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Essential Oils from the Aerial Part and Rhizome of *Amomum muricarpum* Elmer and Their Antimicrobial Activity

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Abstract: Chemical compositions in essential oils of *Amomum muricarpum* Elmer were determined using GC-MS/GC-FID. In the hydro-distilled oils of aerial part and rhizome, twenty-eight and thirty-three constituents were identified, accounting for 98.73 and 97.88%, respectively. *A. muricarpum* essential oils were associated with the presence of monoterpenes, sesquiterpenes, and their oxygenated types. Monoterpenes (> 80.00%) were major compounds, in which the main compound α-pinene reached the highest percentage of 62.94-74.97%. *A. muricarpum* essential oils showed antimicrobial activity against the positive Gram bacterium *Staphylococcus aureus* and filamentous fungus *Aspergillus niger* with the respective MIC values of 400 and 100 µg/mL. This study provides new information that Vietnamese medicinal plant *A. muricarpum* are a rich resource of monoterpene α-pinene

Keywords: *Amomum muricarpum* Elmer; Aerial part; Rhizome; Essential oils; Antimicrobial

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1. Introduction

Zingiberaceae is a family of essential oil-bearing plants. Genus *Amomum* in this family consisted of about 150-180 species and was native to Asia, Africa, and Australia [1-6]. All parts of *Amomum* plants are notable for their pungency and aromatic features, which have been employed as pharmaceutical and food chemical agents [7-15]. The plants of this genus are also well-known in traditional uses for toothache, dysentery, gastrointestinal, nervous system strengthen, pregnancy-related diseases [16-18]. Phytochemical analyses, mostly based on GC-MS procedures, have identified the presence of a vast amount of terpenoids in *Amomum* essential oils [19-21].

A. muricarpum Elmer is a medicinal plant 2.0-3.0 m tall, which its name was accepted at a high level of confidence [22]. It can be gathered from dense forests in China, the Philippines, and Vietnam [23]. Various phytochemical publications evidently concluded that diarylheptanoids were major components in its plant extracts [23,24]. However, GC-MS analysis aimed to determine chemical components in essential oils of this plant is not available to date. Our current paper deals with the chemical compositions in the essential oils of *A.*

muricarpum aerial part and rhizome, collected from Vietnam, together with their antimicrobial activity.

2. Materials and Methods

2.1. Materials.

The fresh aerial part and rhizome of *A. muricarpum* species were collected from Thua Thien Hue, Vietnam, in 04-2021, around 16°07'23.9''N and 107°36'30.3''E. The plant materials were identified by Dr. Vu Tien Chinh, Vietnam National Museum of Nature, VAST. The voucher specimen AM-2021 was deposited at the Institute of Natural Products Chemistry, VAST.

The positive Gram bacteria *Bacillus subtilis* ATCC 27212 and *Staphylococcus aureus* ATCC 12222, the negative Gram bacteria *Escherichia coli* ATCC 8739 and *Pseudomonas aeruginosa* ATCC 25923, fungi *Aspergillus niger* ATCC 9763 and *Fusarium oxysporum* ATCC 48112, and yeasts *Candida albicans* ATCC 10231 and *Saccharomyces cerevisiae* ATCC9763 were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA).

2.1.1. Distillation.

The fresh aerial part (1.0 kg) was chopped and was intermediately hydro-distilled using Clevenger apparatus for 2.5 h, to produce yellow and pleasant smelling oil (0.01% yield, w/w). The oil obtained was dried over anhydrous sodium sulfate and kept in amber-color vials at 4 ^{o}C before use. A similar procedure was applied to the fresh rhizome (1.5 kg), to yield the same oil (0.02%, w/w).

2.1.2. GC-MS analysis.

An HP7890A model GC (Agilent Technologies, USA) linked to an HP5975C MS detector (MSD), and an HP-5 MS column (dimension: 60 m x 0.25 mm, film thickness: 0.25 µm) was used for GC-MS analysis. The injector was set around 250 ^{o}C. The temperature program started at 60 ^{o}C and increased up to 240 ^{o}C with each 4 ^{o}C/min. He was used as a carrier gas (1.0 mL/min). The split ratio was 100:1. The MSD circumstance was contingent upon the full scan modes under electron impact ionization (voltage: 70 eV, emission current: 40 mA, mass range scan: 35-450 amu). The GC-FID analysis was performed with the same condition of GC-MS procedure. Each chemical compound in the oil sample was determined by comparing their RI and MS data with those from the NIST standard database and reference books [25], while the relative percentage was calculated based on the peak area in the GC-FID without any correction factor.

2.2. Antimicrobial assay.

The antimicrobial procedure was carried out according to our previous publications [26-29]. Briefly, the Gram bacteria were cultured in tryptic soy broth, whereas fungal microorganisms were grown in saboraud-2% dextrose with 150 x 10^6 CFU/mL. The oil sample (12.5-400 µg/mL) was loaded into a 96-well plate containing fresh culture and kept at 37 ^{o}C and 24 h. The MIC (Minimum inhibitory concentration) denotes the lowest concentration, at which the tested sample suppressed the visible growth of pathogenic microorganisms after 24 h treatment. Streptomycin and tetracycline served as positive controls for the positive and negative Gram bacteria, respectively, whereas nystatin was used as a positive control for fungi and yeasts. DMSO (5%) was used as a negative control. Each experiment was performed in triplicate.

3. Results and Discussion

The gas chromatography on HP-5 capillary column has identified 28 constituents in the essential oil of the *A. muricarpum* aerial part, which accounted for 98.73% (Table 1). Monoterpenes (11 compounds) were major components with the highest percentage of 87.67%, whereas monoterpenoids (6 compounds), sesquiterpenes (9 compounds), and sesquiterpenoids (2 compounds) reached 7.07, 3.00, and 0.99%, respectively. α-Pinene with 74.97% was a principal compound in the monoterpene group, in addition to several significant ones such as camphene (2.96%), myrcene (2.74%), β-phellandrene (2.21%), and limonene (2.17%). Linalool (4.37%) and α-terpineol (1.10%) were major compounds in the monoterpenoid groups. The percentage of each compound in the groups of sesquiterpenes and their oxygenated derivatives was less than 1.00%.

Table 1. Essential oils of *A. muricarpum* aerial part and rhizome.

No	[a]Rt	[b]RI_E	[c]RI_L	Constituents	Classification	Aerial part [%]	Rhizome [%]
1	10.21	928	927	Tricyclene	Monoterpene	0.23	0.21
2	10.57	940	939	α-Pinene	Monoterpene	**74.97**	**62.94**
3	11.03	955	954	Camphene	Monoterpene	2.96	3.66
4	11.91	984	979	β-Pinene	Monoterpene	0.96	2.63
5	12.13	991	991	Myrcene	Monoterpene	2.74	3.22
6	12.73	1010	1003	α-Phellandrene	Monoterpene	0.38	0.75
7	13.13	1022	1017	α-Terpinene	Monoterpene	-	0.24
8	13.39	1029	1026	*O*-Cymene	Monoterpene	0.19	0.53
9	13.55	1034	1029	Limonene	Monoterpene	2.17	3.92
10	13.61	1036	1030	β-Phellandrene	Monoterpene	2.21	2.88
11	14.55	1063	1060	γ-Terpinene	Monoterpene	0.28	1.10
12	15.62	1094	1089	Terpinolene	Monoterpene	0.59	0.76
13	15.86	1101	1097	Linalool	Monoterpenoid	4.37	1.98
14	16.57	1121	1122	*Endo*-fenchol	Monoterpenoid	-	0.18
15	18.46	1175	1168	*Endo*-borneol	Monoterpenoid	0.63	0.48
16	18.82	1185	1177	Terpinen-4-ol	Monoterpenoid	0.62	0.27
17	19.25	1198	1189	α-Terpineol	Monoterpenoid	1.10	1.02
18	20.30	1228	1220	Fenchyl acetate	Monoterpenoid	0.13	5.10
19	21.25	1256	1253	Geraniol	Monoterpenoid	-	0.18
20	22.59	1294	1289	Bornyl acetate	Monoterpenoid	0.22	1.25
21	23.03	1308	1298	Sabinyl acetate	Monoterpenoid	-	0.40
22	23.88	1333	1327	Myrtenyl acetate	Monoterpenoid	-	0.36
23	25.74	1390	1377	α-Copaene	Sesquiterpene	0.36	0.20
24	26.10	1401	1388	β-Bourbonene	Sesquiterpene	0.22	-
25	27.08	1432	1427	α-Santalene	Sesquiterpene	-	0.48
26	27.98	1460	1452	*Epi*-β-santalene	Sesquiterpene	-	0.31
27	28.58	1480	1470	9-*Epi*-(*E*)-caryophyllene	Sesquiterpene	0.35	0.20
28	28.93	1491	1500	γ-Muurolene	Sesquiterpene	0.17	-

No	^{a}Rt	$^{b}RI_E$	$^{c}RI_L$	Constituents	Classification	Aerial part [%]	Rhizome [%]
29	29.17	1498	1485	Germacrene D	Sesquiterpene	0.42	0.26
30	29.37	1505	1490	β-Selinene	Sesquiterpene	-	0.21
31	29.53	1510	1507	(*Z*)-α-Bisabolene	Sesquiterpene	-	0.18
32	29.58	1512	1506	(*E,E*)-α-Farnesene	Sesquiterpene	0.27	-
33	29.65	1515	1500	Bicyclogermacrene	Sesquiterpene	-	0.72
34	29.75	1518	1506	β-Bisabolene	Sesquiterpene	0.80	0.91
35	30.13	1530	1523	γ-Cadinene	Sesquiterpene	0.22	-
36	30.32	1537	1523	δ-Cadinene	Sesquiterpene	0.19	-
37	31.29	1569	1563	(*E*)-Nerolidol	Sesquiterpenoid	0.26	-
38	32.12	1597	1579	Spathulenol	Sesquiterpenoid	0.73	-
39	38.46	1829	1809	γ-Bicyclohomofarnesal	Sesquiterpenoid	-	0.15
40	46.26	2154	2135	Coronarin E	Diterpenoid	-	0.20
				Total		98.73	97.88
				Monoterpenes		87.67	82.84
				Monoterpenoids		7.07	11.22
				Sesquiterpenes		3.00	3.47
				Sesquiterpenoids		0.99	0.15
				Diterpenoids			0.20

^{a}Retention time, ^{b}Retention indices relative to *n*-alkanes (C_7-C_{30}) on HP-5 MS column, ^{c}Retention indices from NIST standard database

Regarding essential oil from the rhizome, 33 constituents were namely identified in a total amount of 97.88%. Monoterpenes, sesquiterpenes, and their oxygenated derivatives were also characteristic of rhizome oil. Once again, monoterpenes (12 compounds, 82.84%) were found to be the main components. Monoterpenoids (10 compounds, 11.22%) were the second class of remarkable compounds in rhizome oil. It is similar to the essential oil of the aerial part, rhizome oil, accompanied by the appearance of minor compounds type sesquiterpenes (3.47%) and sesquiterpenoids (0.15%). α-Pinene in rhizome oil still ranked the highest amount of 62.94%, but less than that of aerial part by 12.03%. The percentages of four monoterpenes, camphene, myrcene, β-phellandrene, and limonene, have increased in the rhizome oil. In contrast to the amount of linalool, two minor compounds β-pinene and fenchyl acetate in the aerial part were being become remarkable compounds in rhizome oil (Table 1). Coronarin E (0.20%) was the only diterpenoid found in rhizome oil.

The difference between two oils is that sesquiterpenes β-bourbonene (0.22%), γ-muurolene (0.17%), γ-cadinene (0.22%), and δ-cadinene (0.19%), sesquiterpenoids (*E*)-nerolidol (0.26%) and spathulenol (0.73%) were only detected in the essential oil of aerial part, whereas monoterpene α-terpinene (0.24%), monoterpenoids *endo*-fenchol (0.18%), geraniol (0.18%), sabinyl acetate (0.40%), myrtenyl acetate (0.36%), sesquiterpenes α-santalene (0.48%), *epi*-β-santalene (0.31%), β-selinene (0.21%), (*Z*)-α-bisabolene (0.18%), and bicyclogermacrene (0.72%), sesquiterpenoid γ-bicyclohomofarnesal (0.15%), and diterpenoid coronarin E were only observed in the rhizome oil.

Significantly, our study demonstrated that monoterpene α-pinene possessed such a high amount of over 60.00% in *A. muricarpum* species, collected from Vietnam. In comparison with previous reports, this compound was also found in several *Amomum* species, such as essential oils of *A. villosum* leaf and root bark (11.6-22.1%), *A. rubidium* rhizome (7.70%), *A. subulatum* seed and rind (4.8-6.4%) [30-32]. Therefore, it can be concluded that *A. muricarpum* is likely to be a rich resource of α-pinene.

Table 2. Antimicrobial activity of tested samples.

Microbial strains		Minimum Inhibitory Concentration (MIC: mg/mL)				
		Aerial part	Rhizome	Streptomycin	Tetracyclin	Nystatin
Gram (+)	*B. subtilis*	(-)	(-)	7.20		
	S. aureus	400	400	14.38		
Gram (-)	*E. coli*	(-)	(-)		5.5	
	P. aeruginosa	(-)	(-)		11.0	
Fungi	*A. niger*	100	100			23.13
	F. oxysporum	(-)	(-)			11.57
Yeasts	*C. albicans*	(-)	(-)			11.56
	S. cerevisiae	(-)	(-)			5.78

Two oil samples have been further to subject biological assay. Both of them showed antimicrobial activity against the positive Gram bacterium *S. aureus* and filamentous fungus *A. niger* with the MIC values of 400 and 100 µg/mL, but failed to control the remaining pathogenic microorganisms (Table 2). Amomum essential oils were recognized to be potential agents against pathogenic micro bacteria. For example, Geogre *et al.* (2006) also identified that essential oils of *A. cannicarpum* rhizome were capable of inhibiting the growth of the positive Gram bacteria *B. subtilis* and *S. aureus* and the negative Gram bacterium *E. coli* [33]. In the same manner, terpenoids in *A. tsao-ko* essential oils response for its moderate inhibitory antimicrobial activities against *S. aureus, Staphylococcus epidermidis, B. subtilis, Propionibacterium acnes, E. coli, Pseudomonas aeruginosa*, and *C. albicans* [34].

4. Conclusions

For the first time, our study reported chemical compositions of the Vietnamese *A. muricarpum* essential oils. Monoterpenes with over 80.0% were a main chemical class of compounds in essential oils of both aerial part and rhizome. α-Pinene was a principal component in these oils, which ranged from 62.94 to 74.97%. The oils of this plant were also characterized by the presence of monoterpenoids, sesquiterpenes, sesquiterpenoids, and diterpenoids. *A. muricarpum* essential oils successfully inhibited filamentous fungus *A. niger* growth with the MIC value of 100 µg/mL. This paper can be seen as a basic guide for future research.

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Conflicts of Interest

The authors declare no conflict of interest.

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