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 occurrence 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 monoterpen 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. albicans 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        | RI determine | RI literature | Oil yielded (%) |
|-----------------|--------------|---------------|-----------------|
| 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-p- Methene    | 983          | 984           | 0.18            | 0.03            |
| Furfuryl acetate| 986          | 987           | 0.21            | 0.05            |
| Myrcene         | 993          | 988           | 0.74            | 0.93            |
| Compound                        | Amount   | Amount | Amount   |
|--------------------------------|----------|--------|----------|
| 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-Octenol                     | 1065     | 1065   | 0.66     |
| Camphenilone                   | 1078     | 1078   | 0.05     |
| p-Mentha-2,4(8)-diene          | 1085     | 1085   | 0.11     |
| Terpinolene                    | 1095     | 1095   | 0.31     | 0.16     |
| Linalool                       | 1107     | 1114   | 0.15     |
| Fenchol                        | 1116     | 1119   | 0.05     | 0.10     |
| Myrcenol                       | 1128     | 1128   | 2.26     | 0.11     |
| trans-p-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-Cubebe                       | 1353     | 1345   | 0.08     | 0.38     |
| α-Terpinnyl acetate            | 1357     | 1346   | 0.03     |
| Neryl acetate                  | 1361     | 1359   | 0.07     | 0.21     |
| α-Copaene                      | 1380     | 1374   | 0.03     | 0.83     |
| β-Panasinsene                  | 1384     | 1381   | 0.18     | 0.17     |
| β-Cubebe                       | 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     |
Table 2. Antimicrobial Activity of the Leaves and Pseudo Stems Oils of A. malaccensis

| Microorganism       | Antibiotic       | Leaf | Pseudo stem | SS   |
|---------------------|------------------|------|-------------|------|
|                     |                  | DD   | DD          | SS   |
|                     |                  | MIC  | MIC         | MIC  |

| Microorganism       | Antibiotic       | Leaf | Pseudo stem | SS   |
|---------------------|------------------|------|-------------|------|
|                     |                  | DD   | DD          | SS   |
|                     |                  | MIC  | MIC         | MIC  |

**Gram-positive bacteria**

- *Bacillus subtilis*
  - MIC: 15.0
  - MIC: 31.25
  - MIC: 15.0
  - MIC: 7.81
  - MIC: ND
  - MIC: ND

- *Staphylococcus aureus*
  - MIC: 14.0
  - MIC: 7.81
  - MIC: 14.0
  - MIC: 31.25
  - MIC: 15.0
  - MIC: 7.81
  - MIC: ND
  - MIC: ND

**Gram-negative bacteria**

- *Pseudomonas aeruginosa*
  - MIC: 15.0
  - MIC: 125
  - MIC: 18.7
  - MIC: 7.81
  - MIC: ND
  - MIC: ND

*Order of elution on Ultra-1 column.*

*Retention index calculated from retention times in relation to those of a series of C9-C24 n-alkane.*

*Retention index takes from Adams [22].

Identified compounds

- Monoterpene hydrocarbons: 46.97
- Oxygenated monoterpenes: 25.17
- Sesquiterpene hydrocarbons: 13.41
- Oxygenated sesquiterpenes: 6.06
- Esters: 8.29
- Long chain hydrocarbons: 0.00
- Total: 99.90

Monoterpene hydrocarbons: 30.22
Oxygenated monoterpenes: 49.76
Sesquiterpene hydrocarbons: 13.21
Oxygenated sesquiterpenes: 1.92
Esters: 0.86
Long chain hydrocarbons: 0.00
Total: 95.97

| Microorganism       | Antibiotic       | Leaf | Pseudo stem | SS   |
|---------------------|------------------|------|-------------|------|
|                     |                  | DD   | DD          | SS   |
|                     |                  | MIC  | MIC         | MIC  |

| Microorganism       | Antibiotic       | Leaf | Pseudo stem | SS   |
|---------------------|------------------|------|-------------|------|
|                     |                  | DD   | DD          | SS   |
|                     |                  | MIC  | MIC         | MIC  |

*Order of elution on Ultra-1 column.*

*Retention index calculated from retention times in relation to those of a series of C9-C24 n-alkane.*

*Retention index takes from Adams [22].
Pseudomonas putida

|          |   |   |   |   |   |   |   |
|----------|---|---|---|---|---|---|---|
|          | 16.0 | 125 | 15.0 | 125 | 17.3 | 7.81 | ND |

Escherichia coli

|          |   |   |   |   |   |   |   |
|----------|---|---|---|---|---|---|---|
|          | 16.7 | 15.63 | 16.0 | 62.50 | 15.3 | 7.81 | ND |

Yeast

|          |   |   |   |   |   |   |   |
|----------|---|---|---|---|---|---|---|
|          | 14.3 | 125 | 14.3 | 125 | ND | ND | 15.0 |

Candida albicans

|          |   |   |   |   |   |   |   |
|----------|---|---|---|---|---|---|---|
|          |   |   |   |   | ND | ND | 7.81 |

Fungi

|          |   |   |   |   |   |   |   |
|----------|---|---|---|---|---|---|---|
| Aspergillus niger | - | - | 15.0 | 125 | ND | ND | 15.0 |

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-streptomycin 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 MgSO₄ 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 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 C₆-C₂₄ 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 Pseudomonas putida (ATCC49128). The yeast used was Candida albicans (ATCC10231) while the fungi used was Aspergillus 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). Inoculum 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 sulfate (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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