Supporting Information

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Antimicrobial, Larvicidal Activities and Composition of the Leaf Essential Oil of *Magnolia coco* (Lour.) DC

Nguyen T. Chung[1], Le T. Huong[2] and Isiaka A. Ogunwande[3,*]

[1] Graduate University of Science and Technology, Vietnam Academy of Science and Technology,18-Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam
[2] School of Natural Science Education, Vinh University, 182 Le Duan, Vinh City, Nghệ An Province, Vietnam
[3] Foresight Institute of Research and Translation, Ibadan, Nigeria

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S1. Collection of *M. coco* Leaves

The leaves of *M. cocco* were collected from Pù Mát National Park, Nghệ An Province, Vietnam, in August 2018. Botanical identification was conducted by Dr. Huong LT. A voucher specimen NTC 759 was deposited at the Museum of the Institute of Tropical Biology, Vinh City, Vietnam, for future reference.

S2. Hydrodistillation of Essential Oil from *M. coco* **Leaves**

Before hydrodistillation, the leaves were air dry under laboratory shade for two weeks. Thereafter, they were grinded into coarse particle using locally made grinder. The sample was loaded into 5L flask after which distilled water was added until it covered the entire surface. Five hundred gram of air-dry stem was used for the experiment which was conducted in a Clevenger apparatus at normal pressure for 3 h according to the Vietnamese Pharmacopoeia [1] and as described in a previous study [2-4]. The distilled oil was collected at the receiver arm of the distillation unit and preserved until the moment of analysis.

S3. Analysis of Essential Oil from *M. coco* **Leaf**

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 [2-4].

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 *M. coco* **Leaf Oil**

The identification of constituents from the GC/MS spectra of *M. coco* was performed on the basis of retention indices (RI) determined with reference to a homologous series of *n*-alkanes (C_6-C_{40}), under identical experimental conditions. The mass spectral (MS) fragmentation patterns were checked with those of other essential oils of known composition and with those in the literature [5] and as described in previous studies [2-4].

S5. Microbes

Eight standardized ATCC strains from laboratory stock cultures were used in the teste for the antimicrobial activity of the oil of *M. coco*. The Gram-negative strains were *Enterococcus faecalis* (ATCC 299212), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853). The Gram-positive strains were *Bacillus subtilis* (ATCC 14579), *Staphylococcus aureus* (ATCC 25923) and *Salmonella enterica* ATCC 13076. A strain of yeast, *Candida albicans* (ATCC 10231) was also used for the experiment. Testing media included Mueller-Hinton Agar (MHA) used for bacteria and Sabouraud Agar (SA) used for fungi.

S6. Antimicrobial Study on *M. coco* Leaf Oil

The minimum inhibitory concentration (MIC) values were measured by the microdilution broth susceptibility assay [6,7]. Stock solutions of the oil were prepared in dimethylsulfoxide (DMSO). Dilution series 16384 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) were prepared in sterile distilled water inside the micro-test tubes from where they were transferred separately to 96-well microtiter plates with each of the microbial strains. The plate was then incubated overnight at 37°C. One hundred microlitre of the microbial culture of an approximate inoculums size of 1.0 x 10^6 CFU/mL was added to all well and incubated at 37°C for 24 h. The last row, containing only the serial dilutions of the sample without microorganisms, was used as a negative control, while Streptomycin and Nystatine were used as positive control against bacteria and fungi respectively. The MIC values were determined as the lowest concentration of the oil that completely inhibits the growth of microorganisms.

All experiments were performed in triplicate. After incubation at 37°C for 24 h, the MIC values were determined at well with the lowest concentration of agents completely inhibiting the growth of microorganisms. The IC_{50} values were determined by the percentage of microorganisms inhibited growth based on the turbidity measurement data of EPOCH2C spectrophotometer (BioTeK Instruments, United States) and Rawdata computer software (Belgium) according to the following equations:

$$\%\ inhibition = \frac{\text{ODcontrol}(+) - \text{ODtest agent}}{\text{ODcontrol}(+) - \text{ODcontrol}(-)} \times 100$$

$$IC_{50} = High_{conc} - \frac{(\text{Highinh\%} - 50\%) \times (\text{HighConc} - \text{Low Conc})}{(\text{Highinh\%} - \text{Lowinh\%})}$$

where OD is the optical density, control (+) is the only cells in medium 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.

S7. Mosquito Larvae

Adults of *Culex quinquefasciatus* and *Aedes albopictus* were collected from Hoa Khanh Nam ward**,** Lien Chieu district**,** Da Nang city (16°03'14.9"N, 108°09'31.2"E). Adult mosquitoes were maintained in entomological cages (40 x 40 x 40 cm) and fed a 10% sucrose solution and were allowed to blood feed on mice. Eggs hatching were induced with tap water. Larvae were reared in plastic trays (24×35×5 cm). The larvae were fed on dog biscuits and yeast powder in the 3:1 ratio. All stages were held at 25 ± 2°C, 65-75% relative humidity, and a 12:12 h light: dark cycle at the Center for Entomology and Parasitology Research, Duy Tan University, Vietnam.

S8. Larvicidal Test

The larvicidal activity of the essential oil of *M. coco* was evaluated according to an established protocol [8]. For the assay, aliquots of the essential oil from dissolved in EtOH (1% stock solution) was placed in a 200-mL beaker and added to water that contained 20 larvae (fourth instar). With each experiment, a set of controls using EtOH was also run for comparison. Mortality was recorded after

24 h and again after 48 h of exposure during which no nutritional supplement was added. The experiments were carried out at 25 ± 2°C. The larvicidal test was conducted with four replicates under four concentrations (100, 50, 25 and 12.5 μg/mL).

The mortality rate was calculated according to the formula :

$$Mc = (Mo)/(Mt) \times 100$$

Mo = number of larvae dead in the treated groups, Mt = number of larvae introduced and Mc = calculated mortality

S.9. Ethical Status Statement

Since ethics comittee approval is obligatory parts in all interventional studies on human or animal, there is no ethical approval requirement for larvae in Vietnam.

S10. Statistical Analysis

The data obtained (larvicidal test) were subjected to log-probit analysis [9] to obtain LC_{50} values, LC_{90} values, 95% confidence limits, and chi square values using XLSTAT v. 2018.5 (Addinsoft, Paris, France). 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.

Table S1. Parameters of larvicidal activity of the leaf essential oil of *Magnolia coco*

	Ae. albopictus	
	24 h	**48h**
LC_{50}	11.01 (9.961-12.182)[a]	10.40 (9.091-12.387)
LC_{90}	21.20 (18.491-25.445)	18.42 (16.817-27.192)
Regression equation	y = -4.692 + 4.503x	y = -3.520 + 3.460x
X^2	10.789	0.000
P	7.604	0.000
	Ae. aegypti	
	24 h	**48h**
LC_{50}	46.46 (33.132-101.554)	141.30 (73.807-703.658)
LC_{90}	41.98 (30.979-79.202)	128.98 (71.165-492.730)
Regression equation	y = -4.424 + 2.653x	y = -4.267 + 2.629x
X^2	4.530	0.000
P	4.964	0.000
	Cx. quinquefasciatus	
	24 h	**48h**
LC_{50}	87.61 (64.638-182.017)	230.68 (128.897-1037.211)
LC_{90}	53.86 (45.440-70.017)	134.82 (95.603-249.052)
Regression equation	y = -5.921 + 3.048x	y = -5.569 +3.217x
X^2	4.347	0.000
P	6.592	0.000

[a] values in parentheses are 95% confidence limit between the lower and upper values.

Table S2. Table of Model Summary and ANOVA

Model Summary						
	Model	R	R Square	Adjusted R Square	Std. Error of Estimate	
Ae. aegypti	1	.998[a]	.997	.996	.635	
Ae. albopictus	1	.970[a]	.940	.936	1.243	
Cx. quinquefasciatus	1	.981[a]	.963	.960	1.685	
ANOVA [b]						
	Ae. aegypti					
	Model	Sum of squares	df	Mean Square	F	Sig.
1	Regression	1179.978	1	1179.978	4107.78	.000[a]
	Residual	4.022	14	.287		
	Total	1184.000	15			
	Ae. albopictus					
	Model	Sum of squares	df	Mean Square	F	Sig.
1	Regression	340.111	1	340.111	220.043	.000[a]
	Residual	21.639	14	1.546		
	Total	361.750	15			
	Cx. quinquefasciatus					
	Model	Sum of squares	df	Mean Square	F	Sig.
1	Regression	1021.980	1	1021.980	359.758	.000[a]
	Residual	39.770	14	2.841		
	Total	1061.750	15			

[a] Predictors: (Constant), V2; [b] Dependent Variable

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