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Research Article

Essential oils from the leaves of *Mitrephora calcarea* Diels ex Weeras. & R.M.K.Saunders and *M. maingayi* Hook.f. & Thomson: Chemical analysis, biological activity, and molecular docking

Do Ngoc Dai[1], Le Thi Huong[2], Nguyen Cong Truong[1], Nguyen Huy Hung[3,4], Nguyen Dinh Luyen[5], Nguyen Xuan Ha[5], Nguyen Ngoc Linh[6], Vu Minh Trang[7] and Ninh The Son[8]*

[1] Faculty of Agriculture, Forestry and Fishery, Nghe An College of Economics, 51 Ly Tu Trong, Vinh City, Nghean 4300, Vietnam
[2] Faculty of Biology, College of Education, Vinh University, 182 Le Duan, Vinh City, Nghean 4300, Vietnam
[3] Center for Advanced Chemistry, Institute of Research and Development, Duy Tan University, 03 Quang Trung, Lienchieu, Danang 550000, Vietnam
[4] Department of Pharmacy, Duy Tan University, 03 Quang Trung, Lienchieu, Danang 550000, Vietnam
[5] Institute of Natural Products Chemistry, Vietnam Academy of Science and Technology (VAST), 18 Hoang Quoc Viet, Caugiay, Hanoi 10000, Vietnam
[6] Faculty of Pharmacy, Thanh Do University, Kim Chung, Hoai Duc, Hanoi, 10000 Vietnam
[7] VNU University of Education, Vietnam National University, Hanoi, 144 Xuan Thuy, Caugiay, Hanoi 10000, Vietnam
[8] Institute of Chemistry, Vietnam Academy of Science and Technology (VAST), 18 Hoang Quoc Viet, Caugiay, Hanoi 10000, Vietnam

***Corresponding Author**
Ninh The Son
ntson@ich.vast.vn

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Abstract
The current study first describes the chemical profile of the leaf essential oils from two Annonaceous plants. Hydro-distillation and sequential GC-FID/MS analyses resulted in the identification of (*E*)-caryophyllene (29.35%), γ-curcumene (22.91%), β-pinene (12.37%), and bicyclogermacrene (6.16%) as the major compounds in *Mitrephora calcarea* leaf essential oil. In the meantime, *M. maingayi* leaf essential oil was associated with the appearance of the main compounds (*E*)-caryophyllene (31.94%), (*E*)-β-ocimene (12.23%), α-humulene (9.00%), γ-elemene (6.82%), germacrene B (7.57%), and δ-cadinene (5.79%). These two essential oils had moderate cytotoxicity towards A549 cancer cells with IC_{50} values of 56.85±0.35 - 59.16±0.43 µg/mL. *M. calcarea* leaf essential oil showed strong antimicrobial activity against the yeast *Candida albicans* ATCC 10231 with a MIC value of 32 µg/mL. Both essential oils also strongly exhibited mosquito larvicidal activity against the third-instar stage of *Aedes aegypti* and *Culex quinquefasciatus* larvae with 24-h and 48-h LC_{50} values of less than 50 µg/mL. The molecular docking study highlighted (*E*)-caryophyllene to exert the highest binding affinity for the target protein *C. albicans* sterol 14α demethylase (CYP51), while bicyclogermacrene demonstrated a strong affinity for *Cx. quinquefasciatus* odorant-binding protein (OBP). The key interactions driving these affinities were predominantly hydrophobic.

Keywords
Mitrephora calcarea, *Mitrephora maingayi*, Essential oil, Biological activity, Molecular docking

INTRODUCTION

Mitrephora is a genus of flowering plants in the family Annonaceae. About 47 species were recorded, widely distributed in China, Southeast Asia, India, and Australia[1]. Several plants in this genus have been traditionally used to treat malaria, rheumatism, stomachache, and constipation[2]. Previous evidence indicated that phytochemicals isolated from *Mitrephora* plants included alkaloids, diterpenoids, polyacetylenes, and lignans[3-5]. *Mitrephora* crude plant extracts and their isolated compounds also established useful efficacies in pharmacological examinations. For instance, three undescribed polyacetylenic ester-neolignan derivatives, namely mitrephentosins C, E, and F, exhibited moderate antimalarial activity against the *Plasmodium falciparum* strains TM4/8.2 and K1CB1 with IC_{50} values of 13.3-24.6 µM[5]. *M. sirikitiae* leaf methanolic extract and its isolated lignans showed good anti-inflammatory activity via the suppressions of secretion and synthesis of nitric oxide (NO), prostaglandin E_2 (PGE_2), and tumor necrosis factor-alpha (TNF-α)[6].

There have been several reports on the chemical identification of *Mitrephora* essential oils. By solid-phase microextraction and the GC-MS analysis, essential oil extracted from *M. wangii* flowers was reported to contain *Z*-β-ocimene (23.82%), myrcene (18.09%), limonene (17.07%), β-pinene (13.56%), and α-pinene (8.85%)[7]. The acetylcholinesterase potential of *M. poilanei* leaf essential oil is due to the great role of the major components β-caryophyllene (13.2%), α-humulene (10.5%), germacrene D (8.1%), β-elemene (5.2%), and bicyclogermacrene (5.1%)[2].

M. calcarea Diels ex Weeras. & R.M.K.Saunders, commonly known as "Cây đội mũ", or "Mạo đài móng", belongs to the family Annonaceae. It grows primarily in the wet tropical biome of Vietnam and Laos[8]. In the meantime, *M. maingayi* Hook.f. & Thomson is another Annonaceous flowering plant found in the deciduous forests of Bangladesh, Borneo, Cambodia, Laos, Peninsular Malaysia, Myanmar, Sumatra, and Vietnam[9]. Phytochemical and biological investigations of these plants are insufficient. Zhang et al isolated and structurally elucidated the chemical structure of a new acetogenin, namely mitregenin, from the methanolic extract of *M. maingayi* twigs and leaves[10].

In this communication, we tend to describe new information regarding the chemical profiles of their essential oils using the GC-FID/MS analyses. The obtained essential oils have been subjected to cytotoxic, antimicrobial, and mosquito larvicidal explorations. In addition, the best biological results will be extensively explained by the molecular docking approach.

Materials and Methods

Plant materials

M. calcarea fresh leaves were gathered from Thanh Son, Pu Luong Nature Reserve (20°31'17"N and 105°4'58"E), whereas *M. maingayi* fresh leaves were collected from Chau Ly, Pu Huong Nature Reserve (19°12'56"N and 105°7'51"E). Botanical identification was performed by the first author Dr. Do Ngoc Dai. Two voucher specimens MC-2024 (*M. calcarea*) and MM-2024 (*M. maingayi*) have been deposited at Nghe An University of Economics.

Hydro-distillation

M. calcarea fresh leaves (0.5 kg) were chopped into small pieces, and then subjected to hydro-distillation using a Clevenger-type apparatus for approximately 3.0 h. This procedure was repeated in triplicate. The obtained essential oil was dried over anhydrous Na_2SO_4 before being kept at 5°C. The same manner was applied to the second sample. The mean yield was based on the dried weight material, consisting of MC-2024, 0.20±0.002% v/w, and MM-2024, 0.22±0.0018% v/w.

GC-FID/MS analytical procedures

The Shimadzu GC2010 equipped with the FID detector was used to perform the GC-FID analysis[11,12]. It was done using the HP5-MS column (30 x 0.25 mm, 0.25 m film thickness). Operating parameters included: Helium (99.999%) was used as a carrier gas with a flow rate of 1.0 mL/min; the injection volume was 0.1 µL (split ratio of 1:10); the injector and detector temperatures were 260 and 280°C, respectively; the column temperature increased from 50 to 260°C at a rate of 5°C/min and was maintained at that level for 3.5 min. The normalized peak area (%) was used to determine the relative percentage of each component in essential oil, which was then shown as the mean of three replicates.

The Shimadzu GC2010 was used to perform the GC-MS analysis. The HP5-MS fused silica capillary one column has dimensions of 30 m x 0.25 mm i.d. x 0.25 µm film thickness. At 70 eV, the electron ionization (EI) mode occurred. The carrier gas used was helium at a rate of 1.0 mL/min. 0.1 µL of injection volume (split ratio of 1:20) was used. The temperatures of the ion source and injector were set at 260 and 280°C, respectively. The software used for the oven temperature was the same as the one for the GC. Mass spectra were obtained from *m/z* 50 to 550, with a 0.5 s scan interval. Emission current is 5 µA. Based on their retention indices (RIs), constituents in essential oils were identified using an HP-5MS capillary column and the identical operating parameters as the GC-FID analysis, which employed a homologous series of *n*-alkanes (C_7-C_{30}). The Adams book[13], NIST Chemistry WebBook[14], and W09N08 library were used to match chemical structures.

Cytotoxic assay

Cytotoxicity of essential oil samples was performed using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] method[15]. In brief, A549 cells were loaded in 96-well microplates [200 µL, 5×10^4 cells per well]. The samples at 32-256 µg/mL concentration were incorporated into the cells,

and incubated at 5% CO_2, and 37°C for 48 h. MTT (20 µL) was added, and incubation was continued at 37°C for 4 h. The optical density (OD) was recorded at 540/720 nm using an RNE-9002 Elisa reader. Ellipticine was used as a standard. Each experiment was run in triplicate. Inhibitory percentage (%) = [(1 - OD_{sampl}/OD_{con})] × 100%, where OD_{sampl} and OD_{con} were the OD values of the sample and control, respectively.

Antimicrobial assay

Ten pathogenic strains from ATCC (American Type Culture Collection), encompassing three Gram-positive bacteria *Bacillus subtilis* ATCC 35021, *Staphylococcus aureus* ATCC 43300 and *Clostridium sporogenes* ATCC 11437, two Gram-negative bacteria *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 10145, three fungi *Aspergillus brasiliensis* ATCC 16404, *A. niger* ATCC 1015 and *Fusarium oxysporum* ATCC 48112, and two yeasts *Candida albicans* ATCC 10231 and *Saccharomyces cerevisiae* ATCC 9763, were used in this study. They were cultured on Muller Hilton agar (MHA, Merck) plates for 24 h at 37°C.

Essential oils were diluted by DMSO (5%) to reach the tested concentrations of 4, 8, 16, 32, 64, 128, 256, and 512 µg/mL[16]. A mixture of 180 µL bacterial suspension with 10^6 CFU m/L in MHA and 20 µL essential oil was placed in 96-well microplates. This mixture was incubated at 37°C, and the OD was determined at 600 nm using an RNE-9002 Elisa reader. The minimum inhibitory concentration (MIC) was the lowest concentration at which no bacterial growth was observed. The same actions were used for the negative group (MHA and Tween medium), and positive group (MHA and bacterial suspension without the tested sample). Streptomycin and tetracycline were used as the standards for the Gram-positive and Gram-negative bacteria, respectively, whereas nystatin was used for fungi and yeasts.

Mosquito larvicidal assay

The eggs of *Ae. aegypti* and *Cx. quinquefasciatus* were acquired from the Institute of Biotechnology (VAST), and kept in the Department of Pharmacy of Duy Tan University in Danang, Vietnam. To perform the assay, aliquots of each essential oil sample that had been dissolved in DMSO (1% stock solution) were put in a 500 mL beaker together with 20 larvae (third and early fourth instar)[17]. A set of controls using DMSO was also conducted for comparison with every experiment. Mortalities were recorded after 24-h and 48-h of exposure, during which no nutritional supplement was added. The temperature for the experiments was 25±2°C. Each assay was run in triplicate with the tested concentrations of 0.7-100 µg/mL. Permethrin was used as a positive control. The LC_{50} (median lethal dose) and LC_{90} were obtained by log-probit analysis using XLSTAT v. 2018.5 (Addinsoft, Paris, France).

Statistical analysis

Data was processed using Microsoft Excel and represented as Mean±SD (Standard Deviation). The difference was statistically meaningful with $p < 0.05$.

Computational study

The chemical structures of the four studied compounds were drawn using the ChemOffice v21.0 suite. These structures were then converted to 3D form and optimized using the MM2 algorithm in Chem3D. The protein structures of *C. albicans* sterol 14α demethylase (CYP51) and *Cx. quinquefasciatus* odorant-binding protein (OBP) were downloaded from the Protein Data Bank with PDB IDs: 5TZ1 and 3OGN, respectively[18,19]. These protein structures were then processed by adding polar hydrogen atoms, assigning Kollman charges, and removing unnecessary molecules for docking using Chimera software. The prepared protein and ligand structures were then converted into PDBQT format as input for the docking program using AutoDockTools v1.5.6. AutoDock Vina v1.2.3 was chosen to perform the molecular docking simulations for this study, with default parameters, except for the exhaustiveness value, which was set in the same manner as previous reports[20,21]. The grid box parameters were set with the center coordinates at x = 2.8 Å, y = 31.5 Å, and z = 10.8 Å for *Cx. quinquefasciatus* OBP (PDB ID: 5TZ1) and x = 70.5 Å, y = 65.2 Å, and z = 4.5 Å for *C. albicans* CYP51 (PDB ID: 3OGN), with a size of 22 x 22 x 22 and a spacing of 1. Protein-ligand interactions were visualized using the Discovery Studio Visualizer software.

Results and Discussion

Chemical analysis

Hydro-distillation of *M. calcarea* fresh leaves resulted in yellow essential oil, yielding 0.20±0.002% v/w. From the GC-FID/MS analyses (Table 1 and Fig. S1), this essential oil was recorded to contain 35 identified compounds, which represented

99.37%. Sesquiterpene hydrocarbons were the main phytochemical class with 76.48%, followed by monoterpene hydrocarbons (18.82%) and oxygenated sesquiterpenes (4.07%). This sample was dominated by the presence of the principal compounds (*E*)-caryophyllene (29.35%), γ-curcumene (22.91%), β-pinene (12.37%), and bicyclogermacrene (6.16%). Some other compounds were also found to possess the significant percentages, including α-humulene (4.98%), α-pinene (4.67%), (*Z*)-β-farnesene (2.83%), *trans*-β-bergamotene (2.18%), α-curcumene (2.01%), spathulenol (1.38%), caryophyllene oxide (1.19%), and β-sesquiphellandrene (1.13%).

Considering the second essential oil, 26 compounds were identified, accounting for 92.92% (Table 1 and Fig. S2). In this sample, sesquiterpene hydrocarbons possessed the highest amount of 67.15%, whereas the remaining phytochemical classes consisted of monoterpene hydrocarbons (13.63%), oxygenated sesquiterpenes (9.73%), and non-terpenic compounds (2.21%). *M. maingayi* leaf essential oil was associated with the appearance of the main compounds (*E*)-caryophyllene (31.94%), (*E*)-β-ocimene (12.23%), α-humulene (9.00%), γ-elemene (6.82%), germacrene B (7.57%), and δ-cadinene (5.79%). Some other compounds also possessed significant percentages exceeding 1.00%, including α-(*E*,*E*)-farnesene (2.60%), α-copaene (2.02%), germacrene D (1.91%), *cis*-β-elemene (1.61%), γ-curcumene (1.32%), 9-*epi*-(*E*)-caryophyllene (1.16%), and selina-3,7(11)-diene (1.07%).

γ-Curcumene, β-pinene, and bicyclogermacrene were the main agents in *M. calcarea* leaf essential oil, but they were absent or appeared insignificantly in *M. maingayi* leaf essential oil. In the same manner, (*E*)-β-ocimene, α-humulene, γ-elemene, germacrene B, and δ-cadinene were the principal substances in *M. maingayi* leaf essential oil, but they were absent or present in fewer rates in *M. calcarea* essential oil. As mentioned above, some compounds, such as *Z*-β-ocimene, myrcene, limonene, β-pinene, and α-pinene**,** β-caryophyllene, α-humulene, germacrene D, β-elemene, and bicyclogermacrene, represented as the major compounds in *Mitrephora* essential oils[2,7]. Therefore, the current study matches well and reinforces the previous results.

Cytotoxic activity

Two essential oil samples were considered for their cytotoxicity against the growth of A549 cancer cell line. As shown in Table 2, both studied samples exerted more than 90 and 40% inhibitions at the highest and lowest concentrations of 256 and 32 µg/mL, respectively. It is also indicated that the leaf essential oils of *M. calcarea* and *M. maingayi* showed moderate cytotoxicity towards A549 cancer cells with IC_{50} values of 56.85±0.35 and 59.61±0.43 µg/mL, respectively, when ellipticine was used as a positive control with the IC_{50} value of 8.61±0.12 µg/mL. *Mitrephora* plant extracts and isolated compounds exhibited potential in anticancer treatments. The natural 1-azaanthraquinone alkaloid 6-methoxymarcanine A isolated from the methanolic extract of *M. sirikitiae* leaves and stems

P-388, KB, HT-29, MCF-7, A549, and ASK cancer cell lines with IC_{50} values ranging from 8.33 to 38.69 µM[11]. The methanolic extract of *M. chulabhorniana* leaves suppressed migration and invasion and induced apoptosis in HeLa cancer cells via a caspase-dependent signaling pathway[12].

Antimicrobial activity

The antimicrobial effects of two tested essential oil samples are provided in Table 3. *M. calcarea* leaf essential oil moderately inhibited the Gram-positive bacterium *B. subtilis* with a MIC value of 64 µg/mL. In contrast, the second sample was weak with a MIC value of 512 µg/mL. Both studied samples were inactive to suppress two Gram-positive bacteria *C. sporogenes* and *S. aureus* with the MIC > 512 µg/mL. Regarding the Gram-positive bacteria, two samples showed the same MIC value of 256 µg/mL against the bacterium *P. aeruginosa*, but failed to control the bacterium *E. coli* (MIC > 512 µg/mL). The studied essential oils did not exhibit antimicrobial activity against the fungi *A. niger*, *A. brasiliensis*, and *F. oxysporum* (Table 3). Significantly, *M. calcarea* leaf essential oil strongly controlled the yeast *C. albicans* with a MIC value of 32 µg/mL, compared to that of *M. maigayi* essential oil (MIC 256 µg/mL). In the last case, two samples were inactive against the yeast *S. cerevisiae* (MIC > 512 µg/mL). Accumulating previous evidence displayed the great roles of *Mitrephora* constituents in antimicrobial treatments, especially diterpenoids and polyacetylenes. Two diterpenoids of *M. celebica* stem barks, namely *ent*-trachyloban-19-oic acid and *ent*-kaur-16-en-19-oic acid, were identified as the main compounds responsible for antimicrobial activity of the plant against methicillin-resistant *Staphylococcus aureus*[24]. 9,10-Dihydrooropheolide, a polyacetylenic

Table 1. Essential oil compositions (%) in the leaves of *M. calcarea* and *M. maigayi*

No	Constituents	[a]Rt	[b]RI_E	[c]RI_L	*M. calcarea*	*M. maigayi*	Identification
1	α-Pinene	9.79	939	932	4.67 ± 0.01	0.36 ± 0.02	RI and MS
2	Sabinene	10.98	978	969	0.19 ± 0.00	-	RI and MS
3	β-Pinene	11.16	984	974	**12.37** ± 0.02	0.71 ± 0.01	RI and MS
4	Myrcene	11.39	992	988	0.73 ± 0.00	-	RI and MS
5	Limonene	12.78	1034	1024	0.28 ± 0.01	-	RI and MS
6	β-Phellandrene	12.82	1035	1025	0.18 ± 0.00	-	RI and MS
7	(*Z*)-β-Ocimene	12.93	1038	1032	0.15 ± 0.01	0.33 ± 0.01	RI and MS
8	(*E*)-β-Ocimene	13.31	1049	1044	0.25 ± 0.00	**12.23** ± 0.05	RI and MS
9	Safrole	21.94	1295	1285	-	0.63 ± 0.00	RI and MS
10	δ-Elemene	23.57	1347	1340	0.18 ± 0.00	0.28 ± 0.01	RI and MS
11	α-Cubebene	23.98	1360	1351	0.13 ± 0.01	0.37 ± 0.01	RI and MS
12	Cyclosativene	24.68	1381	1380	0.15 ± 0.00	-	RI and MS
13	α-Copaene	24.93	1388	1384	0.40 ± 0.02	2.02 ± 0.02	RI and MS
14	β-Cubebene	25.36	1401	1391	0.44 ± 0.03	-	RI and MS
15	*cis*-β-Elemene	25.40	1403	1395	-	1.61 ± 0.00	RI and MS
16	α-Cedrene	25.92	1419	1410	0.91 ± 0.00	-	RI and MS
17	(*E*)-Caryophyllene	26.51	1438	1437	**29.35** ± 0.04	**31.94** ± 0.03	RI and MS
18	γ-Elemene	26.73	1445	1440	-	**6.82** ± 0.03	RI and MS
19	α-*trans*-Bergamotene	26.75	1445	1436	0.52 ± 0.02	-	RI and MS
20	Sesquisabinene A	26.96	1452	1443	0.70 ± 0.00	-	RI and MS
21	(*Z*)-β-Farnesene	27.24	1461	1445	2.83 ± 0.01	-	RI and MS
22	α-Humulene	27.55	1471	1462	4.98 ± 0.03	**9.00** ± 0.06	RI and MS
23	9-*epi*-(E)-Caryophyllene	27.78	1478	1468	-	1.16 ± 0.02	RI and MS
24	α-Acoradiene	27.92	1483	1475	0.17 ± 0.00	-	RI and MS
25	α-Curcumene	28.17	1490	1481	2.01 ± 0.00	0.62 ± 0.00	RI and MS
26	γ-Curcumene	28.22	1492	1482	**22.91** ± 0.05	1.32 ± 0.01	RI and MS
27	*trans*-β-Bergamotene	28.37	1497	1488	2.18 ± 0.03	-	RI and MS
28	Germacrene D	28.40	1498	1490	0.75 ± 0.02	1.91 ± 0.04	RI and MS
29	β-Selinene	28.57	1504	1494	-	0.64 ± 0.02	RI and MS
30	Asaricin	28.67	1507	1498	-	1.58 ± 0.03	RI and MS
31	α-(*E,E*)-Farnesene	28.85	1513	1505	-	2.60 ± 0.01	RI and MS
32	Bicyclogermacrene	28.88	1514	1514	**6.16** ± 0.07	-	RI and MS
33	β-Sesquiphellandrene	29.48	1534	1525	1.13 ± 0.02	-	RI and MS
34	δ-Cadinene	29.56	1537	1530	0.24 ± 0.01	**5.79** ± 0.05	RI and MS
35	α-(*E*)-Bisabolene	29.97	1550	1541	0.12 ± 0.00	-	RI and MS
36	α-Cadinene	30.03	1552	1544	0.22 ± 0.00	-	RI and MS
37	Selina-3,7(11)-diene	30.26	1560	1551	-	1.07 ± 0.02	RI and MS
38	Germacrene B	30.83	1578	1569	-	**7.57** ± 0.07	RI and MS
39	Dendrolasin	30.96	1583	1575	0.47 ± 0.00	-	RI and MS
40	Spathulenol	31.36	1597	1588	1.38 ± 0.02	0.53 ± 0.00	RI and MS
41	Caryophyllene oxide	31.57	1604	1589	1.19 ± 0.04	0.83 ± 0.01	RI and MS
42	Humulene epoxide II	32.32	1631	1625	0.25 ± 0.03	-	RI and MS
43	*epi*-α-Cadinol	33.10	1658	1651	0.62 ± 0.01	-	RI and MS
44	α-Muurolol	33.24	1663	1661	0.16 ± 0.04	0.51 ± 0.03	RI and MS

Table 1 ***cont***.

No	Constituents	[a]Rt	[b]RI_E	[c]RI_L	*M. calcarea*	*M. maigayi*	Identification
45	Porosadienol	33.33	1666	1670	-	0.29 ± 0.04	RI and MS
Total					99.37	92.92	
Monoterpene hydrocarbons					18.82	13.63	
Sesquiterpene hydrocarbons					76.48	67.15	
Oxygenated sesquiterpenes					4.07	9.73	
Non-terpenic compounds					-	2.21	

Rt: Retention time; RI_E and RI_L: Retention index from experiment and literature, respectively; bold: major compounds with > 5.00%

Table 2. Cytotoxicity of two tested essential oils

Samples	Concentration (µg/mL)	A549
M. calcarea	256	91.12 ± 0.47
	128	71.43 ± 0.89
	64	53.04 ± 0.96
	32	41.22 ± 0.78
	IC_{50} (µg/mL)	56.85 ± 0.35
M. maigayi	256	97.48 ± 1.11
	128	71.42 ± 0.89
	64	50.12 ± 0.77
	32	40.81 ± 1.98
	IC_{50}	59.16 ± 0.43
Ellipticine	IC_{50}	8.61 ± 0.12

compound separated from *M. glabra* stem barks exhibited antibacterial activity against the fungus *A. niger* with the MIC value of 12 µg/mL, which was better than that of the positive control amphotericin B (MIC 25 µg/mL)[25].

Mosquito larvicidal activity

In this section, we evaluate the potential of two studied essential oils in mosquito larvicidal activities against *Ae. aegypti* and *Cx. quinquefasciatus* larvae in the third instar stage for 24 and 48-h treatments. It has been noted that natural products with LC_{50} < 50 µg/mL possessed strong activity, moderate with 50 < LC_{50} < 100 µg/mL, and weak with 100 < LC_{50} < 750 µg/mL[20,26]. Both essential oil samples showed strong activity with LC_{50} values of 19.81-37.82 µg/mL, and *M. calcarea* leaf essential oil is better than *M. maingayi* leaf essential oil in this action (Table 4). In a mosquito larvicidal assay against *Ae. aegypti* larvae, two studied samples exerted 24-h LC_{50} values of 23.51-24.33 µg/mL and 24-h LC_{90} values of 29.82-30.10 µg/mL, and 48-h LC_{50} values of 22.51-24.07 µg/mL and 48-h LC_{90} values of 30.66-30.82 µg/mL. Of *Cx. quinquefasciatus* larvae, *M. calcarea* leaf essential oil possessed 24-h LC_{50} value of 27.60 µg/mL and 48-h LC_{50} value of 19.81 µg/mL, whereas 24-h LC_{50} value of 37.82 µg/mL and 48-h LC_{50} value of 35.89 µg/mL were assigned to the second sample. Two essential oils also exerted LC_{90} values of 46.18-55.59 µg/mL for 24-h treatment and LC_{90} values of 29.15-54.81 µg/mL for 48-h treatment.

This is the first time *Mitrephora* constituents were considered in mosquito larvicidal potential. Our recent report reported that essential oils of two Annonaceous plants *G. yunnanensis* and *G. touranensis* strongly exhibited mosquito larvicidal activities against four-instar of *Ae. aegypti* and *Ae. albopictus* larvae[20]. Essential oils from *Rollinia leptopetala* leaves and stems were responsible for mosquito larvicidal activity against the third-instar of *Ae. aegypti* with 24-h LC_{50} values of 104.7 and 34.7 ppm, respectively[27]. *Annona squamosa* leaf methanolic extract showed strong activity against the early third instar larvae of *Ae. aegypti* and *Anopheles stephensi* with LC_{50} values of 51.450 and 107.121 ppm, respectively[28]. Collectively, the Annonaceous constituents have therapeutic potential for vector-borne diseases.

Molecular docking

It assumes that the good effects of the leaf essential oil of *M. calcarea* against the yeast *C. albicans* and *Cx. quinquefasciatus* larvae are due to a great role of its major compounds (*E*)-caryophyllene, γ-curcumene, β-pinene, and bicyclogermacrene (Fig. S3). This section tends to exhibit the ligand-protein interactions between these compounds and the potential proteins. Before conducting docking simulations, the protocol

Table 3. Antimicrobial activity of two tested essential oils

Microbial strains		Minimum inhibitory concentration (MIC: mg/mL)				
		M. calcarea	***M. maigay*i**	**Streptomycin**	**Tetracycline**	**Nystatin**
Gram (+)	*B. subtilis*	64	512	4		
	C. sporogenes	> 512	> 512	8		
	S. aureus	> 512	> 512	8		
Gram (-)	*E. coli*	> 512	> 512		4	
	P. aeruginosa	256	256		4	
Fungi	*A. niger*	> 512	> 512			8
	A. brasiliensis	> 512	> 512			8
	F. oxysporum	> 512	> 512			8
Yeasts	*C. albicans*	32	256			4
	S. cerevisiae	> 512	> 512			8

Table 4. Mosquito larvicidal activity of two tested essential oils

Samples	LC_{50} (95% limits)	LC_{90} (95% limits)	$\chi 2$	p
Aedes aegypti (24-h)				
M. calcarea	23.51 (22.16-24.79)	29.82 (27.99-33.05)	0.262	0.967
M. maigayi	24.33 (23.08-25.56)	30.10 (28.18-34.29)	0.106	0.991
Permethrin (control)	0.0094 (0.0082-0.00107)	0.0211 (0.0185-0.0249)	57.6	0.000
Aedes aegypti (48-h)				
M. calcarea	22.51 (20.99-24.14)	30.66 (28.55-34.26)	1.762	0.623
M. maigayi	24.07 (22.74-25.45)	30.82 (28.63-34.40)	0.299	0.960
Culex quinquefasciatus (24-h)				
M. calcarea	27.60 (24.93-30.74)	46.18 (41.43-53.04)	10.345	0.016
M. maigayi	37.82 (34.80-41.18)	55.59 (51.04-61.87)	7.497	0.058
Culex quinquefasciatus (48-h)				
M. calcarea	19.81 (18.24-21.55)	29.15 (26.73-32.63)	5.874	0.118
M. maigayi	35.89 (32.86-39.34)	54.81 (50.00-61.49)	15.297	0.002

The standard deviation (SD) for each sample belongs to 0.00-0.001

was validated by re-docking the co-crystallized ligand 3OG ((1*S*)-1-[(2*R*)-6-oxotetrahydro-2H-pyran-2-yl]undecyl acetate) into its binding site within *Cx. quinquefasciatus* odorant-binding protein (OBP). As shown in Fig. S4, the re-docked ligand structure, and the native ligand showed no significant deviations, with a root mean square deviation (RMSD) of 1.888 Å, both below the 2 Å threshold[29,30]. This result confirms the reliability of the docking protocol for further docking experiments on the main compounds considered in this study.

Sterol 14α-demethylase (CYP51) is a cytochrome P450 enzyme essential for sterol biosynthesis in eukaryotic cells and is a major target of clinical antifungal drugs like fluconazole, voriconazole, ketoconazole, posaconazole, miconazole, and clotrimazole[18,31]. Meanwhile, odorant binding proteins (OBPs) are specialized proteins involved in insect odor recognition. Inhibiting OBPs can shed light on the mechanisms of olfactory perception, potentially leading to the development of drugs, perfumes, or treatments for olfactory disorders[32,33]. Therefore, these two protein targets were selected for the current docking study.

The molecular docking results are detailed in Table 5 and 6 and Fig. 1 and 2. For *C. albicans* sterol 14α demethylase (PDB ID: 5TZ1), the docking scores ranged from -7.148 to -6.751 kcal/mol. Among them,

Table 5. Binding affinity (kcal/mol) and binding modes for principal compounds with *C. albicans* CYP51 (PDB ID: 5TZ1)

Compounds	Binding affinity (kcal/mol)	Interacting amino acid residues	Interaction types
β-Pinene	-5.458	Lys143, Ile304, Ile471, Leu300, Leu139, and Ile131	Alkyl and pi-alkyl
(*E*)-Caryophyllene	-7.148	Ile131, Leu139, Lys143, Ile471, Cys470, and Ile304	Alkyl and pi-alkyl
γ-Curcumene	-6.98	Phe380, Phe233, His377, Pro230, Tyr401, Leu87, Met92, and Leu88	Alkyl and pi-alkyl
		Lys90	Pi-cation
Bicyclogermacrene	-6.751	Met508, Leu376, Val509, Leu121, Phe126, and Phe228	Alkyl and pi-alkyl
		Tyr118	Pi-sigma

Table 6. Binding affinity (kcal/mol) and binding modes for principal compounds with *Cx. quinquefasciatus* OBP (PDB ID: 3OGN)

Compounds	Binding affinity (kcal/mol)	Interacting amino acid residues	Interaction types
β-Pinene	-6.275	Met91, Leu80, Trp114, Leu76, Phe123, and Tyr122	Alkyl and pi-alkyl
(*E*)-Caryophyllene	-7.778	Leu19, Leu15, Phe123, Tyr122, Met91, Leu80, Trp114, Leu76, and Phe59	Alkyl and pi-alkyl
γ-Curcumene	-8.015	Phe123, Leu80, Leu76, His77, Ala88, Leu73, Met89, and Met91	Alkyl and pi-alkyl
		Trp114	Pi-sigma
		Met91	Pi-cation
Bicyclogermacrene	-8.273	Phe123, Tyr122, Met84, Leu80, Met91, Trp114, Leu76, and Phe59	Alkyl and pi-alkyl

(*E*)-caryophyllene exhibited the strongest binding affinity of -7.148 kcal/mol. This compound formed alkyl and pi-alkyl interactions with amino acid residues Ile131, Leu139, Lys143, Ile471, Cys470, and Ile304. Bicyclogermacrene interacted through pi-sigma interactions with Tyr118, while γ-curcumene established a pi-cation interaction with Lys90. Additionally, bicyclogermacrene, β-pinene, and γ-curcumene formed pi-alkyl and alkyl interactions, as shown in Table 5. Notably, these major compounds interacted with crucial residues such as Met508, Leu376, Phe126, Phe228, Tyr118, Ile471, Cys470, Ile304, Lys143, Phe308, and His377[18]. These findings suggest that these studied compounds may serve as effective inhibitors of *C. albicans* CYP51, offering important information for antifungal drug developments.

For *C. quinquefasciatus* odorant-binding protein (PDB ID: 3OGN), the docking stimulation for (*E*)-caryophyllene and bicyclogermacrene were performed in our previous report[21]. γ-Curcumene showed notable binding affinity compared to bicyclogermacrene, with the binding affinity of -8.015 kcal/mol, forming pi-sigma interactions with Trp114 and pi-cation interactions with Met91. Additionally, it established alkyl and pi-alkyl interactions with Phe123, Leu80, Leu76, His77, Ala88, Leu73, Met89, and Met91. β-Pinene also formed similar interactions with Met91, Leu80, Trp114, Leu76, Phe123, and Tyr122, with a binding affinity of -6.275 kcal/mol. Significantly, these major compounds interacted with important residues like Phe123, Met84, Leu80, Met91, Trp114, and Leu15 in the active sites of the *C. quinquefasciatus* OBP protein[19]. This suggests these four compounds could be potential inhibitors of *C. quinquefasciatus* OBP.

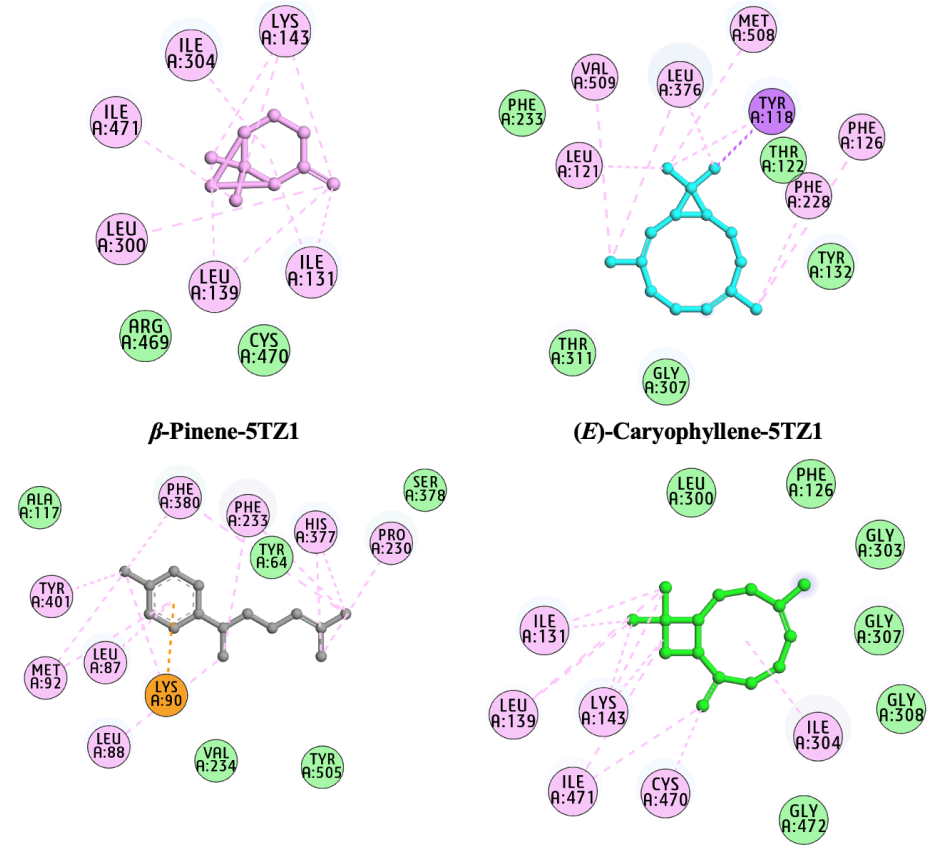

Figure 1. The 2D interaction complexes of the studied major compounds with *C. albicans* CYP51 (PDB ID: 5TZ1)

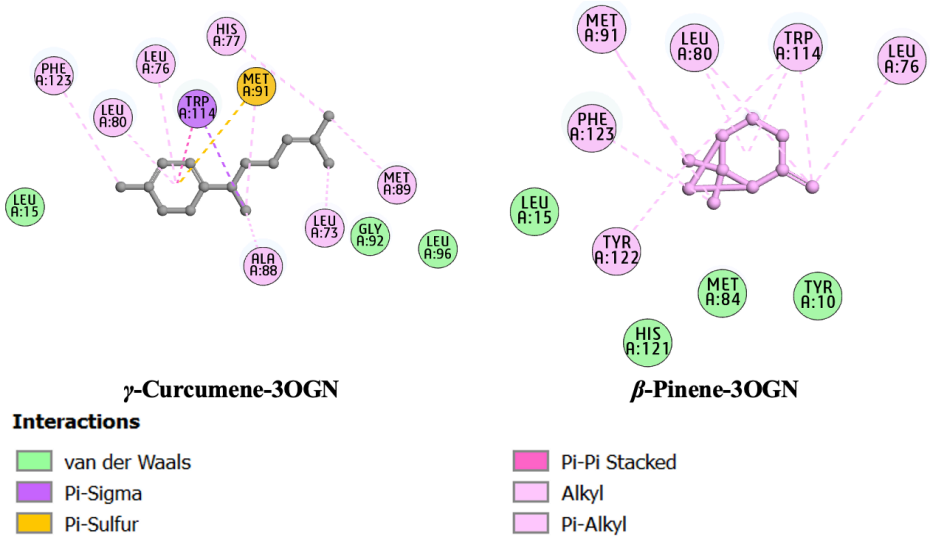

Figure 2. The 2D interaction complexes of the studied major compounds with *Cx. quinquefasciatus* OBP (PDB ID: 3OGN)

Conclusions

The current study first provides the chemical profile of essential oils from two *Mitrephora* plants. *M. calcarea* leaf essential oil was associated with the presence of the principal compounds (*E*)-caryophyllene, γ-curcumene, β-pinene, and bicyclogermacrene.

Meanwhile, (*E*)-caryophyllene, (*E*)-β-ocimene, α-humulene, γ-elemene, germacrene B, and δ-cadinene were the main metabolites in *M. maingayi* leaf essential oil. Both essential oils moderately showed cytotoxic towards A549 cancer cells. The studied essential oils also were found to control the growth of microbial strains *B. subtilis, P. aeruginosa,* and *C. albicans*, as well as they strongly exhibited mosquito larvicidal activity against the third-instar stage of *Ae. aegypti* and *Cx. quinquefasciatus* larvae for 24-h and 48-h treatments. Molecular docking identified the potential of the major compounds to inhibit *C. albicans* CYP51 and *C. quinquefasciatus* OBP, in which two sesquiterpenes bicyclogermacrene and (*E*)-caryophyllene stood out for exhibiting the highest binding affinities. However, it still lacks *in vivo* biological studies on molecular mechanisms of action. The isolation of the major compounds for further biological assays is also encouraged.

Completing interests

No potential conflict of interest was reported by the authors.

Data availability statement

Data available on request from the corresponding author.

Supplementary data

Figures S1 to S4 are provided as supplementary data.

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