SHORT REPORT

A C G publications

Rec. Nat. Prod. 17:3 (2023) 571-576

records of natural products

Essential Oil Compositions and Antimicrobial Activity of the Leaves of *Alphonsea monogyma* Merr. & Chun and *Goniothalamus banii* B. H. Quang, R. K. Choudhary & V.T. Chinh from Vietnam

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(Received Month Day, 202X; Revised Month Day, 202X; Accepted Month Day, 202X)

Abstract: Essential oils from the leaves of *Alphonsea monogyma* Merr. & Chun and *Goniothalamus banii* B. H. Quang, R. K. Choudhary & V.T. Chinh from Vietnam were obtained by hydrodisitllation and the chemical components determined by gas chromatography (GC) and gas chromatography coupled with mass spectrometry (GC/MS). The antimicrobial assay was conducted by microdilution broth method. The respective yields of the essential oils were 0.18% (v/w) and 0.355% (v/w), respectively. The major components of *A. monogyma* were (*E*)-caryophyllene (13.8%), δ-cadinene (12.5%), bicyclogermacrene (12.4%), *cis*-β-elemene (12.1%), and germacrene D (11.4%). However, myrcene (47.1%), α-pinene (9.7%) and (*E*)-caryophyllene (9.1%) were the dominant constituents of the essential oil of *G. banii*. The leaf essential oil of *A. monogyma* displayed potent antimicrobial activity towards the Gram-positive microorganisms of *Enterococcus faecalis* ATCC29212, *Staphylococcus aureus* ATCC25923 and *Bacillus cereus* ATCC14579 with minimum inhibitory concentration (MIC) values of 2.23 µg/mL, 10.45 µg/mL and 10.33 µg/mL, respectively. On the other hand, essential oil from *G. banii* exhibited the most effective antibacterial against Gram-negative *Pseudomonas aeruginosa* ATCC27853 (MIC 5.67 µg/mL), and anti-candidal action towards *Candida albicans* ATCC10231 (MIC 32.67 µg/mL). The chemical constituents and antimicrobial activity of the essential oils of *A. monogyma* and *G. banii* were being reported for the first time.

Keywords: *Alphonsea monogyma; Goniothalamus banii;* essential oil; terpenes; antimicrobial activity; anti-candidal activity.

1. Plant Source

Recently, the authors have published data on compositions and antimicrobial activity of hydrodistilled essential oils from some unexploited flora of Vietnam [1,2]. Also, previously reported were the essential oil composition of some species of *Alphonsea* [3,4] and *Goniothalamus* [6-8] plants harvested in Vietnam. Large quantity of the leaves of *A. monogyna* Merr. & Chun were collected from Pù Mát National Park (GPS: 19º51′1′N; 104º38′7′E) at height of 210 m, in the month August 2020. Moreso, *Goniothalamus banii* (Blume) B. H. Quang, R. K. Choudhary & V.T. Chinh were also harvested at an elevation of 762 m in the month of September 2020 from Pù Hoat Nature Reserve (GPS: 19º42′43′N; 104º49′29′E). The botanical authentication of the plants was performed by Dr. Le Thi Huong. Voucher specimens HNU 820 (*A. monogyma*) and HNU 824 (*G. banii*), were preserved at the plant specimen room, Vinh University.

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The article was published by ACG Publications
http://www.acgpubs.org/journal/records-of-natural-products May-June 2023 EISSN:1307-6167
DOI: http://doi.org/10.25135/rnp.367.2208.2547
Available online: November 26, 2022

2. Previous Studies

Presently, there is no record on the volatile constituents and pharmacological potentials of *A. monogyma* and *G. banii* plants. However, azafluorenone (onychine) alkaloids, 6,7-dimethoxy-5-hydroxy onychinc, as well as cyathocaline, liriodenine, isooncodine anddarienine were isolated from the stem and branch of *A. monogyma* [9]. The research was undertaken due to non-availability of reported data on the essential oil composition and antimicrobial studies on *A. monogyma* and *G. banii*.

3. Present Study

The leaves of *A. monogyma* were analysed for essential oil in accordance with procedures used previously [1,2]. After the hydrodistillation, a light-yellow coloured essential oil was produced from the leaves of the plant. Also, a yield of 0.35% (± 0.01, v/w) essential oil was obtained from the process. In Table 1, the compounds identified from GC/MS spectral of the essential oil were documented. The composition of the essential oil consists mainly of sesquiterpene hydrocarbons (73.5%) and monoterpene hydrocarbons (21.9%). The oxygen-containing monoterpene class of compound was not identified in the essential oil. In addition, the oxygenated sesquiterpenes were identified in the proportion of 4.0%. The main composition of *A. monogyma* were the sesquiterpenes namely β-caryophyllene (13.8%), δ-cadinene (12.5%), bicyclogermacrene (12.4%), *cis*-β-elemene (12.1%), germacrene D (11.4%); and monoterpenes represented by limonene (8.6%) and α-pinene (5.0%). In addition, β-pinene (4.8%), α-copaene (3.4%) and (*E*)-9-*epi*-caryophyllene (2.8%) were the other compounds present in amount >1%. Since this is the first report on the essential oil from any parts of *A. monogyma,* the present data could not be compared with other analyzed samples of the same species. In accordance with reports from other previously studied essential oils of the family *Alphonsea,* terpene compounds were also found to occur in highest quantity predominate in this study. The large amount of β-caryophyllene and β-elemene identified in the oil of *A. monogyma*, was in consistent with data obtained for the leaf oil of *A. tonkinensis* previously analyzed from Vietnam [3]. In addition, bicyclogermacrene, β-caryophyllene and *cis*-β-elemene present in *A. monogyma* were also present in the leaf of *A. philastreana* and *A. gaudichaudiana* [4]. In accordance with chemotaxonomic analysis, the essential oils of *Alphonsea* plants from Vietnam can be classified as belonging to the group which has high content of sesquiterpene hydrocarbons.

A light-yellow coloured essential oil of yield of 0.27% (± 0.01*,* v/w) was obtained from leaves of *G. banii*. The identified compositional patterns of the essential oil are seen in Table 1. The classes of compound namely, monoterpene hydrocarbons (72.9%) and sesquiterpene hydrocarbons (12.5%), predominate in the essential oil. The oxygen-containing terpene compounds were less common in the proportion of 2.7% (oxygenated monoterpenes) and 5.9% (oxygenated sesquiterpenes). The mixture of monoterpenes and sesquiterpenes hydrocarbons represented by myrcene (47.1%), α-pinene (9.7%), β-caryophyllene (9.1%), and o-cymene (8.4%) were the main compounds of the oil. There were significant amount of caryophyllene oxide (3.0%), limonene (2.5%), linalool (2.3%), spathuenol (2.3%), and (*E*)-β-ocimene (2.1%). A comparison of the composition of *G. banii* essential oils revealed some variation with data reported for other *Goniothalamus* plants from Vietnam (Table 2). The comparative results showed sesquiterpene hydrocarbons were the dominant class of compounds in the leaf of *G. banii,* stem of *G. tamirensis* [5], leaf of *G. tamirensis* [6], as well as the leaf and stem of *G. multiovulatus* [7]. Moreover, monoterpene hydrocarbons were found mainly in the leaf of *G. macrocalyx* [5]. The leaf of *G. albiflorus* [8], as well as leaf and stem of *G. takhtajani* [7] contained monoterpene hydrocarbons and oxygenated counterparts. Also oxygen containing monoterpenes and sesquiterpene hydrocarbons were found in the leaf of *G. wightii* [7]. A mixture of monoterpene and sesquiterpene hydrocarbons was found in the stem of *G. albiflorus* [5]. The stems of *G. albiflorus* [8] and *G. wightii* [7] consist of monoterpene hydrocarbons, oxygen containing monoterpenes and sesquiterpene hydrocarbons. Also, the mixture of monoterpene hydrocarbons, sesquiterpene hydrocarbons and oxygenated sesquiterpenes were found in the stem of *G. tamirensis* [5]. Non-terpene compounds were found additionally in the stem of *G. macrocalyx* and the leaf of *G. albiflorus* [5]. Thus, essential oils from the various *Goniothalamus* plants exhibited chemical variability. However, the identities of these terpenes differed from one species to another. A noteworthy observation was that myrcene has not been described as a major compound of essential oils of *Goniothalamus* plants analyzed from Vietnam [5,6,8,11], Malaysia [12-14] and Borneo [15].

Table 1. Constituents of the leaf essential oils of *Alphonsea monogyma* and *Goniothalamus banii*

No.	Compound [a]	RI (Exp.)	Range of RI[b]	*A. monogyma* [c]	*G. banii* [c]
1.	α-Thujene	930	921-939	-	0.1
2.	α-Pinene	937	924-941	5.0	9.7
3.	Camphene	954	933-954	-	0.2
4.	Sabinene	979	944-980	0.5	-
5.	β-Pinene	983	964-985	4.8	0.8
6.	Myrcene	990	981-993	0.5	47.1
7.	α-Phellandrene	1009	995-1011	0.3	-
8.	δ-3-Carene	1016	1010-1020	-	0.8
9.	o-Cymene	1029	1024-1031	-	8.4
10.	β-Phellandrene	1032	1026-1032	0.8	0.6
11.	Limonene	1033	1028-1038	8.6	2.5
12.	1,8-Cineole	1037	1032-1044	-	0.4
13.	(*E*)-β-Ocimene	1049	1041-1054	1.6	2.1
14.	γ-Terpinene	1062	1042-1064	-	0.4
15.	Linalool	1101	1098-1101	-	2.3
16.	Perillene	1104	1102-1111	-	0.2
17.	(*E*)-4,8-Dimethylnona-1,3,7-triene	1118	1116-1120	-	0.1
18.	δ-Elemene	1347	1335-1350	1.1	-
19.	α-Cubebene	1359	1353-1362	0.2	-
20.	γ-Ylangene	1383	1379-1389	0.3	-
21.	α-Copaene	1388	1367-1394	3.4	0.3
22.	*cis*-β-Elemene	1403	1385-1407	12.1	0.1
23.	β-Caryophyllene	1437	1416-1451	13.8	9.1
24.	*trans*-α-Bergamotene	1445	1427-1446	-	0.6
25.	α-Guaiene	1451	1430-1459	0.1	-
26.	Aromadendrene	1455	1437-1460	-	0.3
27.	α-Humulene	1471	1444-1476	1.6	1.7
28.	9-*epi*-(*E*)-Caryophyllene	1477	1457-1480	2.8	0.1
29.	*trans*-Cadina-1(6)-4-diene	1489	1461-1493	0.3	0.3
30.	Germacrene D	1498	1471-1500	11.4	-
31	*trans*-Muurola-4(14)-5-diene	1509	1478-1510	0.5	-
32.	Viridiflorene	1510	1480-1522	-	0.2
33.	Bicyclogermacrene	1513	1483-1525	12.4	0.4
34.	α-Bulnesene	1520	1502-1527	0.3	-
35.	γ-Cadinene	1530	1515-1541	0.6	-
36.	δ-Cadinene	1532	1516-1547	12.5	0.2
37.	α-Calacorene	1558	1531-1567	0.1	-
38.	Elemicine	1561	1538-1560	-	0.2
39.	Elemol	1563	1544-1564	0.2	-
40.	(*E*)-Nerolidol	1568	1549-1569	-	0.1
41.	4,8,12-Trimethyldeca-1,3-7,11-tetraene	1582	1550-1572	-	0.3
42.	Scapanol	1592	1560-1599	0.5	-
43.	Spathulenol	1598	1571-1601	0.7	2.3
44.	Caryophyllene oxide	1605	1578-1613	0.6	3.0
45.	Guaiol (=Champacol)	1610	1595-1616	0.7	-
46.	Cubeban-11-ol	1612	1601-1618	-	0.1
47.	Humulene epoxide II	1629	1610-1636	-	0.4
48.	1-*epi*-Cubenol	1644	1622-1648	0.3	-
49.	*epi*-α-Cadinol	1656	1641-1663	0.2	-
50.	α-Muurolol	1663	1649-1671	0.2	-
51.	α-Cadinol	1672	1642-1680	0.2	-
52.	Bulnesol	1683	1656-1690	0.4	-
	Total			**99.4**	**95.4**
	Monoterpene hydrocarbons (No. 1-11, 13, 14, 16)			**21.9**	**72.9**
	Oxygenated monoterpenes (No. 12, 15)			**-**	**2.7**
	Sesquiterpene hydrocarbons (No. 18-38)			**73.5**	**13.5**
	Oxygenatedsesquiterpenes (No. 39, 40, 42-52)			**4.0**	**5.9**
	Non-terpenes (No. 17, 41)			**-**	**0.4**

[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; **No.** Number

Table 2. Antimicrobial activity of *Alphonsea monogyma* and *Goniothalamus banii* leaf oils

Microorganisms	**MIC (μg/mL)[a,b,c,]**		IC_{50} **(μg/mL) [a]**	
	A. monogyma	***G. banii***	***A. monogyma***	***G. banii***
Enterococcus faecalis ATCC29212	2.20 ± 0.01	8.89 ±0.01	4.00± 0.11	16.00± 0.00
Staphylococcus aureus ATCC25923	10.50 ±0.31	24.56 ±0.01	32.00 ± 0.50	128.0 0± 0.91
Bacillus cereus ATCC14579	10.33 ±0.05	56.78 ± 1.50	32.00± 0.15	128.00 ± 1.00
Pseudomonas aeruginosa ATCC27853	12.45 ±0.01	5.67±0.01	32.00± 0.05	16.00± 0.01
Candida albicans ATCC10231	63.89 ± 1.02	32.67 ± 0.32	128.00± 0.50	64.00 ± 0.10
Escherichia coli ATCC25922	NA	NA	NT	NT
Salmonella enterica ATCC13076	NA	NA	NT	NT

Na: No activity; NT: Not tested; [a] means of three replicate analysis; ± standard deviation; [b] standard Gram-positive antibacterial, streptomycin gave MIC values between 0.42 μg/mL and 4.10 μg/mL; standard Gram-negative ntibacterial, nystatine had MIC value of 1.10 μg/mL; [c] standard anti-fungal, nystatine MIC showed value of 2.20 μg/mL.

This paper reports for the first time the antimicrobial activity of essential oils from the leaves of *A. monogyma* and *G. banii*. Both essential oils displayed antimicrobial activity against the tested gram-positive and gram-negative bacteria with the minimum inhibitory concentrations (MIC) less than 100.0 µg/mL (Table 2). Essential oil from the leaf of *A. monogyma* displayed antimicrobial activity towards four of the tested microorganisms, and anti-candidal activity, with the minimum inhibitory concentration (MIC) values in the range of about 2 μg/mL - 60 μg/mL. The order of antibacterial activity was *E. faecalis* ATCC29212 (MIC, 2.20 μg/mL) > *B. cereus* ATCC14579 (MIC, 10.33 μg/mL) ≥ *S. aureus* ATCC25923 (MIC, 10.45 μg/mL) > *P. aeruginosa* ATCC27853 (MIC, 12.45 μg/mL). The IC_{50} values were obtained in the range of 4.00 μg/mL- 128.00 μg/mL. The essential oil of *A. monogyma* exhibited anti-candidal action towards *C. albican*s ATCC10231 with MIC value of 63.89 μg/mL, with IC_{50} value of 128.00 μg/mL.

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There is no report on the antimicrobial activity of essential oils from any *Alphonsea* plants. However, the antimicrobial properties of essential oils from some *Goniothalamus* plants have been evaluated and reported [17-19]. Comparing the antimicrobial activities of essential oils of *Goniothalamus* plants, the leaf essential oil of *G. banii* was superior to the essential oils from the roots and twigs of *G. macrophyllus* against *S. aureus* (MIC, 0.3 mg/mL and 2.5 mg/mL, respectively), *P. aeruginosa* (MIC > 5.0 mg/mL) and *C. albicans* (MIC 0.3 mg/mL) [17]. Also, *G. banii* leaf oil with MIC value of 24.56 µg/mL was more potent against *S. aureus* than the flower essential oil of *G. macranii* with MIC value of 31.25 µg/mL [18] and the stem oil of *G. cardiopetalus* having MIC value of 1.5 mg/mL [19]. In addition, the leaf oil of *G. banii G. banii* (MIC, 56.78 µg/mL) exhibited greater inhibitory action against *B. cereus* than the stem of *G. cardiopetalus* with MIC value of 1.5 mg/mL. Interestingly, while *G. banii* leaf oil could not inhibit the growth of *E. coli,* essential oils from the twigs of *G. macrophyllus* [17], the flower of *G. macranii* [18] and the stem of *G. cardiopetalus* [19] displayed antimicrobial action with MIC values of 2.5 mg/mL, 15.62 µg/mL, and 1.5 mg/mL, respectively. Overall, *G. banii* essential oil possessed antimicrobial activity comparable with other studied *Goniothalamus* oil.

It is believed that the constituents present in the studied essential oils might have influenced the observed antimicrobial activity of *A. monogyma* and *G. banii* against microorganisms. Previous studies have shown that the biological activities of essential oils from different species of plants are dependent of the major compounds of abundance. In some other cases, synergies between the major and some minor constituents have also enhanced the activity of natural products including essential oils [20]. The antimicrobial potential of *G. macrophyllus* was attributed to α-pinene because its activity was reported to superceeds those of other tested compounds [17]. In addition, *G. macranii* essential oil contained large amount of caryophyllene oxide and (*E*)-caryophyllene, and the antimicrobial action was due to these compounds among others [18]. Moreover, linalool and α-pinene were thought to be responsible for the observed activity of *G. cardiopetalus* [19]. All these compounds were present in one form or the other in the essential oil of *A. monogyma* and *G. banii*. For example, β-caryophyllene demonstrated selective antibacterial activity against *S. aureus* [21] and antifungal effect [22]. In addition, some other compounds identified in the essential oils, including δ-cadinene [23] and germacrene D [24] were previously reported to display broad spectrum of antimicrobial actions. Myrcene was reported to acts as an antibacterial agent against *S. aureus* [25], *E. coli* [24] and *Salmonella enterica* [24]. Moreover, α-and β-pinene, linalool, 1,8-cineol are ubiquitous monoterpenoids in conifer and the other aromatic plants, and each compound were widely tested against many organisms [1, 2, 24].

In conclusion, in this study, the main constituents of the leaf oil of *A. monogyma* were identified as (*E*)-caryophyllene, δ-cadinene, bicyclogermacrene, *cis*-β-elemene and germacrene D, while myrcene, α-pinene and (*E*)-caryophyllene were present in *G. banii*. Also, the essential oils effectively inhibited the growth of standard strains of *E. faecalis, S. aureus, B. cereus, P. aeruginosa* and *C. albicans* with reasonable MIC and IC_{50} thus depicting the antimicrobial activity.

Supporting Information

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

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