SHORT REPORT

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Essential Oils of Lauraceae: Constituents and Antimicrobial Activity of *Dehaasia cuneata* (Blume) Blume and *Caryodaphnopsis tonkinensis* (Lecomte) Airy-Shaw from Vietnam

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Abstract: The herbs, *Dehaasia cuneata* (Blume) Blume and *Caryodaphnopsis tonkinensis* (Lecomte) Airy-Shaw (Lauraceae) were used ethnomedically for the treatment of malaria, inflammation and amelioration of microbial infections. We report herein the chemical constituents and antimicrobial activity of the leaf essential oils of *D. cuneata* and *C. tonkinensis* from Vietnam. The technique of gas chromatography (GC) and gas chromatography coupled with mass spectrometry (GC/MS) was used to analyze the oil samples while the microdilution assay was employed to determine the antimicrobial efficacy. The main constituents of *D. cuneata* were α-pinene (49.0%), camphene (19.5%), *β*-pinene (15.6%) and limonene (7.5%), while α-pinene (26.8%), *β*-pinene (23.0%) and bicyclogermacrene (8.5%). The leaf oil of *D. cuneata* displayed potent antimicrobial activity against Gram-negative bacteria, *Pseudomonas aeruginosa* ATCC27853 with minimum inhibitory concentration (MIC) value of 5.37 µg/mL; and Gram-positive microorganisms of *Staphylococcus aureus* ATCC25923 (MIC, 21.56 µg/mL and *Bacillus cereus* ATCC14579 (MIC, 23.45 µg/mL). The leaf oil of *C. tonkinensis* exhibited good antibacterial activity towards *Enterococcus faecalis* ATCC299212 with MIC value of 15.99 µg/mL, and anti-candidal action against *Candida albicans* ATCC10231 with MIC value of 33.68 µg/mL. the chemical constituents and antimicrobial activity of the essential oils were being reported for the first time.

Keywords: Antimicrobial activity; essential oil composition; terpenes.

1. Plant Source

Dehaasia cuneata Blume (syn. *Cyanodaphne Blume*) is known locally as Tiểu hoa nêm and normally used as culinary, spices and flavoring of foods and meats. The plant has been used in ethnomedicine for the

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treatment of microbial infections and ulcers [1]. *Caryodaphnopsis tonkinensis* (Lecomte) Airy Shaw is a medium-sized tree often referred to in Vietnamese as Cà lồ bắc where it is used to treat microbial infections and act as spices in food condiments [2].

In the study, essential oils from the leaves of *D. cuneata* and *C. tonkinensis* collected Pù Mát National Park (GPS: 19°44′32′N; 3°48′10′E) and Bến En National Park (GPS: 19°35′31′N; 105°22′59′E), Vietnam, respectively, were analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS) techniques, and then screened for antimicrobial efficacy.

2. Previous Studies

To the best of our knowledge, this is the first report on the chemical compositions and antimicrobial activities of essential oils from any parts of *D. cuneata* and *C. tonkinensis* grown in Vietnam or any other parts of the world. Moreover, no report could be seen on the volatile compositions and biological activities of other species in the genus *Dehaasia* and *Caryodaphnopsis*. However, a phytochemical study revealed that lupeol isolated from *D. cuneata* had a moderate inhibition on *Serratia marcescens* ATCC 14756 and low inhibition of *Escherichia coli* ATCC 25922, *Vibrio fluvialis* ATCC 33809, *Bacillus subtilis* ATCC 6633, and Methicillin-resistant *Staphylococcus aureus* ATCC 43300 [3]. Alkaloids were previously isolated from several *Dehaasia* species [4].

3. Present Study

The hydrodistillation of the leaves of *D. cuneata* produced light-yellow coloured essential oil. The average yield of essential oil was 0.35% (w/w). Figure 1 shows the chromatogram of the essential oils of *D. cuneata.*Table 1 presents the compounds as identified by GC/MS. Nineteen compounds accounting for 98.8% of the essential oil contents were identified in the leaves of *D. cuneata*. The composition of the essential oil was dominated by monoterpene hydrocarbon class of compounds (95.9%). The sesquiterpene classes of compounds are less common in the essential oil (ca. 0.9%). The main constituents of the essential oil were α-pinene (49.0%), camphene (19.5%), *β*-pinene (15.6%) and limonene (7.5%). Other compounds except myrcene (2.9%) were identified in amount less than 1%. On the other hand, 46 compounds representing 97.9% of the essential oil contents were identified in the leaves of *C. tonkinensis* (Figure 2), while the yield obtained was 0.54% (w/w). Monoterpene hydrocarbons (71.3%), sesquiterpene hydrocarbons (19.8%) and oxygenated sesquiterpenes (56%) were the quantitatively significant classes of compounds present in the essential oil. The major constituents of the essential oil were α-pinene (26.8%), *β*-pinene (23.0%) and bicyclogermacrene (8.5%). Other significant compounds of the essential oil were camphene (4.9%), sabinene (4.1%), limonene (3.3%) and myrcene (3.2%).

A comparative analysis of the present results with previously data on the essential oils of *D. cuneata* and *C. tonkinensis* could not be performed due to lack of information on the studied species or any other members in the studied genus. The chemical constituents of the essential oils of both *D. cuneata* and *C. tonkinensis* were being reported for the first time.

Table 1. Chemical compositions of the leaf essential oils of *Dehaasia cuneata* and *Caryodaphnopsis tonkinensis* collected in Vietnam

				Percent composition[d]	
No	**Compounds[a]**	**RI[b]**	**Range of RI[c]**	*D. cuneata*	*C. tonkinensis*
1	Tricyclene	928	909-922	0.7	-
2	α-Thujene	930	921-939	-	0.6
3	α-Pinene	939	924-941	49.0	26.8
4	Camphene	955	933-954	19.5	4.9
5	Sabinene	978	944-980	0.4	4.1
6	*β*-Pinene	984	964-985	15.6	23.0
7	Myrcene	992	981-993	2.9	3.2
8	α-Phellandrene	1009	989-1011	-	1.1
9	α-Terpinene	1021	1014-1024	-	1.0

10	o-Cymene	1030	1022-1034	0.3	0.6
11	Limonene	1035	1028-1038	7.5	3.3
12	*β*-Phellandrene	1036	1032-1040	-	0.5
13	1,8-Cineole	1037	1032-1044	0.7	0.3
14	(*E*)- *β*-Ocimene	1049	1041-1054	-	0.3
15	γ-Terpinene	1063	1042-1064	-	1.3
16	Terpinolene	1093	1071-1093	-	0.6
17	Linalool	1101	1098-1106	0.1	-
18	(*E*)-4,8-Dimethylnona -1,3,7-triene	1117	1116-1120	-	0.5
19	*cis*-Sabinol	1148	1138-1152	0.2	-
20	Terpinen-4-ol	1185	1174-1206	-	0.4
21	Myrtenol	1204	1198-1212	0.2	-
22	Myrtenal	1206	1200-1218	0.1	-
23	Sabinyl acetate	1306	1289-1314	0.5	-
24	Methyl genarate	1326	1318-1344	0.2	-
25	δ-Elemene	1347	1339-1354	-	0.5
26	*cis*- *β*-Elemene	1403	1385-1407	-	0.2
27	*β*-Caryophyllene	1437	1416-1448	0.2	0.8
28	*trans*-α-Bergamotene	1445	1420-1450	-	0.7
29	Aromadendrene	1455	1437-1460	0.1	1.5
30	Selina-5,11-diene	1459	1444-1463	-	0.2
31	(*E*)- *β*-Farnesene	1464	1452-1474	-	0.2
32	α-Humulene	1471	1454-1488	-	0.3
33	9-*epi*-(*E*)-Caryophyllene	1477	1458-1477	-	0.2
34	α-Zingiberene	1502	1488-1510	-	0.5
35	δ-Selinene	1504	1491-1518	-	0.6
36	Bicyclogermacrene	1513	1500-1520	-	8.5
37	*β*-Bisabolene	1517	1505-1523	-	1.8
38	*β*-Curcumene	1520	1520-1533	-	0.2
39	(*Z*)-γ-Bisabolene	1526	1522-1535	-	1.7
40	*β*-Sesquiphellandrene	1533	1532-1547	-	0.3
41	δ-Cadinene	1535	1534-1540	-	0.2
42	(*E*)-γ-Bisabolene	1541	1537-1551	-	0.9
43	(*E*)-α-Bisabolene	1550	1548-1558	-	0.2
44	(*E*)-Nerolidol	1569	1551-1569	-	1.3
45	Germacrene B	1576	1563-1580	-	0.3
46	Palustrol	1587	1569-1593	-	0.2
47	Spathulenol	1593	1571-1601	0.5	1.6
48	Caryophyllene oxide	1605	1578-1613	0.1	-
49	Viridiflorol	1602	1599-1612	-	1.0
50	Cubeban-11-ol	1612	1601-1618	-	0.4
51	Rosifoliol	1620	1620-1632	-	0.2
52	*β*-Eudesmol	1671	1647-1672	-	0.4
53	α-Eudesmol	1672	1664-1684	-	0.3
54	*epi*-α-Bisabolol	1696	1690-1702	-	0.2
	Total			**98.8**	**97.9**
	Monoterpene hydrocarbons			**95.9**	**71.3**
	Oxygenated monoterpenes			**2.0**	**0.7**
	Sesquiterpene hydrocarbons			**0.3**	**19.8**
	Oxygenated sesquiterpenes			**0.6**	**5.6**
	Non-terpenes			**-**	**0.5**

[a] Elution order on HP-5MS column; [b] Experimental retention indices; [c] Range of LRI Literature retention indices on HP-5MS column as seen in NIST [5]; [d] means of three replicate values, SD (±) omitted to avoid congestion; Sr. No, serial number; - not identified

Essential oil compositions and antimicrobial activity of *D. Cuneata* and *C. tonkinensis*

Essential oils from the leaves of both *D. cuneata* and *C. tonkinensis* displayed antimicrobial activity towards five of the tested microorganisms, and anti-candidal activity with varying MIC values in the range of 5.0 – 60.0 µg/mL (Table 2). The leaf oil of *D. cuneata* displayed potent antimicrobial activity against Gram-negative bacteria, *P. aeruginosa* ATCC27853 with MIC value of 5.37 µg/mL. Moreover, the essential oil showed notable activity towards the Gram-positive microorganisms of *S. aureus* ATCC25923 (MIC, 21.56 µg/mL and *B. cereus* ATCC14579 (MIC, 23.45 µg/mL). However, *D. cuneata* leaf oil exhibited lesser inhibitory anti-candidal activity to *C. albicans* ATCC10231 with MIC value of 56.56 µg/mL. The leaf essential oil of *D. cuneata* exhibited higher antibacterial action than the non-volatile lupeol which has low inhibition of *B. subtilis* and *S. aureus* [3]. However, lupeol [3] was more active against *E. coli* than the essential oil. On the other hand, the leaf oil of *C. tonkinensis* exhibited good antibacterial activity towards *E. faecalis* ATCC299212 with MIC value of 15.99 µg/mL, and anti-candidal action against *C. albicans* ATCC10231 with MIC value of 33.68 µg/mL. Also, the essential oil showed lesser antimicrobial action towards *S. aureus* ATCC25923 (MIC, 56.78 µg/mL), *B. cereus* ATCC14579 (MIC, 56.54 µg/mL) and *P. aeruginosa* ATCC27853 (MIC, 57.78 µg/mL). On the whole, the leaf oil of *D. cuneata* showed greater antimicrobial potential, with lower MIC values than the leaf oil of *C. tonkinensis*. Conversely, the leaf oil of *C. tonkinensis* exhibited twice greater anti-candidal action than the leaf oil of *D. cuneata*.

Table 2. Antimicrobial activity of *Dehaasia cuneata* and *Caryodaphnopsis tonkinensis* leaves essential oils

	MIC (µg/mL) [a,b,c,d]		**IC_{50} (µg/mL) [a]**	
Microorganisms	***D. cuneata***	***C. tonkinensis***	***D. cuneata***	***C. tonkinensis***
Enterococcus faecalis ATCC299212	16.72	15.99	32.0	32.0
Staphylococcus aureus ATCC25923	21.56	56.78	64.0	128.0
Bacillus cereus ATCC14579	23.45	56.54	64.0	128.0
Escherichia coli ATCC 25922	-	-	-	-
Pseudomonas aeruginosa ATCC27853	5.37	57.78	16.0	128.0
Salmonella enterica ATCC13076	-	-	-	-
Candida albicans ATCC 10231	65.56	33.68	128.0	64.0

[a]Standard deviation in the range ± 0.00-0.01; - No activity; [b]Streptomycin, MIC values in the range 0.28 µg/mL to 3.20 µg/mL; [c]Nystatine MIC value of 8.0 µg/mL; [d]Cycloheximide MIC value of 3.20 µg/mL.

The MIC and IC_{50} values provided evidence that the leaf essential oils of both *D. cuneata* and *C. tonkinensis* displayed potent antimicrobial and anti-candidal activities against the tested microorganisms except *E. coli* ATCC 25922 and *S. enterica* ATCC13076. Recent findings indicated that substances with MIC values ≤ 100 µg/mL may be considered to be of good antimicrobial activity [6]. Thus, *D. cuneata* and *C. tonkinensis* essential oils should be considered a promising antimicrobial agent because the essential oil displayed antibacterial activity with most MIC < 100 µg/mL. Streptomycin, the standard antimicrobial agent for gram-positive bacteria displayed antimicrobial activity with MIC values in the range 0.28 µg/mL to 3.20 µg/mL. In addition, nystatine used as standard antimicrobial agent had MIC value of 8.0 µg/mL, with cycloheximide, an anti-candidal agent, showing activity at MIC of 3.20 µg/mL. This is the first report on the antimicrobial activity of essential oils of *D. cuneata* and *C. tonkinensis*.

The antimicrobial activities of the essential oils of *D. cuneata* and *C. tonkinensis* can be related to their main constituents or some synergy between the major and minor compounds. Essential oil constituents were previously reported to inhibit significantly the growth and cell viability of potential infectious of broad spectrum microorganisms. Nevertheless, the antibacterial effect can be sum up as cumulative actions of several compounds and not to a specific compound [7-11]. Further, due to the complexity of the composition of the essential oils, it is also difficult to explain the mechanism of action of these blends, but is important to underline that the wide variety of composition is a positive factor that may limit the development of resistance which is otherwise very common for synthetic drug. The major constituents of essential oils *D. cuneata* and *C. tonkinensis* such as α-pinene and *β*-pinene have shown antimicrobial activity against infection causing microorganisms such as *S. aureus* [12,13]. Essential oil with large contents of bicyclogermacrene and germacrene D have displayed antimicrobial activity against organisms such as *P. aeruginosa*, *C. albicans* and *S. aureus* with MIC value of 125 mg/mL [14], as well as *B. cereus* and *E. coli* with MIC value of 64 µg/mL [15]. Limonene and camphene were reported to exhibit moderate antimicrobial action against *E. faecalis*, *S.*

aureus, B. cereus and *C. albicans* [16]. The antimicrobial activities of some other compounds present in the essential oils have been reported [7-11, 17], and likely to account for the observed antimicrobial activity.

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Supporting Information

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