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Chemical constituents and antibacterial activity of essential oils in *Amomum longiligulare* from Vietnam

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This paper reports the chemical constituents and the antibacterial activity of essential oils from the leaves, rhizomes, and fruits of *Amomum longiligulare* T.L. Wu (Zingiberaceae) obtained by microwave-assisted hydrodistillation. The essential oils were analyzed by gas chromatography–mass spectrometry techniques. The minimum inhibitory concentration (MIC) values were measured by the broth microdilution assay. The oil yields of leaves, rhizomes and fruits from *A. longiligulare* were 0.23%, 0.27% and 1.93% (v/w), respectively, calculated on a dry weight basis. The leaf essential oil comprised mainly *α*-humulene (28.4%), *α*-pinene (24.9%), *β*-caryophyllene (17.3%), humulene epoxide II (7.3%), and *β*-pinene (4.7%). The major compounds of the rhizome essential oil were *β*-caryophyllene (28.7%), bicyclogermacrene (17.1%), humulene epoxide II (10.5%), camphene (7.9%), and *α*-pinene (5.7%). Camphor (40.7%) and bornyl acetate (34.2%) were the main constituents of the fruit oil. The essential oils demonstrated antimicrobial activities against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, and *Pseudomonas aeruginosa* with the MIC values ranging from 200 to 400 μg/mL. In summary, the *A. longiligulare* essential oils are a source of promising antibacterial agents. This is the first report on the chemical composition and antibacterial activity of *A. longiligulare* essential oil obtained by microwave-assisted hydrodistillation.

Keywords: Zingiberaceae, essential oil, antibacterial activity, microwave-assisted hydrodistillation

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Химический состав и антибактериальная активность эфирных масел *Amomum longiligulare* из Вьетнама

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В этой статье сообщается о химическом составе и антибактериальной активности эфирных масел из листьев, корневищ и плодов *Amomum longiligulare* T.L. Wu (Zingiberaceae), полученных путем гидродистилляции с помощью микроволновой печи. Эфирные масла анализировали методами газовой хроматографии – масс-спектрометрии. Значения минимальной ингибирующей концентрации (МИК) измеряли с помощью анализа микроразведений в бульонной среде. Выход масла из листьев, корневищ и плодов *A. longiligulare* составлял 0,23%, 0,27% и 1,93% (об./вес.) соответственно в расчете на сухую массу. Эфирное масло листьев состоит в основном из α-гумулена (28,4%), α-пинена (24,9%), β-кариофиллена (17,3%), эпоксида гумулена II (7,3%) и β-пинена (4,7%). Основными соединениями эфирного масла корневища были β-кариофиллен (28,7%), бициклогермакрен (17,1%), эпоксид гумулена II (10,5%), камфен (7,9%) и α-пинен (5,7%). Камфора (40,7%) и борнилацетат (34,2%) были основными составляющими масла плодов. Эфирные масла продемонстрировали антимикробную активность в отношении *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* и *Pseudomonas aeruginosa* со значениями МИК от 200 до 400 мкг/мл. Таким образом, эфирные масла *A. longiligulare* являются источником многообещающих антибактериальных средств. Это первое сообщение о химическом составе и антибактериальной активности эфирного масла *A. longiligulare*, полученного путем гидродистилляции с помощью микроволновой печи.

Ключевые слова: Zingiberaceae, эфирное масло, антибактериальная активность, гидродистилляция с помощью микроволновой печи

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Introduction

Medicinal and aromatic plant species have been widely exploited as food flavorings, medicinal agents, preservatives, and decorations, as well as aesthetic and personal enjoyment items, becoming natural alternatives that provide dependability, safety, and sustainability (Inoue et al., 2019; Ibáñez et al., 2021). Essential oils are obtained from the different plant parts, and they are extracted by different techniques (Irshad et al., 2020). Numerous efforts are made to explore the usage of essential oils as a supernumerary treatment to pharmaceutical remedies against various infectious diseases (Irshad et al., 2020). Essential oils are used in the aromatherapy and act as antioxidants, antimicrobial and antifungal coutermeasures, pain relievers, anxiety mitigators, and antidepressants (Dosoky, Setzer, 2018; Valdivieso-Ugarte et al., 2019; Irshad et al., 2020).

The genus *Amomum* (family Zingiberaceae) incorporates about 180 species distributed in Africa, tropical Asia, Australia, and the Pacific Islands (Lamxay, Newman, 2012; Chau et al., 2015; Thinh et al., 2021). *Amomum longiligulare* T.L. Wu is a precious medicinal plant of the genus *Amomum*. It is a plant that grows up to 1–1.5 m, with a glabrous petiole and lanceolate leaf (Chau et al., 2015). *A. longiligulare* contains five main classes of chemical components, including saponins, flavonoid glycosides, organic acids, inorganic components and especially volatile oil whose content is about 1.7–3% (Anh et al., 2020). The fruit is used as a spice ingredient in Vietnam, China, and Taiwan. In addition, *A. longiligulare* is used to treat indigestion and stomach burn and other colds, diarrhea, vomiting, threatened abortion, dysentery, toothache, and oedema (Chau et al., 2015). Microwave-assisted hydrodistillation is an emerging technology in the extraction and purification of the volatile fractions of plants, and is far more efficient than classic hydrodistillation (Mollaei et al., 2019; Lamberti et al., 2021). Therefore, the current study was conducted to analyze the chemical composition and antibacterial activity of the essential oils of *Amomum longiligulare* obtained by microwave-assisted hydrodistillation.

Materials and methods

Plant material

Leaves, rhizomes and fruits of *Amomum longiligulare* were used as experimental raw material in this study. The materials were collected in Quang Nam Province, Vietnam, in August 2020. All samples were air-dried for two weeks and then crushed using a laboratory mill.

Essential oils extraction

A Sharp R-205VN microwave oven was connected to a Clevenger apparatus that had been modified for microwave-assisted hydrodistillation. The extraction procedure followed the method previously described (Lamberti et al., 2021). In a typical microwave-assisted hydrodistillation procedure performed at atmospheric pressure, 250 g of each sample was placed in a 1 L flask containing 500 mL of deionized water. The microwave oven was operated at 800 W for 1 h. To remove water, the extracted essential oils were then dried over anhydrous sodium sulfate, weighed and stored in amber vials at 4°C until they were used for analysis. All measurements were performed in triplicate.

Gas chromatography–mass spectrometry (GC–MS)

The essential oil composition was determined by means of gas chromatography–mass spectrometry (GC–MS) using an Agilent 7890A chromatograph coupled with an HP 5973 MSD mass spectrometer. A capillary column (HP-5MS) (30 m × 0.25 mm id, film thickness 0.25 μm) and ionization energy of 70 eV were used. The temperature for the analyses was initiated at 60°C and subsequently increased to 220°C at a rate of 4°C/min. Injector and detector temperatures were 250°C and 260°C, respectively. The volume of the injected sample was 1 μL while the helium carrier gas was maintained at a flow rate of 1.0 mL/min. The sector mass analyzer was set to scan from 35 to 350 amu. The apparatus was controlled by a ChemStation computer system. Identification of chemical components was based on comparing the retention indices from the analysis of the chromatograms obtained for each oil sample with the standards of *n*-alkanes (C_4-C_{40}) with linear interpolation using the Van den Dool and Kratz equation (Van den Dool, Kratz, 1963) and by comparing results from mass spectral data of each peak using a computer library (Wiley-14 and NIST-14 Mass Spectral Library) with recently described results contained in the literature (Adams, 2007; NIST, 2018). Concentration of identified compounds, expressed as a percentage, was directly calculated from respective peak areas.

Antibacterial assay

The bacterial growth inhibition of the essential oils was evaluated using two strains of Gram-positive test bacteria, *Staphylococcus aureus* (ATCC25923) and *Bacillus cereus* (ATCC14579), plus two strains of Gram-negative test bacteria, *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC27853). Minimum inhibitory concentration (MIC) values were measured by the broth microdilution assay as previously described (Huong et al., 2020; Huong et al., 2021). Stock solutions of each of the essential oils were prepared using dimethylsulfoxide. The essential oils were diluted in two folds (400, 200, 100, 50, 25, 12.5, and 6.25 μg/mL) in sterile distilled water in micro-test tubes from where they were transferred to 96-well microtiter plates for the assays. The MIC values were determined as the lowest concentration of each essential oil that completely inhibited the growth of the microorganisms.

Results and discussion

Amomum longiligulare (leaves, rhizomes, and fruits) collected in Quang Nam, Vietnam was extracted by microwave-assisted hydrodistillation to obtain essential oil. The essential oils with a light-yellow color were analyzed by gas chromatography–mass spectrometry (GC–MS). The oil yields of leaves, rhizomes and fruits from *A. longiligulare* were 0.23%, 0.27% and 1.93% (v/w, ± 0.01), respectively, calculated on a dry weight basis. The essential oil profile and retention indices (RI) of each compound are presented in Table 1.

In the essential oil extracted from the *A. longiligulare* leaf, 34 compounds were identified, corresponding to 96.1% of the total oil (Table 1). The leaf essential oil was composed mostly of sesquiterpene hydrocarbons (50.5%), monoterpene hydrocarbons (34.6%), oxygenated sesquiterpenes (7.9%), and oxygenated monoterpenes (3.0%). The main constituents in the *A. longiligulare* leaf essential oil were *α*-humulene (28.4%), *α*-pinene (24.9%), *β*-caryophyllene (17.3%), humulene epoxide II (7.3%), and *β*-pinene (4.7%).

A total of 35 compounds amounting to 94.3% in the *A. longiligulare* rhizome essential oil were identified (Table 1). Among these, 52.0% were sesquiterpene hydrocarbons, 22.9% were monoterpene hydrocarbons, and it also contained 14.5% of oxygenated sesquiterpenes and 4.7% of oxygenated monoterpenes. The major constituents in the

Table 1. **Qualitative and quantitative composition of *Amomum longiligulare* essential oils, %**
Таблица 1. **Качественный и количественный состав эфирных масел *Amomum longiligulare*, %**

Compound [a]	RI [b]	Percentage composition [c]		
		Leaves	Rhizomes	Fruits
Tricyclene	926	0.1 ± 0.0	0.3 ± 0.1	–
α-Pinene	939	24.9 ± 0.3	5.7 ± 0.3	0.9 ± 0.2
Camphene	955	1.5 ± 0.1	7.9 ± 0.2	3.4 ± 0.3
Sabinene	978	0.2 ± 0.0	–	-
β-Pinene	984	4.7 ± 0.1	2.3 ± 0.1	1.2 ± 0.1
Myrcene	992	0.4 ± 0.1	0.1 ± 0.0	2.1 ± 0.2
α-Phellandrene	1010	0.2 ± 0.0	1.9 ± 0.1	–
δ-3-Carene	1011	–	0.1 ± 0.0	–
α-Terpinene	1022	0.3 ± 0.0	–	–
p-Cymene	1030	0.7 ± 0.0	0.2 ± 0.1	0.3 ± 0.1
Limonene	1035	0.4 ± 0.1	0.3 ± 0.2	4.8 ± 0.3
β-Phellandrene	1036	0.1 ± 0.0	3.4 ± 0.3	–
1,8-Cineole	1038	0.7 ± 0.1	1.2 ± 0.1	–
(*E*)-*β*-Ocimene	1049	–	0.4 ± 0.1	–
γ-Terpinene	1063	0.9 ± 0.1	0.3 ± 0.0	–
Terpinolene	1094	0.2 ± 0.0	–	0.7 ± 0.1
Linalool	1105	1.4 ± 0.2	0.5 ± 0.2	
Camphor	1156	0.5 ± 0.1	1.9 ± 0.2	40.7 ± 0.4
Borneol	1178	0.1 ± 0.0	0.3 ± 0.1	–
Terpinen-4-ol	1187	–	0.1 ± 0.0	–
α-Terpineol	1200	0.2 ± 0.1	–	0.2 ± 0.0
Bornyl acetate	1294	0.1 ± 0.0	0.7 ± 0.1	34.2 ± 0.3
α-Copaene	1389	0.8 ± 0.0	0.5 ± 0.1	–
β-Elemene	1403	2.3 ± 0.1	0.4 ± 0.0	–
β-Caryophyllene	1437	17.3 ± 0.3	28.7 ± 0.3	2.7 ± 0.2
trans-*α*-Bergamotene	1445	0.1 ± 0.0	0.1 ± 0.0	–
α-Humulene	1452	28.4 ± 0.4	3.6 ± 0.1	–
allo-Aromadendrene	1457	0.2 ± 0.0	0.1 ± 0.0	–
β-Selinene	1489	0.1 ± 0.0	0.3 ± 0.0	–
trans-*β*-Bergamotene	1496	-	0.4 ± 0.1	–
Germacrene D	1498	0.8 ± 0.1	0.6 ± 0.2	1.7 ± 0.1
Bicyclogermacrene	1513	0.2 ± 0.0	17.1 ± 0.4	0.1 ± 0.0
β-Bisabolene	1517	–	0.2 ± 0.1	2.4 ± 0.2
δ-Cadinene	1537	0.3 ± 0.0	–	–
(*E*)-Nerolidol	1570	–	0.7 ± 0.1	1.6 ± 0.2

Table 1. The end
Таблица 1. Окончание

Compound [a]	RI [b]	Percentage composition [c]		
		Leaves	Rhizomes	Fruits
Caryophyllene oxide	1583	0.2 ± 0.0	2.2 ± 0.2	–
Viridiflorol	1593	0.1 ± 0.0	0.5 ± 0.1	–
Humulene epoxide II	1600	7.3 ± 0.2	10.5 ± 0.3	–
α-Bisabolol	1683	0.3 ± 0.1	0.6 ± 0.1	1.4 ± 0.1
Phytol	2125	0.1 ± 0.0	0.2 ± 0.1	–
Monoterpene hydrocarbons		34.6	22.9	13.4
Oxygenated monoterpenes		3.0	4.7	75.1
Sesquiterpene hydrocarbons		50.5	52.0	6.9
Oxygenated sesquiterpenes		7.9	14.5	3.0
Others		0.1	0.2	-
Total		96.1	94.3	98.4

Note: [a] – elution order on a HP-5MS column; [b] – retention indices on a HP-5MS column; [c] –each value is expressed as means ± SD (n = 3); (–) – not identified

Примечание: [a] – порядок элюирования на колонке HP-5MS; [b] – индексы удерживания на колонке HP-5MS; [c] – каждое значение выражено в виде среднего ± SD (n = 3); (–) – не определено

A. longiligulare rhizome essential oil were β-caryophyllene (28.7%), bicyclogermacrene (17.1%), humulene epoxide II (10.5%), camphene (7.9%), and α-pinene (5.7%).

In the essential oil of the *A. longiligulare* fruit, only 16 compounds were identified, accounting for 98.4% of the total essential oil content (Table 1). The fruit essential oil was mostly made up of oxygenated monoterpenes (75.1%), monoterpene hydrocarbons (13.4%), sesquiterpene hydrocarbons (6.9%) and oxygenated sesquiterpenes (3.0%). Camphor (40.7%) and bornyl acetate (34.2%) were the most abundant constituents found in the essential oil from fruits. In addition, in the essential oil of fruits there were other abundant compounds, including limonene (4.8%), camphene (3.4%), β-caryophyllene (2.7%), and β-bisabolene (2.4%).

There were some reports on the chemical composition of the essential oil extracted from *A. longiligulare*. For example, the essential oil from *A. longiligulare* leaves comprised mainly β-caryophyllene (26.6%), α-pinene (15.6%), humulene epoxide II (14.8%), and α-humulene (12.5%) (Chau et al., 2015). The major compounds of essential oil from the *A. longiligulare* stem were β-caryophyllene (37.4%), α-humulene (16.5%), and hexahydrofarnesyl acetone (10.0%) (Chau et al., 2015). Camphene (15.7%), hexadecanoic acid (10.0%), octadecanoic acid (8.6%), and bornyl acetate (7.8%) were the main constituents of the root oil of *A. longiligulare* (Chau et al., 2015). The research of Anh et al. (2020) identified that D-camphor (46.714%) and bornyl acetate (31.809%) were the main compounds in the essential oil from *A. longiligulare* fruits (Anh et al., 2020). Overall, there was a difference in the content of the main components of the *A. longiligulare* essential oil in the present study comparing with results the obtained by other researchers. The variations in chemical constituents can likely be attributed to the different collection sites, development stages, farming, and genetic characteristics as well as extraction methods (Özcan, Chalchat, 2005; Kayode, Afolayan, 2015).

A microdilution broth assay was employed to study the antibacterial activities of essential oils of *A. longiligulare*. The data in Table 2 show that all essential oils exhibited activity against *B. cereus* and *E. coli* with MIC values of 400 and

Table 2. Antibacterial activity of *Amomum longiligulare* essential oils
Таблица 2. Антибактериальная активность эфирных масел *Amomum longiligulare*

Microorganisms	MIC (μg/mL)		
	Leaves	Rhizomes	Fruits
Staphylococcus aureus ATCC25923	–	–	400
Bacillus cereus ATCC14579	400	400	400
Escherichia coli ATCC 25922	200	200	200
Pseudomonas aeruginosa ATCC27853	–	400	–

Note: (–) – no activity

Примечание: (–) – активность отсутствует

200 μg/mL, respectively. In addition, the rhizome and fruit essential oil manifested antibacterial action against *P. aeruginosa* and *S. aureus*, respectively, with MIC values of 400 μg/mL. The observed antibacterial result of *A. longiligulare* essential oils was in agreement with previous information that *Amomum* essential oils selectively inhibited the growth of different microorganisms (Huong et al., 2020; Huong et al., 2021).

Conclusions

Our research on *A. longiligulare* essential oils showed a high variation in the chemical composition of oils extracted from different organs. The highest essential oil yield was recorded for fruits. The essential oil from the *A. longiligulare* leaf comprised mainly *α*-humulene (28.4%), *α*-pinene (24.9%), *β*-caryophyllene (17.3%), humulene epoxide II (7.3%), and *β*-pinene (4.7%). The major compounds of essential oil from the *A. longiligulare* rhizome were *β*-caryophyllene (28.7%), bicyclogermacrene (17.1%), humulene epoxide II (10.5%), camphene (7.9%), and *α*-pinene (5.7%). Camphor (40.7%) and bornyl acetate (34.2%) were the main constituents of the fruit oil of *A. longiligulare*. Also, *A. longiligulare* essential oils are a source of promising antibacterial agents.

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