	Liebermann Burchard test/ Raymond's test, Xanthydrol's test	-
Saponins	Foam test	-
Coumarins	Lactone ring test	-
Polyphenols	$FeCl_3$ test	+
Flavonoids	Cyanidin's test	+
Tannins	Gelatin's test	+
Note: "+" indicates the presence and "-" indicates the absence of the phytocompounds.		