Supporting Information

***Rec. Nat. Prod*. 16:4 (2022) 387-392**

Annonaceae Essential Oils: Antimicrobial and Compositions of the Leaves of *Uvaria hamiltonii* Hook. f. & Thoms. and *Fissistigma kwangsiensis* Tsiang & P. T. Li

Le Thi Huong [1], Nguyen Thanh Chung [2], Dao Thi Minh Chau [3], Do Ngoc Dai [2,4] and Isiaka A. Ogunwande [5]

[1]*School of Natural Science Education, Vinh University, 182 Le Duan, Vinh City, Nghệ An Province 4300, Vietnam*

[2]*Graduate University of Science and Technology, Vietnam Academy of Science and Technology, 18-Hoang Quoc Viet, Cau Giay, Hanoi, 10072, Vietnam*

[3]*Institute of Biochemical Technology and Environment, Vinh University, 182 Le Duan, Vinh City, Nghệ An Province, Vietnam*

[4]*Faculty of Agriculture, Forestry and Fishery, NgheAn College of Economics, 51-Ly Tu Trong, Vinh City, NgheAn Province, Vietnam*

[5]*Foresight Institute of Research and Translation, Eleyele, Ibadan, Nigeria*

S1: Collection of the Leaves of *U. hamiltonii* and *F. kwangsiensis*

The leaves of *U. hamiltonii* were collected from Pù Hoạt Nature Reserve, Nậm Giải Commune (GPS: 19º41′ 38′ ′ N, 104º49 ′ 31′ ′ E) at an elevation of 662 m, on August 2020. Also, *F. kwangsiensis* were obtained from Pù Hoạt Nature Reserve, Hạnh Dịch Commune (GPS: 19º42′ 39′ ′ N, 104º50′ 42′ E), Vietnam, at an elevation of 511 m. Botanical identification was conducted by Dr. Huong LT. Voucher specimens LTH 908 and LTH 909, respectively, were deposited at Specimen room, Vinh City, Vietnam.

S2 : Hydrodistillation of Essential Oils from the Plant specimens

Prior to hydrodistillation, the leaves of *U. hamiltonii* and *F. kwangsiensis* were cleaned by handpicking of debris and other undesirable materials to obtain 2 kg of each sample. Afterwards, the leaves were grinded into coarse particles using a locally made grinder. Each of the collected plant materials was divided into three parts to ensure that each of the hydrodistillation was repeated three times. The samples were separately introduced into a 5 L flask after which distilled water was added until it covered the sample completely. Essential oils were obtained by hydrodistillation which was carried out in a Clevenger-type distillation unit designed according to an established procedure [1] as described in previous studies [2-5]. The distillation time was 3 h and conducted at normal pressure. The volatile oils which distilled over water were collected separately by running through the tap in the receiver arm of the apparatus into clean and previously weighed sample bottles. The oils were kept under refrigeration (4ºC) until the moment of analyses. The experiment was conducted in triplicate. The essential oils yield (%) was calculated by mass (g) of the essential oil divided by the mass (g) of the dried rhizomes of the plant.

S3 : Instrumental Analysis of the Hydrodistilled Essential Oils

Gas chromatography (GC) analysis was performed on an Agilent Technologies HP 7890A Plus Gas chromatograph equipped with a FID and fitted with HP-5MS column (30 m x 0.25 mm, film thickness 0.25 μm, Agilent Technology). The analytical conditions were: carrier gas H_e (1 mL/min), injector temperature, 250ºC; detector temperature 260ºC; column temperature programmed from 40ºC (held 2 min isothermally) and rise to 220ºC (10 min hold) at 4ºC/min. Samples were injected by splitting and the split ratio was 10:1. The volume of the oil injected was 1.0 μL. Inlet pressure was 6.1 kPa. Each analysis was performed in triplicate. The relative amounts of individual components were calculated based on the GC peak area (FID response) as described in previous studies [2-5].

An Agilent Technologies HP 7890A Plus Chromatograph fitted with a fused silica capillary HP-5 MS column (30 m x 0.25 mm, film thickness 0.25 μm) and interfaced with a mass spectrometer HP 5973 MSD was used for the GC/MS analysis, under the same conditions as those used for GC analysis. The conditions were the same as described above with He (1 mL/min) as carrier gas. The MS conditions were as follows: ionization voltage 70eV; emission current 40 mA; acquisitions scan mass range of 35-350 amu at a sampling rate of 1.0 scan/s.

S4 : Identification of the Constituents of the Leaves Oil of *U. hamiltonii* and *F. kwangsiensis*

The identification of constituents of essential oils from the GC/MS spectra of *U. hamiltonii* and *F. kwangsiensis* was performed on the basis of comparison of retention indices (RI Exp.) with reference to a homologous series of *n*-alkanes (C_6-C_{40}), under identical experimental conditions. In some cases, co-injection with known compounds under the same GC conditions was employed. The mass spectral (MS) fragmentation patterns were checked with those of other essential oils of known composition in literature [6] as described recently [2-5].

S5: Antimicrobial Activity Assays

The antimicrobial activity of the essential oils was evaluated using three strains of Gram-positive test bacteria, *Enterococcus faecalis* ATCC299212, *Staphylococcus aureus* ATCC25923, *Bacillus cereus* ATCC14579, three strains of Gram-negative test bacteria, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC27853, *Salmonella enterica* ATCC13076 and one strain of yeast, *Candida albicans* ATCC10231. The minimum inhibitory concentration (MIC) and median inhibitory concentration (IC_{50}) values were measured by the microdilution broth susceptibility assay as previously described [2-5].

Stock solutions of the oil were prepared in dimethylsulfoxide. The choice of investigated concentrations was based on our previous reports on similar investigations where essential oils have been found to be active within specific concentration range [2-5, 7-9]. Dilution series were prepared from 16,384 to 2 µg/mL (2^{14}, 2^{13}, 2^{12}, 2^{11}, 2^{10}, 2^{9}, 2^{7}, 2^{5}, 2^{3} and 2^{1} µg/ mL) in sterile distilled water in micro-test tubes from where they were transferred to 96-well microtiter plates. Bacteria were grown in double-strength Mueller-Hinton broth or double-strength tryptic soy broth, and fungi grown in double-strength Sabouraud dextrose broth were standardized to 5×10^5 and 1×10^3 CFU/mL, respectively. The last row, containing only the serial dilutions of the sample without microorganisms, was used as a positive (no growth) control. Sterile distilled water and medium served as a negative (no antimicrobial agent) control. Streptomycin was used as the antibacterial standard, while nystatin and cycloheximide were used as anticandidal standards. After incubation at 37 °C for 24 h, the MIC values were determined to be well with the lowest concentration of agents completely inhibiting the growth of microorganisms. The IC_{50} values were determined by the percentage of microorganisms that inhibited growth based on the turbidity measurement data of EPOCH2C spectrophotometer (BioTeK Instruments, Inc Highland Park Winooski, VT, USA) and Rawdata computer software (Brussels, Belgium) according to the following equations:

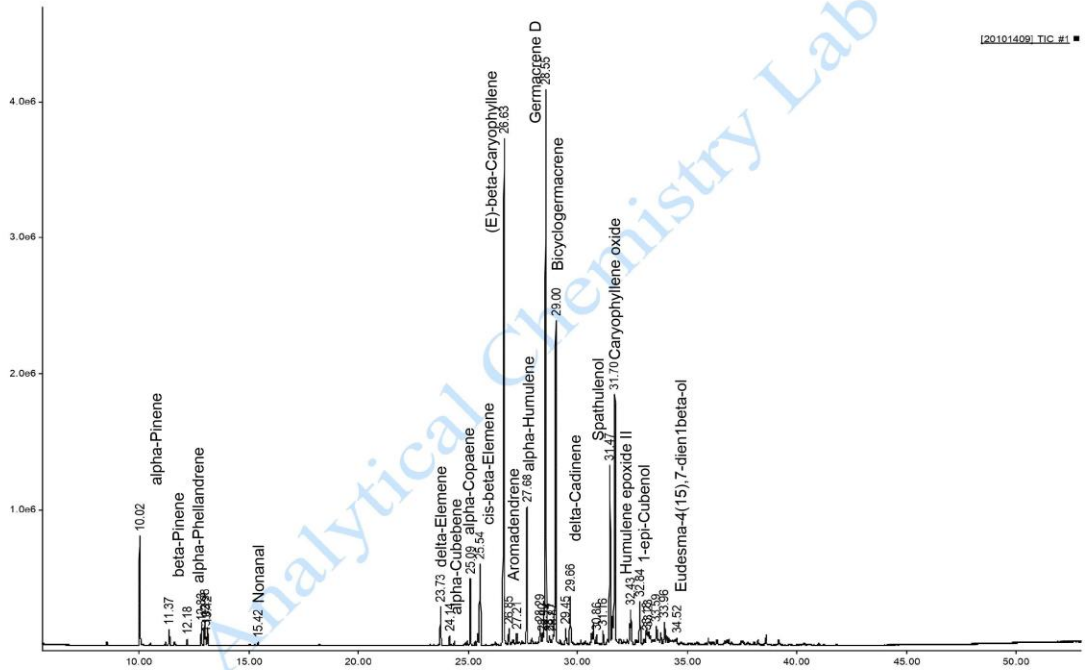
$$\% \text{ Inhibition} = \frac{OD_{control(-)} - OD_{test\ agent}}{OD_{control(-)} - OD_{control(+)}} \times 100\%$$

$$IC_{50} = High_{conc} - \frac{(High_{inh\%} - 50\%) \times (High_{conc} - Low_{conc})}{(High_{inh\%} - Low_{inh\%})}$$

where OD is the optical density, control(–) are the cells with medium but without antimicrobial agent, test agent corresponds to a known concentration of antimicrobial agent, control(+) is the culture medium without cells, $High_{conc}/Low_{conc}$ is the concentration of test agent at high concentration/low concentration, and $High_{inh\%}/Low_{inh\%}$ is the % inhibition at high concentration/% inhibition at low concentration).

S6: Statistical Analysis

Statistical analysis (ANOVA) of the differences between mean values obtained for experimental groups were calculated as a mean of standard deviation (SD) of three independent measurements using Microsoft Excel program 2003.



Chromatogram of Uvaria hamiltonii on HP-5MS column

Figure S1: GC Chromatogram of *U. hamiltonii*

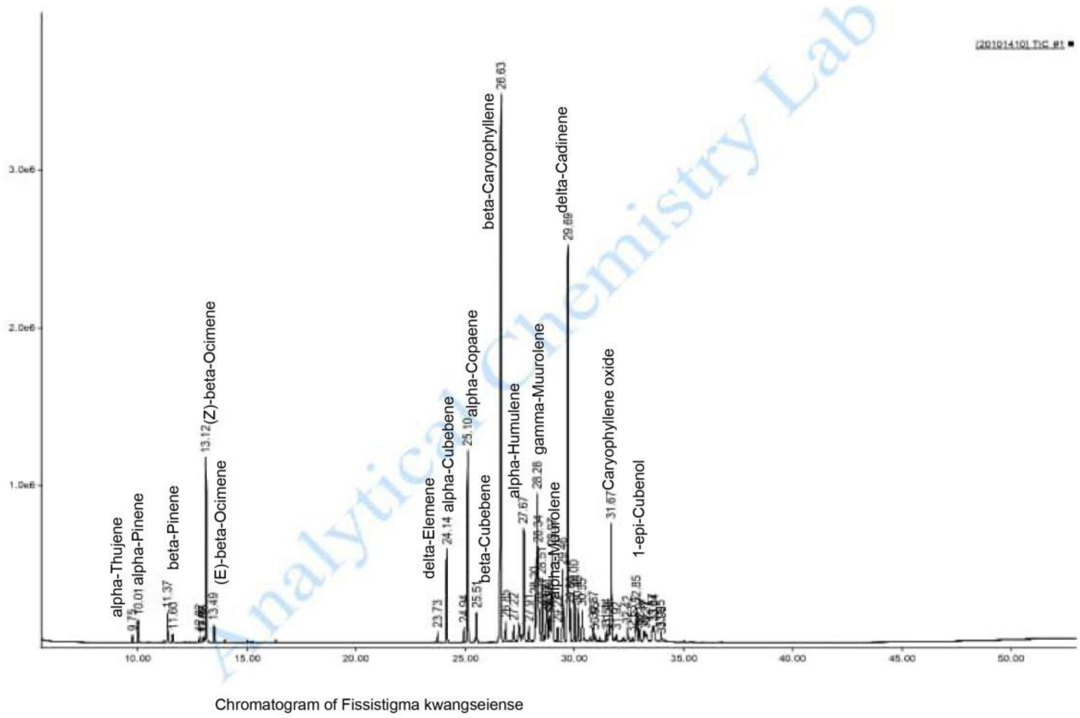


Figure S2: GC Chromatogram of *F. kwangsiensis*

References

[1] Vietnamese Pharmacopoeia (2009). Medical Publishing House, Hanoi, Vietnam.

[2] N.T. Chung, L.T. Huong and I.A. Ogunwande (2020). Antimicrobial, larvicidal activities and composition of the leaf essential oil of *Magnolia coco* (Lour.) DC, *Rec. Nat. Prod.* **14**, 372-377.

[3] B.T. Bui, V.D. Roman, L.D. Chac, V.H. Chinh, N.T.M. Hong and I.A. Ogunwande (2021). Chemical compositionand antimicrobial activity of essential oils from the leaves and stems of *Tinomiscium petiolare* Hook.f. & Thomson from Vietnam, *J. Essent. Oil Bearing Plants*. **24**, 461-468.

[4] T.M. Hoi, N.T. Chung, L.T. Huong and I.A. Ogunwande (2021). Studies on Asteraceae: chemical compositions of essential oils and antimicrobial activity of the leaves of *Vernonia patula* (Dryand.) Merr. and *Grangea maderaspatana* (L.) Poir. from Vietnam, *J. Essent. Oil Bearing Plants*. **24**, 500-509.

[5] L.T. Huong, L.N. Sam, B.D. Thach, D.N. Dai and I.A. Ogunwande (2021). Chemical compositions ofessential oilsand antimicrobial activity of *Amomum cinnamomeum* (Zingiberaceae) from Vietnam,*Chem. Nat. Compd*. **57**, 574-577.

[6] National Institute of Science and Technology (2011). Chemistry Web Book. Data from NIST Standard Reference Database 69.

[7] L.T. Huong, N.T. Viet, L.Y. Sam, C.N. Giang, N.H. Hung, D.N. Dai and I.A. Ogunwande (2021).Antimicrobial activity of essential oils from the leaves and stems of *Amomum rubidum* Lamxay & N. S. Lý. *Bol. Latinoam, Caribe Plantas Med. Aromá*, **20**, 81-89.

[8] L.T. Huong, L.D. Linh and I.A. Ogunwande (2021). Chemical compositions of essential oils and antimicrobial activity of *Elettariopsis triloba* from Vietnam. *J. Essent. Oil Bear. Plants*, **24**, 201-208.

[9] D.N. Dai, L.T. Huong, N.H. Hung, H.V. Chinh and I.A. Ogunwande (2020). Biological potentials of essential oil: Antimicrobial activity, larvicidal efficacy and chemical compositions of essential oils from *Alpinia malaccensis* (Zingiberaceae) from Vietnam. In: What to Know about Essential Oils (M. Kjeldsen, editor). Nova Science Publisher, New York, pp. 103-128.