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Antimicrobial activity of the essential oils from the leaves and stems of *Amomum rubidum* Lamxay & N. S. Lý

[Actividad antimicrobiana de los aceites esenciales de las hojas y tallos de *Amomum rubidum* Lamxay & N. S. Lý]

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Abstract: This paper described the chemical compositions and antimicrobial activity of the essential oils from the leaves and stem of *Amomum rubidum* Lamxay & N. S. Lý, collected from Bidoup Nui Ba National Park, Lam Dong, Vietnam. The essential oils were obtained by hydrodisitllation method while antimicrobial activity was evaluetd by microdilution broth susceptibility assay. The main constituents of the leaf essential oil were identified as 1,8-cineole (37.7%), δ-3-carene (19.5%) and limonene (16.3%) while δ-3-carene (21.9%), limonene (17.8%) and β-phellandrene (14.6%) dominated in the stem essential oil. The leaf and stem essential oils displayed stronger inhibition of *Pseudomonas aeruginosa* with MIC of 25 µg/mL and 50 µg/mL respectively. The stem essential oil was active against *Candida albicans* (MIC, 50 µg/mL) while both essential oils inhibited the growth of *Fusarium oxysporum* (MIC 50 µg/mL). This is the first report on chemical constituents and antimicrobial activity of the essential oils of *A. rubidum*.

Keywords: *Amomum rubidium*; Essential oil; Monoterpenes; Antimicrobial activity

Resumen: Este artículo describe la composición química y la actividad antimicrobiana de aceites esenciales de las hojas y el tallo de *Amomum rubidum* Lamxay & N. S. Lý recolectados del Parque Nacional Bidoup Nui Ba, Lam Dong, Vietnam. Los aceites esenciales se obtuvieron mediante el método de hidrodisitilación, mientras que la actividad antimicrobiana se evaluó mediante un ensayo de susceptibilidad de caldo de microdilución. Los principales componentes del aceite esencial de la hoja se identificaron como 1,8-cineol (37,7%), δ-3-careno (19,5%) y limoneno (16,3%), mientras que δ-3-careno (21,9%), limoneno (17,8 %) y β-felandreno (14,6%) dominaron en el aceite esencial del tallo. Los aceites esenciales de hoja y tallo mostraron una inhibición más fuerte de *Pseudomonas aeruginosa* con un MIC de 25 µg/mL y 50 µg/mL, respectivamente. El aceite esencial del tallo fue activo contra *Candida albicans* (MIC, 50 µg/mL) mientras que ambos aceites esenciales inhibieron el crecimiento de *Fusarium oxysporum* (MIC 50 µg/mL). Este es el primer informe sobre los componentes químicos y la actividad antimicrobiana de los aceites esenciales de *A. rubidum*.

Palabras clave: *Amomum rubidium*; Aceite esencial; Monoterpenos; Actividad antimicrobiana

INTRODUCTION

The aim of the present study was to examine the chemical constituents and antimicrobial activity of essential oils from the leaves and stem bark of *Amomum rubidium*, grown in Vietnam. This is in reference to our continued interest in the analysis of the chemical constituents of essential oils from *Amomum* plants (Chau *et al*., 2015; Dai *et al.*, 2018; Huong *et al*., 2018) and other poorly studied species of Vietnamese flora (Thai *et al*., 2018). *Amomum* is a genus of plants native to China, the Indian subcontinent, Southeast Asia, New Guinea, and Queensland (Lamxay & Newman, 2012). *Amomum rubidum* Lamxay & N. S. Lý (Syn. *Conamomum rubidum*) is included in the list of threatened and endanger species (Lamxay & Newman, 2012). The plant has been used in traditional medicine for the treatment of mental disorder, inflammation related ailment, microbial infections and also act as insect repellant (Mahood & Hung, 2008).

The chemical constituents of volatiles of several *Amomum* plants have been documented (Chau *et al*., 2015; Dai *et al*., 2018; Huong *et al*., 2018). In addition, the biological activities of essential oils from other species of *Amomum* have been reported. These activities includes larvicidal (Chansang *et al*., 2018; Cotchakaew & Soonwera, 2018; Govindarajan *et al*., 2018), antimicrobial (Supriya & Wakode, 2010*;* Noumi *et al*., 2018), antimutagenic (Pulbutr *et al*., 2012), cytotoxic (Yang *et al*., 2010; Pulbutr *et al*., 2012) and antioxidant (Yang *et al*., 2010).

Our previous investigations into the volatile constituents of *Amomum* plants grown in Vietnam have produced results which have been published. The main class of compounds in the leaves of *A. maximum* (Huong *et al*., 2015), *A. longiligulare* (Chau *et al*., 2015) and *A. aculeatum* (Huong *et al*., 2014) were monoterpene hydrocarbons and sesquiterpene hydrocarbons. The essential oil of *A. gagnepainii* (Huong *et al*., 2018) and the stem of *A. longiligulare* (Chau *et al*., 2015) were dominated by sesquiterpene hydrocarbons and oxygenated counterparts. Monoterpene hydrocarbons occurred in higher quantity in *A. repoense* (Huong *et al*., 2018) and *A. villosum* (Dai *et al*. 2016). Oxygenated monoterpenes and sesquiterpene compounds constituted the bulk of essential oil from the leaves and fruits of *A. muricarpum* (Huong *et al*., 2015). However, fatty acids were identified in quantity in the root of *A. longiligulare* (Chau *et al*., 2015). Information obtained from data on the previous analysis of the chemical constituents of essential oils some *Amomum* plants growing all over the world (Sabulal *et al*., 2006; Yang *et al*., 2010; Li *et al.*, 2011; Satyal *et al.*, 2012) revealed that monoterpene and sesquiterpene compounds predominate. However, the identities of these terpene compounds differed from one species to another.

Till moment no information could be seen on either the constituents of volatile and non-volatile fractions or biological activity of *A. rubidum*. This prompted our interest in the present study.

MATERIALS AND METHODS

Plants collection

The leaves and stem of *A. rubidium* were harvested from mature plants growing in Bidoup Nui Ba National Park, Lam Dong Province on October 2018. Botanical identification was achieved by Dr. Dai, D.N. A voucher specimen DND 749 was deposited at the Botany Museum, NghệAn College of Economics, Vietnam.

Hydrodistillation of the essential *oils*

For this experiment, 3 kg of each of the air-dried and pulverized leaves and stems of *A. rubidum* was used. Essential oils were obtained by separate hydrodistillation conducted in a Clevenger-type apparatus for 3 h at normal pressure according to the Vietnamese Pharmacopoeia (2009). The volatile oils were collected separately into clean weighed sample bottles. The experiment was done in triplicate. The oils were kept under refrigeration (4ºC) until the moment of analyses.

Analysis of the essential *oil samples*

Gas chromatography (GC) analysis was performed on an Agilent Technologies HP 7890A Plus Gas chromatograph equipped with a flame ionization detector (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_2 (1 mL/min); injector temperature and detector temperature were 250ºC and 260ºC respectively; column temperature programmed from 60ºC (held 2 min isothermally) and rises to 220ºC (with 10 min hold) at 4ºC/min. Samples were injected by splitting and the split ratio was 10:1. The volume injected was 1.0 μl. Inlet pressure was 6.1 kPa. The relative proportions of the essential oil constituents were percentages obtained by FID peak area normalization.

An Agilent Technologies HP 7890A Plus Chromatograph fitted with a fused silica capillary

HP-5MS 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 gas chromatography-mass spectrometry (GC/MS) analysis. The GC analysis conditions were the same as described above with He (1 mL/min) as carrier gas. The mass spectrometry (MS) conditions were as follows: ionization voltage 70 eV; emission current 40 mA; acquisitions scan mass range of 35-350 amu at a sampling rate of 1.0 scan/s.

Identification of the constituents of the essential ***oil***

The identification of the constituents of the essential oil was based on comparison of their retention times with those of authentic samples, comparing their linear indices relative to a series of *n*-alkanes (C_6-C_{40}). Further identifications were also made possible by the use of a homemade library of mass spectra built up from pure substances and components of known oils, and MS literature data (NIST, 2011) as described previously (Thai *et al*., 2018).

Antimicrobial activity assay

Eight standardized ATCC strains from laboratory stock cultures were used in the evaluation of the antimicrobial activity of the oils of *A. rubidum*. The Gram negative strains were *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 25923). The Gram positive strains were *Bacillus subtilis* (ATCC 11774) and *Staphylococcus aureus* subsp. *aureus* (ATCC 11632), *Aspergillus niger* (ATCC 9763) and *Fusarium oxysporum* (ATCC 48112). Two strains of yeast, *Candida albicans* (ATCC 10231) and *Saccharomyces cerevisiae* (ATCC 16404) were also used for the experiment. Testing media included Mueller-Hinton Agar (MHA) used for bacteria and Sabouraud Agar (SA) used for fungi. The Minimum inhibitory concentration (MIC) values were measured by the microdilution broth susceptibility assay (van den Bergher & Vlietinck, 1999; Vlietinck, 1999). For the assays, the essential oil was diluted with DMSO and loaded into the microtiter plate with each of the microbial strains. The plate was then incubated overnight at 37ºC. One hundred microlitre of microbial culture of an approximate inoculums size of 1.0 x 10^6 CFU/mL was added to all well and incubated at 37ºC for 24 h. The last row, containing only the serial dilutions of sample without microorganisms, was used as a negative control. Sterile distilled water and DMSO served as a positive control. The MIC values were determined as the lowest concentration of the test sample that completely inhibits the growth of microorganisms. All measurements were performed in triplicate.

Statistical analysis

The mean and standard deviation of three experiments were determined. Statistical analysis of the differences between mean values obtained for experimental groups were calculated as a mean standard deviation (SD) of three independent measurements using Microsoft excel program, 2003.

RESULTS AND DISCUSSION

Chemical constituents of the essential oil

The average yields of essential oils were 0.22% and 0.15% (v/w ± 0.01) for the leaves and stems respectively, calculated on a dry weight basis. Both essential oil was light yellow coloured. The volatile compounds were displayed in Table No. 1, along with their percentages and retention indices calculated on HP-5MS column. The thirty compounds present in the leaf essential oil accounted for 99.1% of the essential oil contents. The main classes of compounds identified in the essential oil were monoterpene hydrocarbons (52.1%) and oxygenated monoterpenes (43.2%). The sesquiterpene hydrocarbons were less common (1.5%) while the oxygenated counterpart was not detected. The main constituents of the essential oil were identified as 1,8-cineole (37.7%), δ-3-carene (19.5%) and limonene (16.3%). Few of other significant compounds present in the essential oil include α-pinene (3.0%), myrcene (2.7%), o-cymene (2.5%), α-terpineol (2.5%), α-phellandrene (2.4%) and terpinolene (2.0%). Other significant constituents of the essential oil were α-pinene (5.8%), β-phellandrene (4.7%), o-cymene (3.8%), myrcene (3.4%), α-humulene (2.9%) and terpinolene (2.8%). The abundance of monoterpene hydrocarbon compounds confers qualitative similarity with the components present identified in leaf essential oil of *A. villosum* (Dai *et al*., 2016) and *A. repoense* (Huong *et al*., 2018).

However, the identities of these compounds differ from one another. The abundance of terpene compounds in the essential oil of A. rubidium is in agreement with previous findings on the essential oil compositions of other Amomum plants (Huong *et al*., 2014; Huong *et al*., 2015; Chau *et al*., 2015; Dai *et al*., 2016; Dai *et al*., 2018; Huong *et al*., 2018).

Table N° 1
Compounds identified in the essential oils of *A. rubidum*

Sr. No	RT (min)	Compounds [a]	RI[b]	RI[c]	Percent composition (SD ±)[d]	
					Leaf	Stem
1	8.97	2-Heptanol	900	890	0.1	-
2	10.14	α-Pinene	939	932	3.0	5.8
3	10.56	α-Fenchene	953	946	0.4	0.4
4	10.64	Camphene	955	950	0.6	0.6
5	11.51	β-Pinene	984	978	0.2	1.2
6	11.75	Myrcene	992	988	2.7	3.4
7	12.35	α-Phellandrene	1011	1004	2.4	4.7
8	12.56	δ-3-Carene	1017	1014	19.5	21.9
9	12.75	α-Terpinene	1022	1022	0.9	1.1
10	12.91	p-Cymene	1027	1024	-	0.3
11	13.03	o-Cymene	1030	1026	2.5	3.8
12	13.19	Limonene	1035	1030	16.3	17.8
13	13.25	β-Phellandrene	1037	1032	-	14.6
14	13.32	1,8-Cineole	1039	1036	37.7	1.9
15	13.68	(*E*)-β-Ocimene	1049	1043	0.2	0.7
16	14.16	γ-Terpinene	1064	1060	1.4	1.0
17	15.17	2-Nonanone	1093	1092	0.3	-
18	15.22	Terpinolene	1095	1094	2.0	2.8
19	15.31	Fenchone	1097	1096	0.2	-
20	15.47	2-Nonanol	1102	1100	1.7	0.2
21	15.53	Linalool	1103	1102	0.6	-
22	16.12	p-Ethylanisol	1120	1122	0.2	-
23	16.24	*endo*-Fenchol	1124	1124	0.3	-
24	17.55	Camphene hydrate	1161	1165	0.2	-
25	17.84	p-Mentha-1,5-dien-8-ol	1169	1170	-	0.1
26	18.13	Borneol	1178	1178	0.7	-
27	18.48	Terpinen-4-ol	1187	1180	0.8	-
28	18.80	Cryptone	1197	1192	-	0.3
29	18.94	α-Terpineol	1201	1200	2.5	0.2
30	21.17	(2*E*)-Decenal	1265	1264	-	0.3
31	22.40	Dihydroedulan	1301	1302	0.2	0.3
32	22.59	Terpinen-4-ol acetate	1307	1310	-	0.2
33	24.25	α-Terpinyl acetate	1357	1356	-	0.1
34	25.32	α-Copaene	1389	1387	0.2	1.2
35	26.66	α-Santalene	1431	1431	0.2	0.3
36	26.84	β-Caryophyllene	1437	1435	0.2	1.0
37	27.93	α-Humulene	1472	1475	0.4	2.9
38	28.53	γ-Muurolene	1491	1490	-	0.3
39	28.76	Germacrene D	1498	1498	-	0.2
40	28.87	Aristolochene	1502	1500	-	0.3

41	28.97	β-Selinene	1505	1502	-	0.3
42	29.15	*trans*-Muurola-4(14),5-diene	1511	1512	-	0.5
43	29.20	(*E*,*E*)-α-Farnesene	1513	1513	0.5	0.3
44	29.24	α-Muurolene	1514	1515	-	0.2
45	29.36	β-Bisabolene	1518	1520	-	0.3
46	29.74	γ-Cadinene	1531	1531	-	0.1
47	29.93	δ-Cadinene	1537	1535	-	0.4
48	30.00	*cis*-Calamenene	1539	1540	-	1.7
49	30.27	*trans*-Cadina-1,4-diene	1549	1545	-	0.3
50	30.94	(*E*)-Nerolidol	1571	1569	-	0.4
51	31.77	Spathulenol	1599	1600	-	0.1
52	31.96	Caryophyllene oxide	1605	1602	-	0.6
53	32.73	Humulene Epoxide II	1632	1634	-	1.1
54	33.16	1-*epi*-Cubenol	1648	1650	-	0.2
55	33.58	*epi*-α-Cadinol	1662	1662	-	0.3
56	33.93	α-Cadinol	1675	1670	-	0.2
57	34.02	ar-Turmerone	1678	1678	-	0.5
58	35.07	Curlone	1716	1714	-	0.2
Total					**99.1**	**97.4**
Monoterpene hydrocarbons (Sr. No: 2-13, 15, 16, 18)					**52.1**	**80.1**
Oxygenated monoterpenes (Sr. No: 14, 19, 21, 23-29, 31-33)					**43.2**	**3.1**
Sesquiterpene hydrocarbons (Sr. No: 34-49)					**1.5**	**10.3**
Oxygenated sesquiterpenes (Sr. No: 50-58)					**-**	**3.5**
Non-terpenes (Sr. No: 1, 17, 20, 22, 30)					**2.3**	**0.5**

[a] Elution order on HP-5MS column; RT, Retention time on HP-5MS column; [b] Retention indices on HP-5 column; [c] Literature retention indices (NIST, 2011); [d] Standard deviation were insignificant and excluded from the Table to avoid congestion; Sr. No. serial Number; - Not identified

Antimicrobial test

The essential oils from the leaves displayed stronger inhibition of *P. aeruginosa* with MIC of 25 μg/mL. Likewise the stem bark essential oil was active against *P. aeruginosa* and *C. albicans* (MIC, 50 μg/mL). Both essential oil inhibited the growth of *F. oxysporum* with MIC of 50 μg/mL. MIC values > 50 μg/mL were recorded for other tested microorganisms (Table No. 2). In the test experiment, MIC > 50 μg/mL is considered inactive towards the tested microorganism. The observed antimicrobial result of *A. rubidium* essential oil was in agreement with previous information that some *Amomum* essential oil selectively inhibited the growth of different microorganisms. The results in this study are comparable with data obtained on the antimicrobial actions of other *Amomum* essential oil reported in literature. The leaf and fruit essential oils of *A. subulatum* showed good results against *B. pumilus, S. epidermidis, P. aerugenosa* and *S. cerevisiae* (Supriya & Wakode, 2010; Noumi *et al*., 2018). The rind essential oil of *A. subulatum* was only active against *A. niger* (Satyal *et al*., 2012). Also, essential oil hydrodisitlled from the fruits of *A. cannicarpum* exhibited potent antimicrobial activity against *Salmonella typhi, P. aeruginosa, Proteus vulgaris, C. albicans* and *C. glabrata* (Sabulal *et al*., 2006). In addition, the leaf essential oil showed significant antimicrobial activity against *C. albicans* and *Aspergillus fumigates* (Mathew *et al*., 2006). The essential oil from *A. uliginosum* fruit displayed antibacterial activity against *E. coli* and *S. aureus* (Pulbutr *et al*., 2012). The essential oil of *A. tsao-ko* exerted the strongest bactericidal activity against *S. aureus* (Yang *et al*., 2008; Li *et al*., 2011; Guo *et al*., 2017). There is a report describing the antibacterial activity of essential oil from the fruits of *A. kravanh* against some tested pathogens (Diao *et al*., 2014).

Table No. 2
Antimicrobial activity of the essential oils of *A. rubidium*

Organisms	Minimum inhibitory concentration (MIC, µg/mL)	
	Leaf oil	**Stem oil**
E.coli (ATCC 25922)	> 50	> 50
P. aeruginosa (ATCC 25923)	25 ± **0.10**	50 ± **0.20**
B. subtilis (ATCC 11774)	> 50	> 50
S. aurues subsp. *aureus* (ATCC 11632)	> 50	> 50
A. niger (ATCC 9763)	> 50	> 50
F. oxysporum (ATCC 48112)	50 ± **0.25**	50 ± **0.30**
S. cerevisiae (ATCC 10231	> 50	> 50
C. albicans (ATCC 16404)	> 50	50 ± **0.10**

>50 µg/mL, No activity

The antimicrobial activities *of* the essential oil *of A. rubidium* can be related to its main compounds. Limonene (Bevilacqua *et al*., 2010; Espina *et al*., 2013; Lu *et al*., 2016; Noumi *et al*., 2018; Pathirana *et al*., 2018) and, α-phellandrene (Iscan *et al*., 2012; Zhang *et al*., 2017) and δ-3-carene (Swamy *et al*., 2016), three of the major components in *A. rubidum* essential oils of this study, were previously reported to be able to inhibit significantly the growth and cell viability of potential infectious of broad spectrum microorganisms. 1,8-Cineole also exert and produced antimicrobial effects on many microorganisms (Li *et al*., 2014; Simsek & Duman, 2017; Aldoghaim *et al*., 2018). Essential oils containing high amounts of 1,8-cineole and cineole itself were reported to be potent antimicrobial agents (Mulyaningsih *et al*., 2011).

The efficacy of essential oils derived from Vietnamese plants and other parts of the world as antimicrobial agents have been documented. Essential oil from the pseudo-stem of *Zingiber castaneum* inhibited the growth of *P. aeruginosa* (MIC 12.5 µg/mL), *A. niger* (MIC 50 µg/mL) and *F. oxysporum* (MIC 50 µg/mL) while the leaf of *Z. nitens* showed microbial action against *P. aeruginosa* with MIC of 50 µg/mL (Huong *et al*., 2020). Also, *Z. zerumbet* rhizome and leaf essential oils exhibited potent microbial action against *A. niger* MIC of 50 and 25 µg/mL respectively (Huong *et al*., 2019). The essential oil of *Cyperus distans* from Nigeria showed antimicrobial action against *E. coli* (MIC 0.31 µg/mL), *S. aurues* (MIC 0.63 µg/mL) and *Kliebsiella* spp (MIC 0.63 µg/mL) while MIC of 0.31 µg/mL, 0.63 µg/mL and 0.63 µg/mL respectively were displayed against the pathogens by *C. rotundus* essential oil (Lawal *et al*., 2016). *Pachira glabra* (Lawal *et al*., 2014) was reported to have exhibited strong action towards the growth of *E. coli* (MIC 0.30 mg/mL), *B. subtilis* (MIC 0.61 mg/mL), *E. coli* (MIC 1.3 mg/mL) and *Salmonella* spp (MIC 1.3 µg/mL). *Casuarina equisetifolia* essential oil from Nigeria (Essien *et al*., 2016) exhibited stronger antibacterial effect on *B. cereus* (MIC 39 µg/mL), *S. aureus* (MIC 39 µg/mL) and *E. coli* (MIC 312 µg/mL) while *A. niger* showed more susceptibility to *F. mucosa* volatile oil (MIC 78 µg/mL). The essential oils *Origanum vulgare*, *Thymus vukgaris*, *Citrus limon* and *Lavandula angustifolia* from Romania were most active against a number of pathogens (Man *et al*., 2019). In recent years, greater attention has been paid to the screening of antimicrobial activity from essential oil as source of developing new antimicrobial agents to combat microbial resistance. Review articles describing the antimicrobial potentials of essentials from other parts of the world have been published (Chouhan *et al*., 2017)

CONCLUSION

The present work highlights, for the first time the chemical constituetns and antimicrobial potentials of essential oil hydrodisitlled from the leaves and stems of endemic species, *A. rubidium.* The essential oil exhibited dose dependent activities thus validating and reinforcing the traditional uses ascribed to the plant. The antimicrobial activity and fragrance of these essential oils should make them to be potential for the use in health-care system.

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