Essential oils of leaves and pseudo stems *Alpinia malaccensis* and antimicrobial activities

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Abstract. The essential oils from the leaves, pseudo stems, rhizomes and fruits of *Alpinia malaccensis,* collected from conservation garden, UPM were isolated by hydro distillation. The collected oils were analysed by capillary GC and GC-MS. The leaf, pseudo stem, rhizome and fruit oils afforded 73 and 24 constituents, respectively. The most abundant components in the leaf oil were *β*-pinene (20.02%), 1,8-cineol (16.49%), *trans*- caryophyllene (8.69%) and α-pinene (6.64%), while 1,8-cineole (36.81%), β-pinene (13.95%), α-terpineol (6.85%) , *trans*-caryophyllene (7.49%) and α-terpinolene (6.85%) were the main constituents in the pseudo stems oil. Antimicrobial assay revealed that all the essential oils showed moderate to weak inhibition against the tested microorganisms. The leaf oil was the most active and inhibited both *S. aureus* and *E. coli with* MIC values of 7.81 µg/mL and 15.6 µg/mL, respectively.

Keywords: Essential oils, *Alpinia malaccensis*, Zingiberaceae, Antimicrobial, 1.8- cineole

1. Introduction

Alpinia malaccensis or locally known as “malacca” is one of the 230 species of the ginger family (Zingiberaceae). *Alpinia* is the largest, most wide spread and most taxonomically complex genus in the ginger family throughout tropical and sub- tropical countries [1]. Several *Alpinia* species such as *Alpinia galangal* are used as spices for flavoring foods and as ingredients in traditional medicines. A few species including *Alpinia purpurata* [2] and *Alpinia mutica* [1,3,4] are widely cultivated as ornamental plants due to the beautiful and long-lasting flowers.

The essential oil compositions of Malaysians *Alpinia* have been reported by Sirat et al., [5-8], Ibrahim et al., [9] and Wong et al., [10]. In continuation of our investigation of the essential oils of *Alpinia* species, we investigated the chemical composition of *A. malaccensis* and their antimicrobial activity. To the best of our knowledge, there has been a little report on the essential oil and antimicrobial activity of this plant.

The results of this research showed that the leaf had highest amount of oil (0.07% yield) than the pseudo stem, pseudo stem (0.05%) of oils, respectively. The leaf oil afforded 73 (99.90%) identified

constituents and the most abundant components in the leaf oil were *β*-pinene (20.02%), 1,8-cineole (16.49%), *trans*- caryophyllene (8.69%) and α-pinene (6.64%). The occurance of 1,8-cineole as the major constituent in *Alpinia* essential oils has been reported in *A. officinarum* Hance [11]. *A. conchigera* Griff [10], *A. speciosa* [12], the rhizome and seed oil *A. galanga* [13], the fruit oil of *A. purpurata* [14] and the leaf and rhizome oils of *A. smithae* [15]. *1,8*-cineole, is known for its anti-inflammatory and local anesthetic activities. It is used in spice blends, citrus flavours, soaps, detergents, creams and lotions and also in a variety of food products. It has also been identified as a volatile compound emitted by plants into the atmosphere in response to herbivore attack [16]. Among these compounds were 46.97% monoterpene hydrocarbon, 25.17% oxygenated monoterpene, 13.41% sesquiterpene hydrocarbon, 6.06% oxygenated sesquiterpene and 8.29% ester.

The pseudo stem oil has succesfully yielded 24 identified constituents, accounting for 95.97% of the sample and yielded 0.07% of essential oil. The oil was very rich in monoterpenoid (47.05%), comprising mainly 1,8-cineole (36.81%), hydrocarbon monoterpene which β-pinene (13.95%) and hydrocarbon sesquiterpene *trans*-caryophyllene (7.49%). 1,8-Cineole was also reported to be the major constituents in the flower oils of *A. speciosa* [12], leaf oils *A. purpurata* (Viell.) Schum [12], rhizome oils *A. aquatica* Rosc [8], leaf and stem oils *A. galanga* [17], leaf oils *A. galanga* [18] and rhizome oils *A. zerumbet* (Pers.) B.L. Burtt. & RM. Sm. [19]. Among these compounds were 28.73% monoterpene hydrocarbon, 46.87% oxygenated monoterpene, 15.72% sesquiterpene hydrocarbon, 4.99% oxygenated sesquiterpene, 0.40% ester and 2.49% long chain hydrocarbons.

The antimicrobial activity of the essential oil against Gram-positive (*B. subtillus*, *S. aureus*), Gram-negative bacteria (*P. aeruginosa, P. putida, E. coli*), fungi (*A. niger) and* yeast *(C. albicans*) was assessed both qualitatively and quantitatively by disc diffusion method and MIC values. Table 2 shows the antimicrobial activity of the essential oils of *A. malaccensis.*

The leaf oil inhibited both the Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) each with MIC value of 7.81 µg/mL and 15.6 µg/mL. The pseudo stem oil displayed strong activity against both Gram-positive bacteria, *S. aureus* and *B. subtilis* with MIC 31.25 µg/mL each. The fruit oil was active against *S. aureus* with MIC value 31.25 µg/mL. The rhizome oil was found inactive against all the tested bacteria. All oils were inhibited *C. albican* with MIC 125 µg/mL except the rhizome oil. The rhizome, fruit and pseudo stem oils were found active against *A. niger* with MIC 125µg/mL. The antimicrobial properties of all oils are assumed to be assonated with their oxygenated components [20]. In general, the Gram-positive bacteria seemed to be more sensitive to the observe oils than the Gram-negative bacteria due to the differences in the cell membrane of the bacterial group [21].

Table 1. Constituents Identified in the Leaf, and Pseudo Stem Oils *of A. malaccensis*

Compound[a]	RI determine[b]	RI literature[c]	Oil yielded (%)	
			Leaf	Pseudo stem
α-Pinene	942	932	6.64	5.08
Camphene	947	946	0.16	0.08
Benzaldehyde	951	952	0.11	0.50
Sabinene	957	960	2.26	0.26
trans-p-Menthane	972	973	0.11	
β-Pinene	974	974	20.02	13.95
3-Octanone	977	979	0.19	3.08
3-*ρ*- Menthene	983	984	0.18	0.03
Furfuryl acetate	986	987	0.21	0.05
Myrcene	993	988	0.74	0.93

α-Octanal	995	998	0.04	
δ-2-Carene	996	1001	3.98	3.58
α-Phellandrene	999	1002	4.03	5.26
p-Cymene	1011	1020	5.59	
1,8-Cineole	1032	1026	16.49	36.81
(E)-β-Ocimine	1049	1049	2.26	0.17
γ-Terpinene	1059	1054	0.64	0.62
5Z-0ctenol	1065	1065	0.66	
Camphenilone	1078	1078	0.05	
ρ-Mentha-2,4(8)-diene	1085	1085	0.11	
Terpinolene	1089	1086	0.21	0.26
Linalool	1095	1095	0.31	0.16
Fenchol	1107	1114	0.15	
Myrcenol	1116	1119	0.05	0.10
trans-ρ-Menth-2-en-1-ol	1137	1136	0.11	0.11
trans-pinocarveol	1133	1135	0.77	0.04
Camphor	1143	1141	0.48	
Citronellal	1155	1148	0.13	
Pinocarvone	1161	1160	0.70	
Borneol	1166	1165	0.20	
cis –Linalool oxide	1168	1170	0.07	
Terpinen-4-ol	1175	1174	1.31	2.08
α-Terpineol	1186	1186	0.30	6.85
Myrtenol	1193	1194	1.46	
Myrtenal	1193	1195	1.79	
Linalool formate	1209	1214	3.98	
Citronellol	1220	1223	0.36	
Thymol methyl ester	1241	1232	0.08	
Geraniol	1247	1249	0.05	
Dihydrolinalool acetate	1274	1274	2.98	
Safrole	1281	1285	0.08	0.03
Carvacrol	1299	1298	0.07	
cis-Methyl cinnamate	1304	1299	0.17	0.60
Myrtenyl acetate	1321	1324	0.40	
α-Cubebene	1353	1345	0.08	0.38
α-Terpinenyl acetate	1357	1346		0.03
Neryl aetate	1361	1359	0.07	0.21
α-Copaene	1380	1374	0.03	0.83
β-Panasinsene	1384	1381	0.18	0.17
β-Cubebene	1396	1387	0.05	0.04
α-Gurjunene	1413	1409		0.33
trans-Caryophyllene	1418	1417	8.69	7.49
γ-Elemene	1434	1434	0.06	0.03
β-Humulene	1439	1442	0.04	
Cedrane	1445	1441	0.05	
α-Humulene	1454	1452	0.98	0.82
Aromadendrene	1464	1458	0.04	0.18

γ-Gurjunene	1467	1475	0.16	
α-Amorphene	1473	1483	0.41	0.10
Germacrene D	1480	1485	0.27	0.34
β-Selinene	1496	1489	0.20	0.04
Valencene	1509	1496	0.07	
β-Sesquiphellandrene	1517	1521	0.08	
δ-Cadinene	1520	1522	0.47	2.07
Chavibetol acetate	1526	1524	0.34	
(E)-γ-Bisabolene	1534	1529	0.14	0.27
δ-Cuprenene	1543	1542	0.43	
Elemol	1549	1548		0.08
Germacrene B	1558	1559	0.26	0.09
(E)-Nerolidol	1567	1561	0.07	
Cedrene epoxide	1572	1574	0.52	
Germacrene D-4-ol	1574	1574	1.12	0.08
Caryophellene oxide	1583	1582	3.69	1.36
Guaiol	1594	1600	0.13	0.17
α-Eudesmol	1657	1652	0.33	
Bulnesol	1664	1660		0.23
(2Z,6Z)-Farnesol	1696	1698	0.20	
(2E, 6E)-Farnesyl acetate	1834	1845	0.06	
Identified compounds				
Monoterpene hydrocarbons			46.97	30.22
Oxygenated monoterpenes			25.17	49.76
Sesquiterpene hydrocarbons			13.41	13.21
Oxygenated sesquiterpenes			6.06	1.92
Esters			8.29	0.86
Long chain hydrocarbons			0.00	0.00
Total			99.90	95.97

[a]order of elution on Ultra-1 column.
[b] retention index calculated from retention times in relation to those of a series of C_9-C_{24} *n*-alkane.
[c]retention index takes from Adams [22].

Table 2. Antimicrobial Activity of the Leaves and Pseudo Stems Oils of *A. malaccensis*

Microorganism							Antibiotic	
	Leaf		Pseudo stem		SS		Nystatin	
	DD	MIC	DD	MIC	DD	MIC	DD	MIC
Gram-positive bacteria								
Bacillus subtilis	-	-	15.0	31.25	15.0	7.81	ND	ND
Staphylococcus aureus	14.0	7.81	14.0	31.25	15.0	7.81	ND	ND
Gram-negative bacteria								
Pseudomonas aeruginosa	-	-	15.0	125	18.7	7.81	ND	ND

Pseudomonas putida	16.0	125	15.0	125	17.3	7.81	ND	ND
Escherichia coli	16.7	15.63	16.0	62.50	15.3	7.81	ND	ND
Yeast								
Candida albicans	14.3	125	14.3	125	ND	ND	15.0	7.81
Fungi								
Aspergillus niger	-	-	15.0	125	ND	ND	15.0	7.81

[a] Data represent mean±standard deviation of three independent experiment; DD- disc diffusion method (including the diameter of disc 6 mm); MIC-minimum inhibitory concentration (μg/mL); SS-streptomysin sulfate; ND-not determined.

2. Experimental

Plant materials: Fresh leaves and pseudo stems of *Alpinia malaccensis* were collected from conservation garden Universiti Putra Malaysia in July 2010.

Extraction of essential oils: The essential oils were obtained by hydro distillation for 8 hours using a Clevenger-type apparatus. The lighter than water, transparent oil was re-extracted with diethylether and dried over anhydrous $MgSO_4$ and stored at 4-6°C. The light-yellow oils yielded (w/w) were 0.10%, 0.07%, 0.05% and 0.03% for leaves and pseudo stems respectively, based on their fresh weight.

Gas chromatography (GC): GC analysis was carried out using an Agilent 6890 series II. A gas chromatograph equipped with a flame ionization detector, using a non-polar Ultra-1 fused equipped silica capillary column (25 m long, 0.33 mm thickness and 0.20 μm inner diameter). Helium was used as a carrier gas at a flow rate of 1.5 mL/min and 30 psi inlet pressure; split ratio 1:20. The oven temperature was programmed from 50°C for 5 min, 50-300°C at a rate of 5°C/min, and kept isothermal for 10 min. Injector and detector temperatures were set at 300°C and 310°C. Samples were diluted using (1/100, diethyl ether v/v) of 1.0 μL were injected manually (split ratio 1:50). The injection was repeated three times and the peak area percentage were reported as means ± SD of triplicates. Calculation of peak area percentage was carried out using GC HP Chem station software (Agilent Technologies).

Gas Chromatography–Mass Spectrometry (GC-MS): The qualitative GC-MS analysis was carried out an Agilent Technologies Model 6890N gas chromatography and an Agilent Technologies Model 5973i mass spectrometer. The GC was equipped with Ultra-1 column (25 m long, 0.33 μm thickness and 0.20 mm inner diameter). Helium was used as carrier gas at flow rate 1 mL/min. Injector temperature was 250°C. Oven temperature was programmed from 50°C (5min hold) to 300°C at 5°C/min and finally held isothermally for 10 min. For GC-MS detection, an electron ionization system, with ionization energy of 70 eV was used. A scan rate of 0.5 s (cycle time: 0.2 s) was applied, covering a mass range from 50-400 amu. The individual components of the oil were identified on the basis of their retention indice (RI) with reference to homologous series of C_9-C_{24} n-alkanes and by matching their mass spectra with the MS data from the Wiley library and also by comparing their retention indices with those of either authentic compounds or data in the literature and reference [22].

Antimicrobial assay - Microbial strains: A collection of seven microorganisms were used including the Gram-positive bacteria, *Staphylococcus aureus* (ATCC29737), *Bacillus subtilis* (ATCC6633), Gram-negative bacteria, *Pseudomonas aeruginosa* (ATTC9027), *Escherichia coli* (ATTC10536) and *Pseodomonas putida* (ATCC49128). The yeast used was *Candida albicans* (ATCC10231) while the fungi used was *Aspergilus niger* (ATCC16888). The strains were grown on Nutrient agar (Oxoid, Italy) for the bacteria and Potato dextrose agar (PDA) for fungi and yeast.

Disc Diffusion: Antimicrobial activity of the essential oils of *A. malaccensis* was carried out by the disc diffusion method [23]. The essential oils were dissolved in DMSO (4 mg/mL). Inocolumn of 400 µL suspension containing 10^8 CFU/mL of bacteria and 10^6 CFU/mL of fungi each was spread on the nutrient agar (NA) and potato dextrose agar (PDA) medium. The disc (6 mm diameter) impregnated with 10 µL of the essential oils and DMSO (negative control) were placed on the inoculated agar, and were incubated or 24 h at 37°C (bacterial) and 72 h at 30°C (fungi), streptomysin sulfat (10 µg/mL) and nystatin (100 IU) were used as the positive controls for bacteria and fungi, respectively. Clear inhibition zones around the discs indicated the positive antimicrobial activity. All the test and analysis were carried out in triplicates.

Minimum Inhibitory Concentration (MIC): The minimum inhibitory concentration (MIC) was determined by using the micro dilution method, using 96 wells microtitre plates [24]. The inocula of the microbial strains were prepared from 24 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity [25]. Each essential oil (1 mg) was dissolved in DMSO (1 mL) to get 1000 µg/mL stock solution. A number of wells were reserved in each plate for positive and negative controls. Sterile broth (100 µL) was added to wells at row B to H. The stock solutions of samples (100 µL) were added to wells at row A and B. Then, the mixture of samples and sterile broth (100 µL) at row B were transferred to each well in order to obtain a twofold serial dilution of the stock samples (concentration of 1000, 500, 250, 125, 62.5, 31.3, 15.63 and 7.81 µg/mL). The inoculated bacteria (100 µL) were added to each well. The final volume in each well was 200 µL. Nystatin was used as standard antibiotic comparison with the antifungal activities of the essential oils, while streptomysin sulfate was used as the standard in the antibacterial activity test. Plates were incubated at 37°C for 24 h. Microbial growth was indicated by the turbidity and the presence of pellet at bottom of the well. After an incubation period at 37°C for 24 h, turbidity was taken as indication of growth, thus the lowest concentration which remained clear after macroscopic evaluation was taken as the minimum inhibitory concentration (MIC). The MIC was recorded as mean concentration of triplicates. Data obtained from essential oil analysis and antimicrobial activity were expressed as mean values and the standard deviation values.

Statistical analysis: Data obtained from essential oils analyses and antimicrobial activity are expresses as mean values. Statistical analysis was carried out by employing one way ANOVA ($p>0.05$). A statistical package (SPSS version 11.0) was used for the data analysis.

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References

[1] Burkill IH. (**1966).** *Dictionary of the economic products of the Malay Peninsula*, Vol 2. Ministry of agriculture and co-operatives. Kuala Lumpur. pp 1323-1334.
[2] Sirat MH, Liamen MR. (**1995**). Chemical constituents of *Alpinia purpurata*. *Pertanika Journal of Science & Technology. **3,*** 67-71.
[3] Larsen K, Ibrahim H, Kha, SH & Saw LG. (**1999**) *Gingers of Peninsular Malaysia and Singapore*. Kota Kinabalu: Natural History Publication (Borneo). pp 58.
[4] Criley RA. (**1988**). *Propagation of tropical cut flowers*: *Strelitzia, Alpinia and Heliconia*. Acta Hort. ***226***, 509-517.
[5] Sirat HM, Nordin AB. (**1995**). Chemical composition of the rhizome oil of *Alpinia conchigera* Griff of Malaysia. *Journal of Essential Oil Research, 7*, 195-196.
[6] Sirat HM, Khalid NF, Jani NA, Basar N. (**2009**). Chemical composition of the fruits oil of *Alpinia mutica* Roxb. (Zingiberaceae). *Journal of Essential Oil Research*, ***21***, 457-458.

[7] Sirat HM, Rahman AA. (**1998**) Essential oil constituents of *Alpinia mutica* Roxb. *Journal of Essential Oil Research*, ***10***, 83-84.
[8] Sirat HM, Basar N, Jani NA. (**2011**). Chemical composition of the rhizome oils of two *Alpinia* species of Malaysia . *Natural Product Research*, ***21***, 982-986.
[9] Ibrahim H, Aziz AN, Syamsir DR, Ali NAM, Mohtar M, Ali RA, Awang K. (**2009**) Essential oils of *Alpinia conchigera* Griff. and their antimicrobial activities. *Food Chemistry*. ***113***, 575-577.
[10] Wong KC, Lee BC, Lam NF, Ibrahim H. (**2005**). Essential oil of the rhizomes of *Alpinia conchigera* Griff and *Alpinia Latibraris* Ridl. *Flavour & Fragrance Journal*, ***20***, 431-433.
[11] Tram NL, Ryo Y, Koji K. (**2001**). Volatile components of the essential oils in Galanga (*Alpinia officinarum* Hance) from Vietnam. *Food Science & Technology Research*, *7*, 303-306.
[12] Zoghbi M, Andrade E, Maia J. (**1999**). Volatile constituent from leaves and flowers of *Alpinia speciosa* K. Shum and *Alpinia purpurata* (Viell.) Schum. *Flavour & Fragrance Journal*, ***14***, 411-414.
[13] Jantan I, Ahmad F. (**2004**). Constituent of the rhizome and seed oils of *Galangal* (L.) Willd. from Malaysia. *Journal of Essential Oil Research*, ***16***, 174-176.
[14] Santos GKN, Dutra KA, Baroos RA, da Camara CAG, Lira, Diana D, Norma B. Gusmao, Navarro DMAF. (**2012**). Essential oils from *Alpinia purpurata* (Zingiberaceae): Chemical composition, oviposition deterrence, larvicidal and antibacterial activity. *Industrial Crops & Products*, ***40***, 254-260.
[15] Joseph R, Joseph T, Joseph J. (**2001**). Volatile essential oil constituents of *Alpinia smithiae* (Zingiberaceae), *Revista De Biologîa Tropical*. ***49***, 509-512.
[16] Sabulal B, Dan M, John AJ, Kurup R, Pradeep NS, Valsamma RK, George V. (**2006**) Caryophellene-rich rhizome oil of *Zingiber nimmoni* from South India: Chemical characterization and antimicrobial activity. *Phytochemistry*, 67, 2469-2473.
[17] Jirovetzi L, Buchbaueri G, Shafi MP, Leela NK. (**2003**). Analysis of the essential oils of the leaves, stems, rhizomes and roots of the medicinal plant *Alpinia galanga* from Shorthen India. *Acta Pharmaceutica*. ***53***, 73-81.
[18] Raina VK, Srivasta SK, Syamasunder KV. (**2002**). The essential oil of 'greatear galangal' [*Alpinia galanga* (L.) Willd.] from the lower Himalayan region of India. *Flavour & Fragrance Journal*. ***17***, 358-360.
[19] Elzaawely AA, Xuan TD, Koyama H, Tawatta S. (**2007**). Antioxidant activity and contents of essential oil and phenolic compounds in flowers and seeds of *Alpinia zerumbet* (Pers.) B. L Burtt. & R, M Sm. *Food Chemistry*, ***104***, 1648-1653.
[20] Marzoug HNB, Romdhane M, Lebrihi A, Mathieu F, Couderc F, Abderraba M, Khouja, ML and Bouajila J. (**2011**). *Eucalyptus oleosa* essential oils: Chemical compostion and antimicrobial and antioxidant activities of the oils from different plant parts (Stems, Leaves, Flowers and Fruits). *Molecules*, ***16***, 1695-1709.
[21] Filippi JJ, Lanfranchi DA, Prado S, Baldovini N, Meierhenrich UJ. (**2006**). Composition enantiomeric, distribution and antibacterial activity of the essential oil of *Achillea ligustica* All. from Corsica. *Journal of Agricultural & Food Chemistry*. ***54***, 6308-6313.
[22] Adams RP. (**2007**). *Identification of essential oil components by gas chromatography/ Mass spectrometry, 4th ed.* Allured Publishing Corporation, Carol Stream, Illinois, USA.
[23] Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH. (**1999**). *Manual of Clinical Microbiology 7th ed.* ASM, Washington, DC, USA.
[24] Gulluce M, Ozer H, Baris O, Daferera D, Sahin F, Polissiou M. (**2004**). Chemical composition of the essential oils of *Salvia aethiopis* L. *Turkish Journal of Biology*, ***30***, 231-233.

[25] Vandepitte J, Engback K, Piot P, Heuck CC. (**1991**). *Basic Microbiology Procedures in Clinical Bacteriology*. Geneva. World Health Organization. pp 85.