

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

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Abstract: The chemical constituents, antimicrobial and larvicidal activities of essential oils obtained by hydrodistillation of the leaves of *Magnolia coco* (Lour.) DC. are being reported. The essential oils were analyzed using Gas Chromatography-Flame Ionization Detector (GC-FID) and Gas Chromatography-Mass Spectrometry (GC-MS) while the larvicidal activity was evaluated by the protocol of the World Health Organization (WHO). The microbroth dilution assay was used for the study of antimicrobial activity. The major compounds in the oil were sabinene (35.4%) and β -pinene (16.3%). The oil exhibited 100% mortality towards *Aedes albopictus* at 24 h and concentrate of 50 $\mu\text{g/mL}$ while maximum mortality was shown against *Ae. aegypti* and *Culex quinquefasciatus* at concentration of 100 $\mu\text{g/mL}$. The median lethal concentrations, LC_{50} , values of 11.01, 46.46 and 87.61 $\mu\text{g/mL}$ were obtained against *Ae. albopictus*, *Ae. aegypti* and *Cx. quinquefasciatus* respectively at 24 h. However, the LC_{50} values at 48 h period were 10.40, 41.98 and 53.86 $\mu\text{g/mL}$ respectively. In addition, the oil exhibited antimicrobial activity against *Enterococcus faecalis* ATCC 299212, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 14579 and *Candida albicans* ATCC 10231 with minimum inhibitory concentrations (MIC) values of 128.0, 256.0, 128.0 and 64.0 $\mu\text{g/mL}$ respectively, while the IC_{50} values were 64.33, 128.4, 65.33 and 32.33 $\mu\text{g/mL}$ respectively. These reports, the first of its kind, indicate the potentials of *M. coco* leaf oil as a source of antimicrobial and larvicidal agents.

Keywords: *Magnolia coco*; essential oil; monoterpenes; antimicrobial activity; larvicidal activity. © 2020 ACG Publications. All rights reserved.

1. Plant Source

Vietnam is a country blessed with many plants which are yet to be exploited for their economic and medicinal values. Previously the chemical constituents and biological potentials activities of essential oils from some of the plants have been reported [1-3]. The leaves of *M. coco* were collected from Pù Mát National Park, Nghệ An Province (GPS: 19°20'N 104°50'E), Vietnam, in August 2018. A voucher specimen NTC 759 was deposited at the Museum of the Institute of Tropical Biology, Vietnam.

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2. Previous Studies

The phytochemical compounds isolated from *M. coco* were magnolamide, magnolone *epi*-sesamin, sesamin, magnolol, fargesin, aschantin, *epi*-eudesmin, syringaresinol, syringaresinol-O- β -D-glucopyranoside, scoparone, *oxo*-anolobine, and dicentrinone [4]. Studies on secondary metabolites of *M. coco* yielded alkaloids the liriodenine, magnoflorine, salicifoline, anolobine, stephanine and magnococline from the bark, as well as N-acetylanolobine, dicentrinone and *oxo*-anolobine from the leaves [5]. The polyphenols namely kobusin, (+)-sesamin, *epi*-catechin, chlorogenic acid and rutin were isolated from the flowers [6]. A methanol extract showed antioxidant activity [7]. The procyanidin oligomer and *epi*-catechin isolated from the flower showed remarkable inhibitory effect on advanced glycation end-products (AGE) formation which suggests the potential for the flowers to play a role in prevent of age-related processes and diseases and/or an anti-skin aging cosmeceutical [6]. The main constituents of essential oils of *M. coco* from Guangxi, China were α -pinene, nerolidol, caryophyllene, germacrene D and bicyclogermacrene [8].

3. Present Study

The average yield of the essential oil was $0.63 \pm 0.01\%$ (v/w), calculated on a dry weight basis. Figure 1 indicate the chromatogram of the essential oil obtained on HP-5MS column. Thirty-four constituents accounting for 94.5% of the volatile contents were identified in the oil as seen in Table 1. The main classes of compounds present therein includes monoterpene hydrocarbons (69.5%), oxygenated monoterpenes (2.4%), sesquiterpene hydrocarbons (19.9%), oxygenated sesquiterpenes (2.5%) and non-terpenes (0.2%). The major compounds of essential oil were sabinene (35.4%) and β -pinene (16.3%). Other notable constituents were α -pinene (7.1%), β -elemene (6.2%) and β -caryophyllene (6.2%). The principal components of the oil analyzed from China namely α -pinene, nerolidol, caryophyllene, germacrene D and bicyclogermacrene [8] were identified in the present sample in much lower amount. The composition of *M. coco* was similar to *M. virginiana* L., *M. hypolampra* (Dandy) Figlar and *M. calophylla* (Lozano) Govaerts [9] only in the large content of its β -pinene. However, sabinene, a major constituent of *M. coco* has not been reported previously as significant compound of other *Magnolia* oil samples [9].

Table 1. Constituents of the leaf essential oil of *Magnolia coco*

Sr. No	Compound ^a	RI (Exp.)	Range of RI ^b	<i>M. coco</i> ^c
1.	α -Thujene	930	921-939	0.6
2.	α -Pinene	937	924-941	7.1
3.	Camphene	954	933-954	0.5
4.	Sabinene	979	944-980	35.4
5.	β -Pinene	983	964-985	16.3
6.	Myrcene	990	981-993	2.9
7.	α -Terpinene	1020	1014-1024	1.1
8.	β -Phellandrene	1032	1026-1032	0.6
9.	Limonene	1034	1028-1038	1.5
10.	(<i>Z</i>)- β -Ocimene	1038	1027-1044	1.3
11.	(<i>E</i>)- β -Ocimene	1049	1041-1054	0.2
12.	γ -Terpinene	1062	1042-1064	1.6
13.	<i>cis</i> -Sabinene hydrate	1074	1071-1101	0.1
14.	Terpinolene	1092	1071-1093	0.4
15.	Linalool	1102	1098-1106	0.4
16.	(<i>E</i>)-4,8-Dimethylnona-1,3,7-triene	1118	1116-1120	0.2
17.	Terpinen-4-ol	1187	1174-1206	1.6
18.	α -Copaene	1388	1367-1394	0.3

Table 1 continued..

19.	β -Elemene	1404	1385-1407	6.2
20.	β -Caryophyllene	1437	1416-1451	6.2
21.	<i>trans</i> - α -Bergamotene	1445	1427-1446	0.1
22.	(<i>Z</i>)- β -Farnesene	1458	1428-1457	0.1
23.	α -Humulene	1471	1434-1488	0.2
24.	9- <i>epi</i> -(<i>E</i>)-Caryophyllene	1477	1457-1479	1.3
25.	Germacrene D	1498	1461-1519	0.7
26.	β -Selinene	1503	1464-1509	1.1
27.	Bicyclgermacrene	1513	1483-1532	2.7
28.	β -Bisabolene	1518	1489-1547	0.1
29.	β -Sesquiphellandrene	1534	1513-1536	0.5
30.	δ -Cadinene	1532	1516-1536	0.4
31.	(<i>E</i>)-Nerolidol	1568	1551-1569	1.8
32.	Spathulenol	1598	1571-1601	0.2
33.	Caryophyllene oxide	1605	1578-1613	0.4
34.	Guaiol (=Champacol)	1614	1595-1616	0.1
	Total			94.5
	Monoterpene hydrocarbons			69.5
	Oxygenated monoterpenes			2.4
	Sesquiterpene hydrocarbons			19.9
	Oxygenatedsesquiterpenes			2.5
	Non-terpenes			0.2

^a Elution order on HP-5MS column; RI (Exp.) Retention indices on HP-5MS column; ^b Range of LRI Literature retention indices on HP-5MS column as seen in NIST [10]; ^c Standard deviation were insignificant and excluded from the Table to avoid congestion; Sr. No. serial Number

Larvicidal activity of the essential oil; The mortality (%) and minimum lethal concentrations (LC₅₀) displayed by the essential oils towards the mosquito vectors are shown in Table 2 and Table S1. The leaf oil of *M. coco* exhibited 100% mortality against the larvae of *Ae. albopictus* under tested concentrations of 50 and 100 μ g/mL at 24 h and at concentrations of 25-100 μ g/mL for 48 h period. However, this highest mortality was achieved with *Ae. aegypti* and *Cx. quinquefasciatus* only at concentration of 100 μ g/mL when tested at 24 h and 48 h.

Table 2. Mortality and larvicidal activity of the leaf essential oil of *Magnolia coco*

Mosquito larva	Mortality (%) ^a				
	Concentration (μ g/mL) ^b				
	12.5	25	50	100	
<i>Ae. albopictus</i>					
24 h	48.75 \pm 4.349	93.75 \pm 1.893	100.0 \pm 0.000	100.0 \pm 0.000	
48 h	61.25 \pm 4.349	100.0 \pm 0.000	100.0 \pm 0.000	100.0 \pm 0.000	
<i>Ae. aegypti</i>					
24 h	7.5 \pm 5.777	17.5 \pm 1.291	17.5 \pm 2.000	100.0 \pm 0.000	
48 h	10.0 \pm 0.000	20.0 \pm 0.816	33.75 \pm 2.000	100.0 \pm 0.000	
<i>Cx. quinquefasciatus</i>					
24 h	0	5.0 \pm 0.000	13.75 \pm 1.732	100.0 \pm 0.000	
48 h	0	16.25 \pm 1.258	42.5 \pm 1.258	100.0 \pm 0.000	
Minimum lethal concentration (μg/mL)					
		LC₅₀		LC₉₀	
		24 h	48 h	24 h	48h
<i>Ae. albopictus</i>		11.01	10.40	21.20	18.42
<i>Ae. aegypti</i>		46.46	41.98	141.30	18.98
<i>Cx. quinquefasciatus</i>		87.61	53.86	230.68	134.82

^a n =4; ^bno mortality in the EtOH used as negative control

The ethanol used as negative control did not show any mortality to the insects. It can be inferred that the oil was more susceptible to *Ae. albopictus* than other mosquito vectors. The oil showed increased mortality and inhibition of *Ae. aegypti* and *Cx. quinquefasciatus* as concentration increases. The larvicidal activity of the oil was confirmed further by the level of minimum inhibitory concentrations displayed towards the mosquito vectors. The leaf oil of *M. coco* displayed larvicidal action towards *Ae. albopictus* with LC₅₀ values of 11.01 µg/mL (24 h) and 10.40 µg/mL (48 h) while the observed LC₉₀ values were 21.20 µg/mL and 18.42 µg/mL respectively. In addition, LC₅₀ values of 46.46 µg/mL and LC₉₀ of 141.30 µg/mL was exhibited by the oil towards *Ae. aegypti* at 24 h. The values at 48 h were 41.98 µg/mL and 128.98 µg/mL respectively. Moreover, *M. coco* leaf oil also inhibited *Cx. quinquefasciatus* with LC₅₀ of 87.61 µg/mL and 53.86 µg/mL at 24 h and 48 h respectively. The LC₉₀ values obtained at 24 h and 48 h respectively were 230.68 µg/mL and 134.82 µg/mL. On the other hand, the standard drug, permethrin, which was used as positive control displayed activity at lower LC₅₀ and LC₉₀ values. From Table S2, the model summary indicated that 93.6%, 99.6% and 96.0% of *Ae. albopictus*, *Ae. aegypti* and *Cx. quinquefasciatus*, respectively, were killed. From Table S2, all analytical data are significant at 1% level. There exist a significant relationship between the effectiveness of the essential oil and the mortality of the mosquito vectors. The essential oil was more effective against *Ae. aegypti* with sum of square of 1179.98 and F statistics of 4107.78, followed by *Cx. quinquefasciatus* (1021.98 sum of square and 359.76 F statistics), and lastly *Ae. albopictus* having the sum of square of 340.11, with F statistics of 220.43

There are scanty reports describing the larvicidal activity of genus *Magnolia* L. in particular and the family Magnoliaceae in general. *Magnolia grandiflora* L. exhibited larvicidal activity against *Ae. aegypti* with LD₅₀ values between 51.5 and 54.7 ppm [11]. The seed essential oil of *M. denudata* Desr. exhibited action against *Ae. aegypti*, *Ae. albopictus* and *Cx. p. pallens* larvae with LC₅₀ of 19.30, 21.40 and 19.60 µg/mL respectively [12]. The leaf oil of *Magnolia dandyi* Garnep. showed good mortality and larvicidal activity on *Ae. albopictus* with LC₅₀ values of 29.57 and 29.02 µg/mL respectively at 24 and 48 h [1]. The oil of *Manglietia garrettii* Craib showed good repellent activity towards *Ae. aegypti*, *Ae. albopictus* and *Cx. quinquefasciatus* [13]. The present results showed that *M. coco* oil exhibited mortality and larvicidal action comparable to other oil samples in the family screened for the same purpose.

Antimicrobial activity of the oil; The results of the antimicrobial test on *M. coco* essential oil are shown in Table 3. The oil displayed antimicrobial activity against four of the tested microorganisms with reasonable MIC values. The oil was active against *E. faecalis* ATCC 299212 and *B. cereus* ATCC 14579 with MIC value of 128.0 µg/mL and IC₅₀ values of 64.33 and 65.33 µg/mL, respectively. The oil inhibited the growth of *S. aureus* ATCC 25923 with MIC and IC₅₀ values of 256.0 and 128.4 µg/mL, respectively, which are double the values obtained for *E. faecalis* ATCC 299212 and *B. cereus* ATCC 14579. However, the antimicrobial activity was more pronounced with *C. albicans* ATCC 10231 at MIC and IC₅₀ values of 64.0 and 32.33 µg/mL, respectively. The oil, however, did not show any activity against *Escherichia coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27853 and *Salmonella enterica* ATCC13076. This report was the first of its kind aimed at evaluation of the antimicrobial potentials of *M. coco* leaf oil. The antimicrobial activities of essential oils of some *Magnolia* species were reported previously. The leaf oil of *M. grandifolia* showed MIC of 500 µg/mL against *S. aureus* [14]. The oil of *M. liliiflora* Desr. displayed antifungal action with MIC in the range 125 to 500 µg/mL [15].

Table 3. Antimicrobial activity of *M. coco* leaf oil

Microorganisms	MIC (µg/mL)	IC ₅₀ (µg/mL)	Str	Nst
<i>Enterococcus faecalis</i> ATCC299212	128.0 ± 3.021	64.33 ± 0.912	1.15	NT
<i>Staphylococcus aureus</i> ATCC25923	256.0 ± 2.314	128.4 ± 0.500	0.98	NT
<i>Bacillus cereus</i> ATCC14579	128.0 ± 1.500	65.33 ± 1.500	1.83	NT
<i>Candida albicans</i> ATCC10231	64.0 ± 1.02	32.33 ± 1.100	NT	1.20

- No activity; NT Not tested; Str, Streptomycin; Nst, Nystatine

The leaf oil of *M. hypolampra* Dandy was active against *S. aureus*, *S. enterica*, *E. coli* and *C. albicans* with MIC of 4.1 µg/mL while activity against *B. subtilis* and *P. aeruginosa* were recorded with MIC of 16.4 and 8.2 µg/mL, respectively [9]. A report indicated that the antimicrobial activity of *M. ovata* changed with seasonal variations [16]. It could be seen that *M. coco* exhibited antimicrobial action comparable with data from other *Magnolia* oils screened for their antimicrobial activities.

The significant antimicrobial and larvicidal activities of the essential oil may be due to actions of sabinene and β-pinene, the two major compounds of *M. coco*. β-Pinene has shown antimicrobial activity against infection causing organisms such as *S. aureus* [17] and displayed larvicides against *Ae. aegypti* among others with LC₅₀ of 35.9 ppm [18] and 21.1 ppm [19]. Sabinene exhibited significant larvicidal activity against *Ae. aegypti* and *Ae. albopictus* with LC₅₀ values of 74.1 and 39.5 µg/mL respectively [20]. There are reports describing the antimicrobial activity of sabinene [21]. The synergistic effects of the minor compounds of the essential oil may also be taken into consideration. Some compounds such as limonene, α-terpinene, γ-terpinene, α-terpineol, terpinen-4-ol, β-caryophyllene, α-pinene and nerolidol that were present in the oil have demonstrated potent larvicidal actions against *Aedes* and *Culex* species [12] as well as antimicrobial efficacy [22].

In conclusion, in this study, the main constituents of the leaf oil of *M. coco* were identified as sabinene and β-pinene which differs from a previous analyzed sample. Moreover, the essential oil for the first time was shown to have displayed larvicidal activity against *Ae. albopictus*, *Ae. aegypti* and *Cx. quinquefasciatus*. Also, the essential oil was effectively inhibited the growth of standard strains of *E. faecalis*, *S. aureus*, *B. cereus* and *C. albicans* with reasonable MIC and IC₅₀ thus depicting the antimicrobial activity.

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Supporting Information

Supporting Information accompanies this paper on <http://www.acgpubs.org/journal/records-of-natural-products>

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