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Article

Chemical Compositions of Essential Oils and Antimicrobial Activity of *Alpinia kwangsiensis* from Vietnam

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Abstract: This paper reports the chemical compounds and antimicrobial activity of essential oils isolated from the leaves, stem, and rhizomes of *Alpinia kwangsiensis* T. L. Wu and S. J. Chen (Zingiberaceae). The essential oils were separately isolated using hydrodistillation of the pulverized samples (2 kg each) materials in an all-glass Clevenger-type apparatus and characterized by GC-FID and GC/MS. The yields of the essential oils were 0.16, 0.11 %, and 0.21 % (w/w), respectively, calculated on a dry weight basis. The main constituents of the essential oils (percentages, respectively) were 1,8-cineole (16.9 %, 14.3 % and 5.8 %), terpinene-4-ol (16.0 %, 13.6 % and 14.3 %), (*E*)-methyl cinnamate (9.4 %, 15.4 % and 4.6 %) and β-pinene (7.6 %, 9.9.% and 3.1 %). The study found out that the rhizome essential oil was the most active against *Enterococcus faecalis* ATCC 299212 with a minimum inhibitory concentration (MIC) value of 1.60 µg/mL, while the leaf oil displayed pronounce activity towards *Bacillus cereus* ATCC 14579 (MIC 3.20 µg/mL). All the essential oils inhibited the growth of *Staphylococcus aureus* ATCC 25923 and *Candida albicans* ATCC 10231, with MIC value of 6.40 µg/mL. Therefore, the essential oils obtained from *A. kwangisensis* may have potential applications as antimicrobial agents.

Keywords: *Alpinia kwangsiensis*, essential oil, monoterpenes, antimicrobial activity.

Introduction

In recent years, essential oils have received a great deal of attention as disease control agents. They are typically characterized by low toxicity to humans and animals, high volatility, and toxicity to microorganisms [1]. *Alpinia* Roxb., a member

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of the ginger family (Zingiberaceae) comprises more than 250 species found in Southeast Asia, extending from Japan in the north to Australia in the south and into the Western Pacific [2]. *Alpinia kwangsiensis* T. L. Wu and S. J. Chen (Vietnamese name, Rieng quang tây) is a perennial herb that grows widely in valley forests in Guangdong, Guangxi, Guizhou, Yunnan provinces of China, and the northern mountainous areas of Vietnam [3,4]. In Vietnam, this species is used only for food and medical treatment according to folklore. Its roots can cure abdominal, stomach, vomiting, etc.[4]. Extracts from *A. kwangsiensis* was found to be active against bacteria such as *Staphylococcus aureus* ATCC 13709 and *Bacillus subtilis* ATCC 6633 with the IC_{50} values of 74.65 μg/ml and 80.54 μg/ml, respectively, and also showed antioxidant activity through DPPH test with the EC_{50} value of 87.98 μg/mL[4]. The compounds isolated from the roots of *A. kwangsiensis* grown in Vietnam were described as methyl-*trans*-*p*-coumarate, scopoletin, and (+)-gallocatechin [4]. The main compounds in the essential oil from *A. kwangsiensis* rhizomes collected in China were identified as camphor (17.59 %), eucalyptol (15.16 %), β-pinene (11.15 %), and α-pinene (10.50 %) [5], while another report identified cinnamic acid methyl ester (94.54 %) in abundance [6]. These compounds and essential oil exhibited significant insecticidal activity on *Lasioderma serricorne* [5].

During our mass screening program for new biologically active products from the wild plants grown in Vietnam, essential oils from the leaves, rhizomes, and stems of *A. kwangsiensis* were found to displayed antimicrobial potentials. Although the chemical constituents and biological activities of essential oils from *A. kwangsiensis* grown in Vietnam have not been defined, we have reported recently the volatile constituents and biological potentials of some other *Alpinia* plants. The main constituents of these essential oils consist of monoterpenes and sesquiterpene compounds of diverse structural patterns [7-19]. The essential oil of *A. tonkinensis* inhibited the growth of *Saccharomyces cerevisiae* ATCC 16404 with a minimum inhibitory concentration (MIC) value of 25.0 μg/mL, while both *A. globosa* and *A. tonkinensis* essential oils displayed antimicrobial activity against *Escherichia coli* ATCC 25922, *Staphylococcus aureus* subsp. *aureus* ATCC 11632 and *Fusarium oxysporum* ATCC 48112 with MIC value of 50.0 μg/mL[7]. Also, essential oils from various parts of *A. malaccensis* inhibited the growth of several microorganisms including *Pseudomonas aeruginosa* ATCC 25923 and *Aspergillus niger* ATCC 9763 among others with MIC < 50.0 μg/mL[12]. Essential oils from *A. napoensis* showed activity towards six of the eight tested microorganisms with MIC < 50.0 μg/mL[13]. Considering the plants of the genus *Alpinia* as sources of biologically active compounds, we report herein the chemical constituents and anti-microbial activity of essential oils from the leaves, stem barks, and rhizomes of *A. kwangsiensis* grown in Vietnam.

Material and methods

Plant collection and hydrodistillation of essential oils

Large quantities of the leaves, stems, and rhizomes were collected from wild-growing plants in Vu Quang National Park (GPS: 18°172 153 N, 105°212 393 E), Ha Tinh Province, Vietnam. Sample collection was done by Lindh, L.D., in October 2016 at an elevation of 124 m. The plant samples were identified by Dr. Do Ngoc Dai, Faculty of Agriculture, Forestry and Fishery, Nghe An College of Economics, Vinh City, Vietnam. A voucher specimen number LDL 501 was deposited in the plant specimen room, Faculty Agriculture, Forestry and Fishery, Nghe An, College of Economics, Vietnam. The samples were subjected to cleaning by handpicking to remove unwanted materials to obtained 2 kg each. Two kilograms each of the leaves, stems, and rhizomes of *A. kwangsiensis* was used for the hydrodistillation experiment. Each of the collected plant materials was divided into three parts to ensure that each of the hydrodistillation was repeated three times. The sample was separately introduced into a 5 L flask after which distilled water was added until it covered the sample completely. The essential oil was obtained by hydrodistillation which was carried out in a Clevenger-type distillation unit designed according

to an established procedure as described in the previous studies [7-19]. The distillation time was 3 h and was conducted at normal pressure. The volatile oils distilled over water were collected separately by running through the tap in the receiver arm of the apparatus into clean and previously weighed sample bottles. The oils were kept under refrigeration (4°C) until the moment of analysis. The experiment was conducted in triplicate. The essential oil yield (%) was calculated by mass (g) of the essential oil divided by the mass (g) of the dried rhizomes of the plant.

Analysis of the essential oils

The analysis of the chemical constituents of the leaves stems rhizome essential oil of *A. kwangsiensis* was achieved using GC and GC/MS. Gas chromatographic (GC) analysis was performed on an Agilent Technologies HP 7890A Plus Gas chromatograph equipped with a FID and fitted with HP-5MS column (Agilent Technologies, Santa Clara, California, USA) of dimension 30 m x 0.25 mm with a film thickness of 0.25 µm. The analytical conditions employed in the GC analysis were: carrier gas H_2 with a flow rate of 1 mL/min, while both the injector temperature (PTV: programmable temperature vaporization) and detector temperature were maintained at 250°C and 260°C, respectively. The column temperature was programmed from 60°C, with a 2 min hold, to 220°C (10 min hold) at a rate of 4 C/min. The essential oil (1.0 µL; 10 % *n*-hexane solution) was injected using a split mode with a split ratio of 10:1, at inlet pressure was 6.1 kPa. Quantification was done using the calibration curves generated from the analyses of representative standard compounds from each class.

An Agilent Technologies (Santa Clara, California, USA) HP 7890A Plus Chromatograph fitted with a fused silica capillary HP-5MS column (dimension 30 m x 0.25 mm; film thickness 0.25 µm) and interfaced with a mass spectrometer HP 5973 MSD was used for the GC/MS analysis. The GC conditions were the same as those reported above for the GC analysis. However, He was used as the carrier gas. The MS was operated at an ionization voltage of 70 eV with an emission current of 40 mA, with the acquisitions scan mass range of 35-350 amu at a sampling rate of 1.0 scan/s as described previously [7-19].

The identification of constituents of essential oils from the GC/MS spectra of *A. kwangsiensis* was performed based on a comparison of retention indices (RI Exp.) with reference to a homologous series of *n*-alkanes (C_6-C_{40}), under identical experimental conditions. In some cases, co-injection with known compounds under the same GC conditions was employed. The mass spectral (MS) fragmentation patterns were checked with those of other essential oils of known composition in literature [20-22] as described recently [7-19].

Antimicrobial activity assays

The antimicrobial activity of the essential oils was evaluated using three strains of Gram-positive test bacteria, *Enterococcus faecalis* ATCC 299212, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 14579, three strains of Gram-negative test bacteria, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella enterica* ATCC 13076, and one strain of yeast, *Candida albicans* ATCC 10231. The minimum inhibitory concentration (MIC) and median inhibitory concentration (IC_{50}) values were measured by the microdilution broth susceptibility assay as previously described [7-19].

Stock solutions of the oil were prepared in dimethylsulfoxide. The choice of investigated concentrations was based on our previous reports on similar investigations where essential oils are active within a specific concentration range [7,12,15]. Dilution series were prepared from 16,384 to 2 µg/mL (2^{14}, 2^{13}, 2^{12}, 2^{11}, 2^{10}, 2^9, 2^7, 2^5, 2^3, and 2^1 µg/ mL) in sterile distilled water in micro-test tubes from where they were transferred to 96-well microtiter plates. Bacteria were grown in double-strength Mueller-Hinton broth or double-strength tryptic soy broth, and fungi grown in double-strength Sabouraud dextrose broth were standardized to 5×10^5 and 1×10^3 CFU/mL, respectively. The last row, containing only the serial dilutions of the sample without microorganisms, was used as a positive (no growth) control. Sterile distilled water and medium served as a negative (no antimicrobial agent) control. Streptomycin was used as the antibacterial

standard, while nystatin and cycloheximide were used as anticandidal standards. After incubation at 37°C for 24 h, the MIC values were determined to be well with the lowest concentration of agents completely inhibiting the growth of micro-organisms. The IC_{50} values were determined by the percentage of microorganisms that inhibited growth based on the turbidity measurement data of EPOCH2C spectrophotometer (BioTeK Instruments, Inc Highland Park Winooski, VT, USA) and Raw data computer software (Brussels, Belgium) according to the following equations:

$$\% \text{ Inhibition} = \frac{OD_{control}(-) - OD_{test\ agent}}{OD_{control}(-) - OD_{control}(+)} \times 100$$

$$IC_{50} = High_{conc} - \frac{(High_{inh\%} - 50\%) - (High_{conc} - Low_{conc})}{(High_{inh\%} - Low_{inh\%})}$$

where OD is the optical density, control(–) are the cells with medium but without the antimicrobial agent, test agent corresponds to a known concentration of the antimicrobial agent, control(+) is the culture medium without cells, $High_{conc}/Low_{conc}$ is the concentration of test agent at high concentration/low concentration, and $High_{inh\%}/Low_{inh\%}$ is the % inhibition at high concentration/% inhibition at low concentration).

Statistic analysis

Statistical analysis (ANOVA) of the differences between mean values obtained for experimental groups were calculated as a mean of standard deviation (SD, ±) of three and four independent measurements, respectively for the chemical constituents and antimicrobial test, using Microsoft excel program 2003.

Results and discussion

The percentage yield and colour of the essential oils

The average yields of the essential oils were 0.16 % (1.23 g), 0.11 % (1.19 g), and 0.21 % (1.84 g) (w/w, SD ± 0.01), respectively, for the leaves, stems, and rhizomes of *A. kwangsiensis,* calculated on a dry weight basis. The essential oils were yellow-colored. The yields are consistent with data obtained for essential oils of other *Alpinia* plants from Vietnam and the rest of the world. Previously, *A. globosa* and *A. tonkinensis* leaf essential oils from Vietnam were obtained in yields of 0.16 % (v/w, ± 0.01) and 0.21 % (v/w, ± 0.02), respectively[7], while *A. napoensis* rhizome [13] gave oil in a yield of 0.26 % (v/w, ± 0.01). The yields for *A. malaccensis* leaf, pseudo-stem, rhizomes and fruits essential oils [12] were 0.23 %, 0.19 %, 0.25 % and 0.40 % (v/w). Essential oil from the dried rhizomes of *A. kwangsiensis* grown in China was obtained in a yield of 0.16 % (v/w) [5].

Chemical constituents of the essential oils

The chemical constituents of *A. kwangsiensis* essential oils are shown in Table 1. By using a combination of GC-FID and GC/MS with HP-5 MS column, forty-four, forty-three, and fifty-three compounds representing 96.8 %, 91.6 %, and 85.4 % of the oil contents were identified respectively in the leaf, stem, and rhizome *A. kwangsiensis* (Table 1). Monoterpene hydrocarbons (25.6 %, 21.1 %, and 12.8 %, respectively) and oxygenated monoterpenes (52.3 %, 56.8 %, and 51.7 % respectively) were the main classes of compounds identified in the leaf, stem, and rhizome of *A. kwangsiensis* essential oils. The rhizome oil contained the highest amount of sesquiterpene hydrocarbons (11.6 %) while fatty acids (8.6 %) were identified in larger quantities in the leaf essential oil. Both the stem and rhizome essential oils consist of an almost equal quantity of oxygen-containing sesquiterpenes (5.2 % vs. 5.1 %) and fatty acids (2.1 % vs. 2.2 %). It could be seen that ubiquitous terpenes as defined in some other previously analysed *Alpinia* essential [7-19], were also identified in the present oil sample of *A. kwangsiensis*. The main constituents of *A. kwangsiensis* essential oils (percentages, respectively) were 1,8-cineole (16.9 %, 14.3 % and 5.8 %), terpinene-4-ol (16.0 %, 13.6 % and 14.3 %), (*E*)-methyl cinnamate (9.4 %, 15.4 % and 4.6 %) and β-pinene (7.6 %, 9.9 % and 3.1 %). The leaf oil had a sizeable amount of o-cymene (7.1 %) and octadecane (5.4 %), while (*E*)-cinnamyl alcohol (5.5 %) and bicycloelemene (5.9 %), and ascaridol (4.1 %) could be seen prominently in the rhizome oil.

Table 1. ***Chemical compounds identified in the essential oils of A. kwangsiensis***

No.	RT (min)	Compounds [a]	RI [b]	RI [c]	Concentration (%)[d]		
					Leaves	Stem	Rhizomes
1	9.10	2-Heptanol	900	892	0.3	-	-
2	10.01	α-Thujene	931	926	1.0	0.3	0.3
3	10.28	α-Pinene	940	932	2.7	2.0	1.0
4	10.76	Camphene	956	946	1.6	0.9	0.5
5	11.43	Sabinene	979	974	2.9	2.4	0.8
6	11.57	β-Pinene [e]	985	978	7.6	9.9	3.1
7	11.82	Myrcrene	992	988	0.3	0.2	0.2
8	12.50	α-Phellandrene	1011	1008	-	-	0.3
9	12.72	δ-3-Carene	1016	1014	0.3	0.3	-
10	12.91	α-Terpinene	1022	1020	0.2	0.2	0.5
11	13.01	o-Cymene	1030	1028	7.1	3.6	2.5
12	13.11	Limonene	1034	1032	1.0	0.6	0.5
13	13.32	1,8-Cineole	1038	1036	16.9	14.3	5.8
14	14.02	γ-Terpinene	1064	1056	0.5	0.7	1.1
15	14.56	*trans*-Dihydro-rose oxide [e]	1075	1077	0.2	0.4	1.6
16	15.04	Terpinolene	1094	1089	0.1	-	0.2
17	15.33	2-Nonanol	1102	1100	0.1	-	-
18	15.71	*endo*-Fenchol	1124	1122	0.2	0.3	0.2
19	16.11	*allo*-Ocimene	1128	1128	-	-	1.8
20	16.51	*cis*-p-Menth-2-en-ol	1130	1127	0.2	-	-
21	16.72	Nopinone [e]	1148	1143	-	0.2	-
22	16.83	*trans*-Sabinol	1150	1148	0.6	0.7	-
23	17.31	Camphor	1156	1151	0.8	0.7	0.2
24	17.48	Pinocarvone	1173	1169	0.4	0.6	-
25	18.32	Borneol	1178	1176	1.9	1.6	1.0
26	18.68	Terpinene-4-ol	1187	1187	16.0	13.6	14.3
27	18.69	p-Cymene-8-ol	1194	1193	0.7	0.8	0.8
28	19.02	Cryptone	1197	1195	0.3	0.3	0.2
29	19.12	α-Terpineol	1200	1198	1.2	2.0	2.3
30	19.88	Myrtenol	1207	1206	0.9	1.0	0.2
31	20.06	Fenchyl acetate	1228	1227	1.1	2.2	1.5
32	20.19	Ascaridol	1234	1234	-	0.5	4.1
33	21.50	*trans*-Ascaridol glycol [e]	1282	1282	-	0.4	1.2
34	22.34	Bornyl acetate	1294	1294	0.2	0.5	-
35	22.53	Thymol	1301	1301	0.3	0.7	0.3
36	22.93	Dihydroedulane	1310	1309	-	-	1.2
37	23.19	Carvacrol	1312	1311	0.3	0.6	0.8
38	23.27	Undecanal	1313	1313	1.0	2.0	1.9
39	23.30	(*E*)-Cinnamyl alcohol	1315	1314	-	-	5.5
40	23.38	*iso*-Ascaridol [e]	1316	1314	-	-	3.0
41	23.47	Myrtenyl acetate	1332	1330	-	-	1.8
42	23.81	Bicycloelemene	1335	1335	-	-	5.9
43	24.22	α-Terpinyl acetate	1357	1353	-	-	1.1

table 1. (continued).

No.	RT (min)	Compounds [a]	RI [b]	RI [c]	Concentration (%)[d] Leaves	Stem	Rhizomes
44	25.20	*cis*-Carvyl acetate	1384	1383	0.5	-	-
45	25.32	α-Copaene	1390	1389	0.3	-	-
46	25.66	(*E*)-Methyl cinnamate [e]	1394	1394	9.4	15.4	4.6
47	25.78	*cis*-β-Elemene	1408	1407	-	-	1.9
48	26.84	β-Caryophyllene	1437	1437	-	0.4	1.2
49	28.77	Germacrene D	1497	1498	3.2	2.8	2.3
50	28.95	β-Selinene	1505	1505	-	0.3	-
51	29.12	α-Selinene	1513	1511	-	0.6	-
52	29.34	Bicyclogermacrene	1516	1513	-	-	1.2
53	29.93	γ-Cadinene	1530	1532	-	0.3	0.1
54	30.75	Elemol	1565	1568	-	-	0.3
55	30.93	(*E*)-Nerolidol	1571	1572	0.1	0.4	0.2
56	32.15	Caryophyllene oxide	1605	1604	1.1	1.3	-
57	32.23	Carotol [e]	1619	1618	-	0.4	-
58	32.37	Widdrol [e]	1625	1622	-	-	0.2
59	32.664	Cedrol	1628	1628	1.3	2.7	2.7
60	32.72	Humulene epoxide II	1632	1630	0.3	-	-
61	33.22	*epi*-α-Muurolol	1662	1672	-	-	0.2
62	33.90	β-Eudesmol	1674	1674	0.2	0.4	0.7
63	33.97	α-Eudesmol	1677	1676	-	-	0.2
64	34.18	neo-Intermedeol [e]	1678	1678	-	-	0.3
65	36.21	Isoclamendiol [e]	1771	1769	-	-	0.3
66	39.97	Octadecane	1901	1900	5.4	2.1	1.9
67	41.27	1,2-Benzenedicarboxylic acid [e]	1917	1916	2.4	-	-
68	42.31	*n*-Hexadecanoic acid [e]	1965	1966	-	-	0.3
69	42.90	Palmitic acid	1972	1972	3.2	-	-
70	44.22	Octadecanal [e]	2025	2024	-	-	0.1
	Total				96.8	91.6	85.4
	Monoterpenes hydrocarbons (Sr. No. 2-12, 14, 16, 19)				25.6	21.1	12.8
	Oxygenated monoterpenes (Sr. No. 13, 15, 18, 20-37, 39-41, 43, 44, 46)				52.3	56.8	51.7
	Sesquiterpenes hydrocarbons (Sr. No. 42, 45, 47-53)				3.5	4.4	11.6
	Oxygenated Sesquiterpenes (Sr. No. 54-65)				3.0	5.2	5.1
	Fatty acids (Sr. No. 66, 68, 69)				8.6	2.1	2.2
	Others (Sr. No. 1, 17, 38, 67, 70)				3.8	2.0	2.0

[a] Elution order on HP-5MS column

[b] Retention Index calculated with respect to a homologous series of *n*-alkanes on a HP-5MS column

[c] Retention Index from the databases

[d] Standard deviation was insignificant and excluded from the Table to avoid congestion

[e] Further identification by co-injection with known compounds

- Not identified

To the best of the authors' knowledge, this is the first report on the chemical constituent of essential oils from *A. kwangsiensis* grown in Vietnam. Monoterpenes represent the main class of compounds in the essential oils of *A. kwangsiensis*, in agreement with the composition of previously analysed essential oils from China [5,6]. However, the identities of these compounds differ from each other. In addition, camphor [5], α-pinene [5,] and cinnamic acid methyl ester [6], the principal compounds of the essential oils analyzed from China were present in much lower quantities in the essential oils of *A. kwangsiensis* from Vietnam. The contents of 1,8-cineole and β-pinene in the present study compete favorably with data obtained from a previous study [5]. A noteworthy observation is that terpinene-4-ol one of the major compounds of the studied essential oils in this study was not previously reported to be of significant quantity in the essential oils from *A. kwangsiensis*. This variation in the chemical constituents of essential oils of *A. kwangsiensis* may be possibly due to differences in the place of origin affected by ecological conditions and plant parts used.

Result of the antimicrobial test on the essential oils

The essential oils from *A. kwangsiensis* were screened against a panel of microorganisms (Table 2). The MIC was to determine the minimum concentration that prevented the growth of test microbes. However, IC_{50} is that concentration that achieved 50 % growth inhibition. In the present work, it may be useful in drug formulation for some applications including the use of essential oils as a component of antimicrobial packaging. The essential oil showed good antibacterial activity against the Gram-positive bacteria *E. faecalis* ATCC 299212, *B. cereus* ATCC 14579, and *S. aureus* ATCC 25923, and anticandidal activity against *C. albicans* ATCC 10231. In this report, the rhizome essential oil was the most active against *E. faecalis* ATCC 299212 with a minimum inhibitory concentration (MIC) value of 1.60 μg/mL, while the leaf oil displayed pronounce activity towards *Bacillus cereus* ATCC 14579 (MIC 3.20 μg/mL). All the essential oils inhibited the growth of *S. aureus* ATCC 25923 and *C. albicans* ATCC 10231, with MIC value of 6.40 μg/mL. The essential oils did not exhibit any activity towards the Gram-negative bacteria of *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, and *S. enterica* ATCC 3076. The potency of the essential oils against the tested microorganisms defined by IC_{50} is shown in Table 2. The rhizome oil exhibited activity towards *E. faecalis* and *S. aureus* with the least inhibitory concentration of 10.67 μg/mL and 24.78 μg/mL, respectively. The stem oil showed good activity towards *B. cereus* and *C. albicans* with IC_{50} values of 19.89 μg/mL and 20.56 μg/mL, respectively. Overall, all the essential oils displayed significant activity towards *E. faecalis*, *B. cereus, S. aureus,* and *C. albicans* The reference compounds namely Streptomycin for gram-positive bacteria exhibited activity with MIC values in the range of 0.5-1.0 μg/mL, while cycloheximide used as antifungal had MIC values in the range of 1.2-3.7 μg/mL. Also, Nystatin an anticandidal compound displayed activity with MIC values in the range of 0.8-2.3 μg/mL. The IC_{50} values have also been considered in the range of 10-120 μg/mL[7-19]. No previous information exists on the antimicrobial activity of essential oils from *A. kwangsiensis.*

The inactivity of the essential oils against Gram-negative bacteria was not unexpected. Several previous studies have shown Gram-positive bacteria to be more susceptible to essential oils than Gram-negative organisms [7-19,23,24]. This may be due to cell wall lipopolysaccharides in the Gram-negative organisms that inhibit the lipophilic essential oil components from diffusing into the cells. However, the ability of essential oils to disrupt the permeability barrier of cell membrane structures and the accompanying loss of chemiosmotic control is the most likely reason for its lethal action [25].

The observed antimicrobial result of *A. kwangsiensis* essential oils was in agreement with previous information that *Alpinia* essential oils from Vietnam and other parts of the world selectively inhibited the growth of different microorganisms. For example, essential oils from the leaves of *A. globosa* and *A. tonkinensis* [7] from Vietnam displayed activity against *E. coli,*

Table 2. Antimicrobial activity of the essential oils of *A. kwangsiensis*

Sample	Gram (+)			Gram (-)			Yeast
	Enterococcus faecalis ATCC 299212	*Staphylococcus aureus* ATCC 25923	*Bacillus cereus* ATCC 14579	*Escherichia coli* ATCC 25922	*Pseudomonas aeruginosa* ATCC 27853	*Salmonella enterica* ATCC 13076	*Candida albicans* ATCC 10231
				MIC (μg/mL)			
Leaf	6.40±0.500	6.40±0.500	3.20±0.100	-	-	-	6.40±0.500
Stem	6.40±0.100	6.40±0.500	6.40±0.100	-	-	-	6.40±0.500
Rhizome	1.60±0.200	6.40±0.500	6.40±0.120	-	-	-	6.40±0.500
				IC_{50} (μg/mL)			
Leaf	25.56±0.500	39.67±0.100	20.45±0.000	-	-	-	26.53±0.500
Stem	18.99±0.500	32.87±0.200	19.89±0.001	-	-	-	20.56±0.500
Rhizome	10.67±0.500	24.78±0.500	30.24±0.001	-	-	-	22.34±0.500

-No activity

S. aureus subsp. *aureus* and *F. oxysporum* with MIC of 50 μg/mL. The rhizome of *A. galangal* was also active towards *Salmonella typhi* [26]. The essential oils of *A. nieuwenhuizii* and *A. ligulata* oils exhibited antimicrobial properties against *S. aureus* var. *aureus* and *E. coli* with MIC values of 2.0-3.2 μg/mL[27]. Essential oils from the rhizome of *A. pahangensis* inhibited several strains of *S. aureus* (MIC values between 0.08 and 0.31 μg/mL), *C. albicans* (MIC of 1.25 μg/mL), and *C. galabrata* (MIC 2.50 μg/mL) [28]. Likewise, the leaf essential oils of *A. rafflesiana* were active against *E. coli* (MIC 15.6 μg/mL) and *S. aureus* (MIC 7.81 μg/mL)[29].

The major components of the essential oil namely 1,8-cineole, terpinene-4-ol, and (*E*)-methyl cinnamate and β-pinene have shown antimicrobial activities against microorganisms, and likely account for the observed antimicrobial activity of *A. kwangsiensis* essential oils. 1,8-Cineole was reported to have demonstrated synergistic antimicrobial effect on *S. aureus*, *E. faecalis*, and *C. albicans* among others but not *P. aeruginosa* [30]. Terpinen-4-ol was active against some tested organisms including *P. aeruginosa, C. albicans, E. coli,* and *S. aureus* [1]. (*E*)-Methyl cinnamate exhibited strong growth inhibition towards *Saccharomyces cerevisiae*, *C. lipolytica, C. albicans,* and *Microsporum canis* [31,32]. The other minor constituents of *A. kwangsiensis* essentials oil either alone or in combination with others may have provided synergistic antimicrobial effects [7-19].

Conclusions

This study found out that significant quantities of 1,8-cineole, terpinene-4-ol, (*E*)-methyl cinnamate, and β-pinene were present in the essential oils of *A. kwangsiensis*. The essential oils displayed a varying degree of antimicrobial activity with the rhizome essential oil being the most active against *E. faecalis* ATCC 299212 (MIC, 1.60 μg/mL) and the leaf with pronounced activity towards *B. cereus* ATCC 14579 (MIC 3.20 μg/mL). Moreover, all the studied essential oils showed antimicrobial activity against *S. aureus* ATCC 25923 and anti-candidal potential towards *C. albicans* ATCC 10231 with MIC value of 6.40 μg/mL.

Therefore, the essential oils obtained from *A. kwangsiensis* may have potential applications as antimicrobial agents.

Competing interests

The authors declare that they have no competing interests.

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