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Chemical composition and antimicrobial activity of essential oils extracted from *Amomum muricarpum* Elmer from North Vietnam

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Abstract. *Recent years have seen the development of bacterial resistance to currently available antibiotics, which necessitates a search for new antimicrobial agents. Amomum muricarpum Elmer is a widely used medicinal plant species in the genus Amomum (family Zingiberaceae) that is commonly found in Laos, the Philippines, China, and Vietnam. The present article describes the chemical composition and antimicrobial activity of essential oils extracted from the leaves and rhizomes of A. muricarpum from North Vietnam. The hydrodistilled essential oil was analyzed using gas chromatography and gas chromatography-mass spectrometry, with the broth microdilution method designed to evaluate its antimicrobial efficacy. The absolute yield of essential oils amounted to 0.11% and 0.13% (v/w) for leaves and rhizomes, respectively, on a dry weight basis. It was found that the leaves and rhizomes of A. muricarpum produce oils abounding in monoterpenes. Of the total identified volatile components in the leaf oil (97.18%), three main constituents include α-pinene (40.45%), linalool (12.34%), and β-pinene (10.31%). In the rhizome oil, the main constituents include α-pinene (48.10%), β-pinene (20.32%), and linalool (7.56%) of the total identified volatile components (98.08%). An antimicrobial activity test indicates that essential oils from the leaves and rhizome of A. muricarpum inhibit the growth of Staphylococcus aureus ATCC 25923, with a minimum inhibitory concentration (MIC) of 200 μg/ml. In addition, the rhizome essential oil also exhibits antimicrobial activity against Bacillus cereus ATCC 14579, with a MIC value of 200 μg/ml. The results indicate the potential of essential oils extracted from A. muricarpum as a source of antimicrobial agents.*

Keywords: *Amomum muricarpum, Zingiberaceae, essential oil, monoterpenes, α-pinene, antimicrobial activity*

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ФИЗИКО-ХИМИЧЕСКАЯ БИОЛОГИЯ

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Химический состав и антимикробная активность эфирных масел *Amomum muricarpum* Elmer из Северного Вьетнама

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Аннотация. *В последние годы развитие устойчивости бактерий к антибиотикам потребовало поиска новых противомикробных средств. Amomum muricarpum Elmer – это вид рода Amomum семейства Zingiberaceae (Имбирные), распространенный в Лаосе, Филиппинах, Китае и Вьетнаме, широко используемый как лекарственное растение. В данной статье описан химический состав и антимикробная активность эфирных масел из листьев и корневищ A. muricarpum, собранных в Северном Вьетнаме. Эфирное масло, полученное гидродистилляцией, было проанализировано с использовани-*

ем газовой хроматографии и газовой хроматографии-масс-спектрометрии, тогда как анализ с микроразбавлением бульона был разработан для оценки его антимикробной эффективности. Абсолютный выход эфирных масел составил 0,11% и 0,13% (об/масс.) соответственно для листьев и корневищ в пересчете на сухой вес. Анализ масел из листьев и корневищ A. muricarpum показал, что в масле преобладают монотерпены. В масле листьев из общего количества идентифицированных летучих компонентов (97,18%) три составляющих – α-пинен (40,45%), линалоол (12,34%) и β-пинен (10,31%), являлись основными. В масле корневища основными из общего количества идентифицированных летучих компонентов (98,08%) являлись следующие: α-пинен (48,10%), β-пинен (20,32%) и линалоол (7,56%). Тест на антимикробную активность показал, что эфирные масла из листьев и корневища A. muricarpum подавляют рост Staphylococcus aureus ATCC 25923 со значением минимальной ингибирующей концентрации (МИК) 200 мкг/мл. Кроме того, эфирное масло корневища проявляло антимикробную активность и в отношении Bacillus cereus ATCC 14579 со значением МИК 200 мкг/мл. Результаты указывают на потенциал эфирных масел A. muricarpum как источника противомикробных агентов.

Ключевые слова: *Amomum muricarpum, семейство Имбирные, эфирное масло, монотерпены, α-пинен, антимикробная активность*

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INTRODUCTION

Since immemorial times, it has been known that the chemical constituents of essential oils of various plants are biologically and pharmacologically active natural substances [1, 2]. Therefore, greater attention has been paid to the screening of essential oils for their biological activity, as a source of developing new therapeutic agents for the prevention and amelioration of natural ailments caused by microorganisms [3, 4]. In continuation of our research on the chemical compounds and biological activities of essential oils from Vietnamese Zingiberaceae plants, we report our findings on the chemical composition and antimicrobial activity of essential oils of *Amomum muricarpum*.

Amomum is a large genus in the Zingiberaceae family distributed in Asia, Africa, and Australia with about 180 species [5]. *Amomum* plants have been described as sources of biologically active components [4, 6–8]. *A. muricarpum* is a medicinal plant that can grow up to 2.5 m tall[1]. Phytochemical investigation of *A. muricarpum* led to the iden-tification of diarylheptanoids [9, 10]. Previously, the compositions and biological activities of essential oils from various parts of *A. muricarpum* from Central Vietnam were determined and reported [11–14]. Studies noted the effects of geographical and envi-ronmental factors, on the composition and quality of the essential oil [15–17]. This article will provide new data on the chemical composition and antimicrobial activety of essential oils extracted from *A. mu-ricarpum*, which was grown in North Vietnam.

MATERIALS AND METHODS

Plant material. The plant parts used for this study namely the leaves and rhizomes of *A. muricarpum* were collected from Na Hang, Tuyen Quang Province, Northern Vietnam in July 2018. Botanical identification was performed by Assoc. Prof. Dr. Dau Ba Thin. Leaves and rhizomes of *A. muricarpum* were dried at room temperature (25 ºC) for one week before hydrodistillation.

Hydrodistillation of essential oils. Essential oils were obtained from leaves and rhizomes of *A. muricarpum* (two kilogram for each extraction) by hydrodistillation using a Clevenger-type apparatus for 4 h at normal pressure according to the pro-cedure of the Vietnamese Pharmacopoeia[2]. The process of hydrodistillation using a Clevenger type apparatus has obtained a mixture of oil with a quantity of water. To remove water, the extracted essential oils were then dried by adding anhydrous sodium sulfate-Na_2SO_4. The absorption of water into the sodium sulfate is complete in seconds, causing the grains to coagulate. If additional sodium sulfate is added, and the grains do not coagulate, then the oil is essentially anhydrous. The obtained oils were stored in dry amber vials at 4 ºC until analysis. All measurements were performed in triplicate.

Analysis of essential oils. Gas chromatography (GC) analysis was performed on Agilent GC 7890A equipped with a FID and fitted with HP-5MS column (30 m × 0.25 mm, film thickness 0.25 µm, Agilent Technology). The analytical conditions were: carrier gas Helium (1 ml/min), injector temperature (PTV) 250 ºC, detector temperature 260 ºC, column temperature programmed from 60 ºC (2 min hold) to 220 ºC (10 min hold) at the heating rate 4°C/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.

[1]Nguyen T.B. *Flora of Vietnam. Vol. 1*. Hanoi: Science and Technology Publishing House, 2000.
[2]Vietnamese Pharmacopoeia. Medical Publishing House, 2nd Edition, Hanoi, Vietnam, 2009.

An Agilent GC 7890A chromatograph fitted with a fused silica capillary HP-5MS column (30 m × 0.25 mm, film thickness 0.25 μm) and interfaced with a mass spectrometer HP 5973 MSD was used for the GC/MS analysis, under the same conditions as those used for GC analysis. The conditions were the same as described above with Helium (1 ml/min) as carrier gas. The MS conditions were as follows: ionization voltage 70 eV; emission current 40 mA; scan mass range of 35–350 amu at a sampling rate of 1.0 scan/s.

The identification of constituents from the GC/MS spectra of *A. muricarpum* was performed on the basis of retention indices (RI) determined with reference to a homologous series of *n*-alkanes, under identical experimental conditions, co-injection with standards (Sigma-Aldrich, St. Louis, MO, USA) or known essential oil constituents, MS library search[3] and as described in previous studies [11–14].

Antimicrobial screening. Antimicrobial activity of *A. muricarpum* essential oil was carried out on three Gram-negative bacteria, Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, and Salmonella enterica ATCC 13076; three Gram-positive bacteria, Enterococcus faecalis ATCC 299212, Staphylococcus aureus ATCC 25923, and Bacillus cereus ATCC 14579; and the yeast, Candida albicans ATCC 10231, using the microdilution broth susceptibility assay as previously described [18]. Testing media included Mueller-Hinton Agar (MHA) used for bacteria and Sabouraud Agar (SA) used for fungi. The minimum inhibitory concentration (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. All results of chemical composition and antimicrobial experiments were repeated three times and are expressed as mean ± standard deviation (SD).

RESULTS AND DISCUSSION

Yields and chemical constituents of essential oils. Hydrodistilled essential oils from the leaves and rhizomes of *A. muricarpum* are analyzed by GC/MS. The yields of the essential oils were 0.11 and 0.13% (v/w, ±0.01) respectively for the leaf and rhizome of *A. muricarpum*. All the essential oils were yellow coloured. The identities of the compounds of *A. muricarpum* oils, their per cent compositions and retention indices on HP-5MS column could be seen in Table 1.

A total of 45 compounds amounting to 97.18% in the *A. muricarpum* leaf essential oil were identified (Table 1). Among these 59.53% were monoterpene hydrocarbons, 17.71% were oxygenated monoterpenes, and it also contained 17.59% sesquiterpene hydrocarbons and 2.14% oxygenated sesquiterpenes. The major constituents in the *A. muricarpum* leaf essential oil were α-pinene (40.45%), linalool (12.34%), and β-pinene (10.31%). Comparing our results with those obtained by previous studies showed that all the leaf essential oils extracted are similar with α-pinene and β-pinene predominating [11–13]. However, although 1,8-cineole was the second major component in the previous studies [12, 13], this component was not detected in the leaf oil of *A. muricarpum* in the present study. Furthermore, linalool (12.34%) was found at relatively high amounts in the leaf oil of *A. muricarpum* in the present study (Table 1), while this component was in much lower amounts in the previous studies [12, 13]. The variations in chemical constituents can likely be attributed to the different geographical collection sites as well as climatic factors.

In the essential oil extracted from *A. muricarpum* rhizome, 44 compounds were identified, corresponding to 98.08% of the total oil (Table 1). It is comprised of monoterpene hydrocarbons (75.83%), oxygenated monoterpenes (10.76%), sesquiterpene hydrocarbons (10.52%), and oxygenated sesquiterpenes (0.81%). The main constituents in the *A. muricarpum* rhizome essential oil were α-pinene (48.10%), β-pinene (20.32%), and linalool (7.56%). To the best of our knowledge, there are several reports on the chemical composition of *A. muricarpum* rhizome oil [11–14]. Most of these reports indicate that α-pinene and β-pinene are the main and/or characteristic constituents of rhizome oil. The findings on the major components of *A. muricarpum* rhizome oil were in agreement with the previous reports except for linalool, which was found to be 7.56% in our study. As highlighted previously, this difference can also be attributed to growth, genetics, and climatic conditions.

Antimicrobial Activity. The antimicrobial activities of essential oils from the leaf and rhizome of *A. muricarpum* were estimated by means of the microdilution broth method and the results are expressed as the minimum inhibitory concentration (MIC) in Table 2. The rhizomes oil had moderate bactericidal activities against *S. aureus* and *B. cereus* with the MIC value of 200 μg/mL. The leaves oil only exhibited antimicrobial action against the growth of *S. aureus* with a MIC value of 200 μg/mL. The observed antimicrobial result of *A. muricarpum* essential oils was in agreement with previous information that Amomum essential oils from Vietnam and other parts of the world selectively inhibited the growth of different microorganisms [4, 6, 7, 14].

In general, the antibacterial activities of essential oils could be attributed to the most abundant components or the synergistic effects between its major components and minor ones in the oils

[3]National Institute of Science and Technology. NIST Chemistry Webbook // Data from NIST Standard Reference Database 69, 2018.

Table 1. Chemical constituents of essential oils from the leaves and rhizomes of *A. muricarpum*

Таблица 1. Химический состав эфирных масел из листьев и корневищ *A. muricarpum*

Compound name[a]	RI[b]	RI[c]	Percentage composition[d]	
			Leaves	Rhizomes
Tricyclene	928	927	0.15	0.21
α-Pinene	939	932	40.45	48.10
Camphene	955	954	1.15	0.96
β-Pinene	980	979	10.31	20.32
β-Myrcene	990	988	3.05	1.46
α-Phellandrene	1006	1003	0.42	0.22
δ-3-Carene	1010	1007	–	0.32
α-Terpinene	1017	1014	–	0.21
p-Cymene	1026	1020	0.15	–
β-Phellandrene	1028	1024	1.52	0.41
Limonene	1032	1024	0.45	0.23
1,8-Cineole	1035	1030	–	0.14
(*Z*)-β-Ocimene	1045	1032	0.17	–
(*E*)-β-Ocimene	1051	1044	–	0.23
γ-Terpinene	1061	1056	0.50	0.85
α-Terpinolene	1091	1086	1.21	2.31
Linalool	1100	1095	12.34	7.56
Borneol	1166	1165	0.21	0.30
Terpinen-4-ol	1177	1174	–	0.51
α-Terpineol	1189	1187	–	0.23
Fenchyl acetate	1228	1225	0.54	0.11
Geraniol	1253	1249	1.24	0.23
Bornyl acetate	1289	1287	0.12	0.56
Bicycloelemene	1327	1325	0.98	–
α-Cubebene	1351	1345	0.35	–
α-Copaene	1377	1374	0.62	0.59
Geranyl acetate	1381	1380	3.26	1.12
β-Bourbonene	1385	1384	–	0.28
β-Elemene	1391	1398	–	0.53
α-Gurjunene	1412	1409	0.13	0.11
β-Caryophyllene	1419	1417	1.95	0.72
α-Santalene	1427	1427	0.61	–
γ-Elemene	1430	1437	0.27	0.47
trans-α-Bergamotene	1435	1431	0.23	–
Aromadendrene	1441	1439	–	0.35
(*Z*)-β-Farnesene	1443	1440	0.56	0.89
α-Humulene	1454	1452	0.42	0.12
β-Santalene	1457	1457	–	0.15
Valencene	1473	1470	0.60	–
γ-Gurjunene	1477	1475	0.15	–
Germacrene D	1490	1484	0.27	0.89
α-Selinene	1493	1498	1.93	1.24
Bicyclogermacrene	1500	1500	3.47	0.95
(*E,E*)-α-Farnesene	1508	1505	2.24	1.22
γ-Cadinene	1514	1513	0.24	0.54
trans-γ-Bisabolene	1516	1514	0.21	0.14
α-Panasinsene	1518	1518	0.17	–
β-Sesquiphellandrene	1524	1521	0.56	–
δ-Cadinene	1525	1522	1.63	0.23
Calacorene	1546	1540	–	0.28
Germacrene B	1561	1559	–	0.84
(*E*)-Nerolidol	1563	1561	0.27	0.49
Spathulenol	1578	1577	0.31	–
Guaiol	1601	1601	0.34	–
α-Cadinol	1654	1652	0.52	–
(*E,E*)-Farnesol	1718	1718	0.70	0.32
Phytol	2125	2124	0.21	0.16
Total			97.18	98.08
Monoterpene hydrocarbons			59.53	75.83
Oxygenated monoterpenes			17.71	10.76
Sesquiterpene hydrocarbons			17.59	10.52
Oxygenated sesquiterpenes			2.14	0.81
Others			0.21	0.16

[a]Elution order on HP-5MS column; [b]Retention indices on HP-5MS column; [c]Literature retention indices; [d]Standard deviation were insignificant and excluded from the Table to avoid congestion; "–" – Not identified.

Table 2. Antimicrobial activity of *A. muricarpum* essential oils

Таблица 2. Антимикробная активность эфирных масел *A. Muricarpum*

Microorganisms	Minimum inhibitory concentration (MIC, μg/mL)	
	Leaves	Rhizomes
Escherichia coli ATCC 25922	na	na
Pseudomonas aeruginosa ATCC 27853	na	na
Salmonella enterica ATCC 13076	na	na
Enterococcus faecalis ATCC 299212	na	na
Staphylococcus aureus ATCC 25923	200.0±0.231	200.0±0.147
Bacillus cereus ATCC 14579	na	200.0±0.325
Candida albicans ATCC 10231	na	na

Note: na – no activity.

[19, 20]. As mentioned above, α-pinene, β-pinene and linalool were found to be the most important components in the leaf and rhizome oils of *A. muricarpum*. The individual components the essential oils such as α-pinene, β-pinene and linalool have been determined for antimicrobial activity, and the results indicated that these compounds exhibited inhibitory effects against microorganisms [20–22]. In addition, the *A. muricarpum* essential oil showed better antimicrobial activity against the Gram-positive bacteria than the Gram-negative bacteria. According to previous studies, this is attributed to the existence of cell wall lipopolysaccharides in the Gram-negative bacteria, which can inhibit the hydrophobic essential oil constituents from diffusing into the cells [22, 23].

CONCLUSIONS

In summary, this study provides information on the chemical composition and antimicrobial activity of essential oils from the leaves and rhizomes of *A. muricarpum*. According to GC/MS analyses the major components of leaf oil were α-pinene (40.45%), linalool (12.34%), and β-pinene (10.31%), while rhizome oil consists mainly of α-pinene (48.10%), β-pinene (20.32%), and linalool (7.56%). Also, these compounds may be thought of as the contributing factor to the observed antimicrobial activity of the essential oils against *S. aureus* and *B. cereus*. Thus, the *A. muricarpum* essential oils may be sources of promising antimicrobial agents.

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