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Biological Activities of Essential Oils Hydrodistillated from Two Closely Related Ginger Species: *Alpinia malaccensis* var. *nobilis* and *Alpinia latilabris* leaves

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**Abstract:** The essential oils of *A. malaccensis* var. *nobilis* and *A. latilabris* were initially screened for antimicrobial activity against eleven microbes using the qualitative BacTiter Glo™ kit followed by IC₅₀ determination using the quantitative protocol of the same kit. The antioxidant activities were determined using DPPH radical scavenging test, ABTS and FRAP analysis. *A. malaccensis* var. *nobilis* showed inhibition of growth of 10 out of 11 microbes tested, with the most significant result observed for *C. tropicalis* and *C. neoformans* having IC₅₀ of 1.75 mg/mL and 1.97 mg/mL, respectively. *A. latilabris* inhibited the growth of 8 out of 11 microbes, with the highest inhibition against *K. pneumonia*, having IC₅₀ of 18.83 mg/mL. *A. malaccensis* var. *nobilis* also had a better antioxidant activity compared to *A. latilabris*. The IC₅₀ for *A. malaccensis* var. *nobilis* was 32.67 mg/mL while 54.33 mg/mL for *A. latilabris*, using DPPH free radical scavenging assay. Measurements by ABTS and FRAP assays provided GAE value of 26.59 mg GAE/g and TE value of 24.56 M TE/g, respectively for essential oil of *A. malaccensis* var. *nobilis* while for *A. latilabris*, 14.47 mg GAE/g and 17.51 M TE/g, respectively. GC analysis of the essential oil showed high presence of methyl cinnamate (60.26 %) and thymol (16.04 %) in essential oil of *A. malaccensis* var. *nobilis* and phytol (91.75 %) in essential oil of *A. latilabris*. In conclusion, *A. malaccensis* var. *nobilis* established to have generally better antimicrobial and antioxidant activities to its closely related species, *A. latilabris*.

**Key words:** *Alpinia*; GC-FID; GC-MS; wild ginger; anti-yeast.

**Introduction**

In Malaysia, plants from the family Zingiberaceae or alternatively termed as gingers, had been traditionally and still commonly used for medicinal purposes 1. Many species such as, *Alpinia mutica*, *A. conchigera* and *Zingiber cassumunar* were proven to have antimicrobial and antioxidant properties 1-3. Similar biological properties have been explored in this study on two closely related wild gingers endemic to Malaysia and its surroundings. *A. malaccensis* var. *nobilis* and *A. latilabris* are morphologically similar with stems 2-4 meter tall, with upright, relatively huge inflorescences and long creeping rhizomes. *A. malaccensis* var. *nobilis* can be differentiated from *A. latilabris* by its much taller stems which can reach up to 4 meters tall, longer and bigger leaves and presence of fine hairs within the underneath of its leaf. The inflorescence of *A. malaccensis* var. *nobilis*
is almost twice as long as that of *A. latilabris*. The floral parts and floral inner colour also differentiated the former from the latter.

*A. malaccensis* var. *nobilis*, also locally known as “puar” is a medicinal plant that is commonly found in South East Asian countries such as Malaysia, Vietnam, Myanmar and Thailand 4,5. The plant is easily identified from its white flower with yellow interior arranged in an upright conical shape. Although currently this species is popular as an ornamental plant, the natives used to apply the rhizome of this species on wounds and sores to prevent infection 6. There are few studies that have been done on methanol leaf extracts of *Alpinia malaccensis* that showed the antioxidant properties of the leaf 5. The chemical composition of leaves of *A. malaccensis* was analyzed and confirmed with the presence of mostly α-phellandrene, eucalyptol and cymene 6. However, unlike the work of Bhuiyan *et al.*, the sample selected for this study was a variety of that particular *malaccensis* species, referred here as *nobilis* which is a more robust plant with bigger compact inflorescence 6.

*A. latilabris* is another Zingiberaceous species that is commonly found wild in Malaysia and closely allied to *A. malaccensis* var. *nobilis* 7. The plant can be identified by its yellow flower with crimson spots. A study of the antimicrobial activity of the essential oils extracted from unripe and ripe fruits of *A. latilabris* revealed moderate activity against selected bacteria, while the chemical composition analysis revealed the presence of cineole and β-pinene 7.

In the current study, the essential oils (EO) of two closely related species i.e. *A. malaccensis* var. *nobilis* and *A. latilabris* were extracted and their antimicrobial and antioxidant activities were compared.

**Material and method**

**Sample collection and preparation**

Samples *A. latilabris* and *A. malaccensis* var. *nobilis* were collected in Janda Baik, Pahang, Malaysia and authenticated by Prof Dr Halijah Ibrahim, an expert who specializes on the family of Zingiberaceae. The leaves were separated from the plants and were cut into small pieces. The leaves were dried in an oven with convection at a set temperature of 50°C for a maximum of 3 days or until found to be brittle on applying pressure by hand. The dried leaves were ground into powder form and stored in airtight containers.

**Extraction of essential oils**

The leaf powder was weighed to 100 g and soaked in 500 mL of distilled water overnight. The soaked sample was transferred to a Clevenger apparatus and hydro distilled for 9 hours per day for two days. The steam carrying EO of the sample condenses to fall into a small burette with water (on bottom) and hexane (on top). The hexane containing EO was collected and aerated carefully with nitrogen to remove residue of hexane.

**Antimicrobial activity**

**Screening for antimicrobial activity**

The screening was performed using the suggested qualitative protocols of the Promega BacTiter-Glo™ Microbial Cell Viability Assay (Promega, U.S.A) kit and done in triplicates. The technique was adapted with very few modifications from the BacTiter-Glo™ protocol meant specifically for antimicrobial screening. The selected microbes were cultured for a total of 15 hours using the selected broth (Potato-Dextrose for microbes of *Candida* and *Cryptococcus*; while Mueller-Hinton for others) in temperature 37°C. The cultured microbe solution was diluted to 100-fold, and 245 μL of the diluted culture was transferred to Eppendorf tubes. 5 μL of 500 mg/mL of EO in DMSO was added into the culture. A control of 245 μL of culture and 5 μL of dH2O were also prepared. The culture was incubated for 5 hours in 37°C. Into each Eppendorf tube, 250 μL of BacTiter-Glo™ Reagent was added and left for 5 minutes. A volume of 200 μL of the solution was next transferred to an opaque micro plate, and the luminescence emitted from the solution was read using Infinite Pro M2000 Series micro plate reader. The percentage of inhibition of the EO for different microbes was calculated using the formula below:

\[
\% \text{ Inhibition} = \frac{(\text{Abs}_{\text{control}}) - (\text{Abs}_{\text{sample}})}{\text{Abs}_{\text{control}}} \times 100 \%
\]
where, $\text{Abs}_{\text{control}}$ = Luminescence reading of inoculums with negative control, $\text{Abs}_{\text{sample}}$ = Luminescence reading of inoculums with sample.

The culture that exhibited more than 50% inhibition was considered to have antimicrobial activity against the microbe tested.

Determining $IC_{50}$ value

The value was determined based on the quantitative protocol of the BacTiter-Glo™ Microbial Cell Viability Assay (Promega, U.S.A) kit and done in triplicates. The selected microbes were cultured 15 hours using the selected broth (Potato-Dextrose for *Candida* and *Cryptococcus*; Mueller-Hinton for other microbes) in 37°C. The cultured microbe solution was diluted to 100-fold, and 245 $\mu$L of the diluted culture was transferred to clean Eppendorf tubes. Next, added with 5 $\mu$L of different concentrations of EO dissolved appropriately in DMSO. A control of 245 $\mu$L of culture and 5 $\mu$L of dH$_2$O were also prepared. The Eppendorf tubes were incubated for 19 hours at 37°C followed by addition of 250 $\mu$L of BacTiter Glo™ reagent and left for 5 minutes. A total of 200 $\mu$L of the solution was next transferred to an opaque microplate. The luminescence emitted by the solution was read using Infinite Pro M2000 Series microplate reader. The percentage of viable microbe was calculated using the formula below:

$$\% \text{ Microbial Activity} = \frac{\text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100 \%$$

where, $\text{Abs}_{\text{control}}$ = Luminescence reading by inoculums with negative control, $\text{Abs}_{\text{sample}}$ = Luminescence reading by inoculums with sample.

Next, a graph of percentage of microbial activity against the concentration of EO was plotted and the $IC_{50}$ (the concentration at which there is 50% inhibition of microbial activity) was determined.

Determining the minimum inhibitory concentration (MIC)

The MIC of the selected EO was determined using well-diffusion method. The microbe inoculums was prepared using selected broth (Mueller-Hinton for bacteria, Potato Dextrose for yeast) and were incubated until the absorbance obtained in 600 nm was 0.1, approximately $10^8$ colony forming units (CFUs). Wells were punched in selected agar plates using an immunodiffusion cutter. EO of various serially diluted concentrations was prepared using DMSO. 2 mL of the microbe culture was pipetted onto the agar surface and allowed to stand briefly. The excess culture was carefully discarded and 30 $\mu$L of EO was filled into the punched well. The plate was allowed to stand momentarily before being turned upside down to be incubated in an oven at 37°C for 15 hours. The minimum concentration at which there was no visible inhibition zone was recorded as the MIC.

Monitoring the inhibition of microbial growth over incubation time

The selected microbe culture was incubated until the absorbance of the microbe culture was 0.1 at 600 nm. 1 mL of the microbe culture was transferred to two different cuvettes. In one of the cuvette, 15 $\mu$L of 500 mg/mL of EO dissolved in DMSO was added, while the other cuvette served as control. The absorbance of the culture was measured every 30 minutes for 5 hours. Graph of absorbance against time plotted.

Antioxidant activity

2,2-Diphenyl,1-picrylhydrazyl (DPPH) radical scavenging activity

The method was adapted from Cheung *et al.*, with few modifications. A total of 200 $\mu$L of different concentrations of EOs were prepared in ethanol. A blank and negative control of 200 $\mu$L of ethanol was also prepared. 15 $\mu$L of 0.004% DPPH prepared in ethanol was added into the sample solution and the negative control. The sample was incubated in the dark for 30 minutes, and the absorbance was determined at 517 nm using Infinite M 2000 Series microplate reader. The radical scavenging activity of the sample was calculated using the formula below:

$$\% \text{ Scavenging activity} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100 \%$$

where, $\text{Abs}_{\text{control}}$ = Absorbance of negative control.
A graph of free radical scavenging activity against concentration of EO was plotted, and the IC_{50} was determined as the concentration at which the free radical scavenging activity was 50%.

Ferric ion reducing antioxidant power (FRAP) assay
The method was adapted from Cheung et al., with few modifications. A 50 μL of 0.1M Potassium Phosphate Buffer (pH 6.6) was added with 50 μL of 1% w/v potassium ferricyanide. A volume of 20 μL of different concentration of EO dissolved in ethanol was added to the mixture. A blank mixture consisting of 20 μL of ethanol was also prepared. The reaction mixture was incubated at 50°C for 20 min. 50 μL of 10% w/v trichloroacetic acid was added to the mixture. 50 μL of water and 10 μL of 0.1% w/v ferric chloride were added to the 50 μL of the reaction mixture. The solution was incubated at room temperature for 30 min. The absorbance was measured at 700 nm using Infinite M2000 Series microplate reader. The absorbance of the samples was compared with 0 μg - 0.2 μg Gallic acid standard curve. The FRAP value was expressed in mg GAE/g.

2,2’-Azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) antioxidant assay
The ABTS antioxidant activity of the EO was determined using Sigma-Aldrich Antioxidant Assay Kit and performed in triplicates. ABTS Substrate Working Solution was prepared by adding 10 mL of ABTS Substrate Solution with 25 μL of 3% H₂O₂. The assay was prepared by adding 10 μL of different concentrations of EO dissolved in ethanol with 20 μL of Myoglobin Working Solution. A blank of 10 μL of ethanol added with 20 μL of Myoglobin Working Solution was also prepared. A volume of 150 μL of ABTS Substrate Working Solution was added to the mixture, and it was incubated for 5 minutes. 100 μL of Stop Solution was added to each mixture and the absorbance was measured at 405 nm using Infinite M2000 Series microplate reader. The absorbance obtained was compared with standard curve obtained using 0mM-0.42mM Trolox standards. The ABTS value was expressed as M TE/g.

Gas chromatography analysis
Sample preparation
The essential oil samples were diluted 1:100 in hexane and prepared fresh the day of the analysis.

Gas chromatography- Mass spectrometry (GC-MS)
GC-MS analysis were performed on Agilent Technology 7890A GC System fitted with HP-5 MS UI (30 m × 0.25 mm inner diameter, i.d., film thickness 0.25 μm) column using He as carrier gas. The oven was programmed for 50°C for 5 minutes, to 150°C at 4°C/minute and held for 5 minutes, to 250°C at 4°C/minute and held for 10 minutes. The chemical constituents of the essential oil were separated and identified using the GC-MS NIST libraries.

Gas chromatography-Flame ionization detector (GC-FID)
GC-FID analysis were performed on Agilent Technology 7890A GC System fitted with HP-5 MS UI (30 m × 0.25 mm inner diameter, i.d., film thickness 0.25 μm) column using He as carrier gas and equipped with a Flame Ionization Detector (FID). The oven was programmed for 50°C for 5 minutes, to 150°C at 4°C/minute and held for 5 minutes, to 250°C at 4°C/minute and held for 10 minutes. Kovats index (KI) is used to identify the compounds detected by comparing it with peaks of alkane standard solution C₈ – C₂₀ (Sigma-Aldrich, USA).

Results
The EO of A. malaccensis var. nobilis showed that it had inhibition against selected bacteria and yeast (Table 1) at a standardized dose of 500 mg/mL. The culture that exhibited more than 50% inhibition for A. malaccensis var. nobilis included ten microbes (inclusive of the different accession numbered microbe of the same species). In contrast, A. latilabris showed capabilities against only total of eight microbes with more than 50% inhibition. In both, a total of eleven types of microbes have been screened using the Promega BacTiter-GloTM Microbial Cell Viability Assay (Promega, U.S.A) kit. Any microbial
inhibitions above 50 % were considered to have significant activity, and selected for IC₅₀ determination (Table 2). Based on Table 2, it can be observed that *A. malaccensis* var. *nobilis* had the lowest IC₅₀ against *C. tropicalis* and *C. neoformans*. In *A. latilabris* it can be observed that it worked most efficiently against *K. pneumoniae* with IC₅₀ of 18.83 mg/mL. Overall, it was observed that both EO worked on a broad spectrum of microbes with *A. malaccensis* var. *nobilis* shown to be most effective against the two yeasts tested based on IC₅₀ values obtained. The MIC of the three selected microbes was also determined as shown in Table 3. It can be observed that a

### Table 1. Antimicrobial screening of *A. malaccensis* var. *nobilis* and *A. latilabris*

<table>
<thead>
<tr>
<th>Microbe</th>
<th>ATCC</th>
<th><em>A. malaccensis</em> var. <em>nobilis</em></th>
<th>Percentage of inhibition</th>
<th><em>A. latilabris</em></th>
<th>Percentage of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. subtilis</em></td>
<td>11774</td>
<td>99.35±0.01</td>
<td>98.85±0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>25923</td>
<td>98.55±0.01</td>
<td>95.43±0.55</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>43300</td>
<td>98.37±0.05</td>
<td>94.86±0.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>29213</td>
<td>97.54±0.03</td>
<td>89.75±0.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. baumanii</em></td>
<td>19606</td>
<td>n.a</td>
<td>99.24±0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>10536</td>
<td>98.17±0.02</td>
<td>34.36±1.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>BA-1114</td>
<td>93.27±0.09</td>
<td>97.72±0.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>15442</td>
<td>80.22±0.54</td>
<td>70.15±0.71</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. typhii</em></td>
<td>13311</td>
<td>98.24±0.02</td>
<td>80.59±0.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. neoformans</em></td>
<td>14116</td>
<td>79.56±1.11</td>
<td>n.a</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. tropicalis</em></td>
<td>1369</td>
<td>79.55±0.24</td>
<td>n.a</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: n.a = no activity

The anti-microbial screening done in triplicates using Promega BacTiter-Glo™ Microbial Cell Viability Assay (Promega, U.S.A) kit. The culture that exhibited more than 50 % inhibition was considered to have antimicrobial activity against the microbe tested.

### Table 2. IC₅₀ Values of *A. malaccensis* var. *nobilis* and *A. latilabris*

<table>
<thead>
<tr>
<th>Microbe</th>
<th>ATCC</th>
<th>IC₅₀ (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. subtilis</em></td>
<td>11774</td>
<td>85.67±0.67</td>
</tr>
<tr>
<td></td>
<td>25923</td>
<td>22.33±1.45</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>43300</td>
<td>32.00±1.16</td>
</tr>
<tr>
<td></td>
<td>29213</td>
<td>17.33±0.67</td>
</tr>
<tr>
<td><em>A. baumanii</em></td>
<td>19606</td>
<td>87.33±0.17</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>10536</td>
<td>21.33±0.67</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>BA-1114</td>
<td>13.67±0.60</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>15442</td>
<td>13.67±0.60</td>
</tr>
<tr>
<td><em>S. typhii</em></td>
<td>13311</td>
<td>1.97±0.32</td>
</tr>
<tr>
<td><em>C. neoformans</em></td>
<td>14116</td>
<td>1.75±0.03</td>
</tr>
<tr>
<td><em>C. tropicalis</em></td>
<td>1369</td>
<td>-</td>
</tr>
</tbody>
</table>

IC₅₀ as determined in triplicates using the BacTiter-Glo™ Microbial Cell Viability Assay (Promega, U.S.A) protocol.
similar trend in the strength of inhibition obtained with the order being highest for *C. tropicalis* followed by *C. neoformans* and lastly by *K. pneumoniae*.

The inhibition of EO on the growth of these selected microbes over duration of 300 minutes of incubation is as shown in Fig. 1 to Fig. 3. Fig. 1 shows the growth curve of *C. neoformans* in the presence of *A. malaccensis* var. *nobilis* and Fig. 2 shows the growth curve of *C. tropicalis* in the presence of *A. malaccensis* var. *nobilis*. It can be observed that in the yeast cultures tested, there are no discernible changes for both test solutions, i.e. culture only (control) and the culture with EO for the initial 150 minutes. This is expected of yeast due to its slower growth compared to bacteria or fungi. However, after 150 minutes, the absorbance of culture with EO remained unchanged while the absorbance of control culture increased in its absorbance measurements.

Fig. 3 shows the growth curve of *K. pneumoniae* in the presence of *A. latilabris*. The growth pattern indicates that the control culture has a logarithmic growth pace, while the culture solution utilizing EO of *A. latilabris* was found to have lower as well as a constant absorbance, indicating inhibition of growth.

Additionally, the antioxidant capabilities of the EOs were tested using three different methods: DPPH radical scavenging activity, FRAP and ABTS tests. The DPPH radical scavenging activity was obtained as shown in Fig. 4 for both EOs. The IC$_{50}$ of *A. malaccensis* var. *nobilis* obtained was 32.67±4.37 mg/mL, while that of *A. latilabris*.

### Table 3. MIC against selected microbe

<table>
<thead>
<tr>
<th>Plant type</th>
<th>Microbe</th>
<th>MIC (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. malaccensis</em> var. <em>nobilis</em></td>
<td><em>C. tropicalis</em></td>
<td>3.91</td>
</tr>
<tr>
<td></td>
<td><em>C. neoformans</em></td>
<td>15.63</td>
</tr>
<tr>
<td><em>A. latilabris</em></td>
<td><em>K. pneumoniae</em></td>
<td>250</td>
</tr>
</tbody>
</table>

The MIC (concentration at which there was no visible inhibition zone) of the serially diluted EO was determined using well-diffusion method.

![Figure 1. Growth curve of *C. neoformans* culture in the absence (control) (♦) and presence (■) of *A. malaccensis* var *nobilis* as measured every 30 minutes for 5 hours](image-url)
was higher i.e. 54.33±5.48 mg/mL. The Gallic acid equivalents (mg GAE/g) of the EO was determined using FRAP assay as shown in Fig. 5. The calculated GAE value of A. malaccensis var. nobilis is 26.59±3.56 mg GAE/g, while for A. latilabris oil is 14.47±2.15 mg GAE/g. The Trolox equivalent values of the EOs was obtained using ABTS assay as shown in Fig. 6. The Trolox equivalent value of EO of A. malaccensis var. nobilis was approximately 24.56± 1.85 M TE/g while the value of A. latilabris was only 17.51±1.79 M TE/g. As such overall it can be concluded that both A. malaccensis var. nobilis and A. latilabris have shown antioxidant capa-
Figure 4. Graph of DPPH free radical scavenging activity of different EO based on n=3

Figure 5. Graph of Gallic acid equivalency (GAE) value of the different EO based on n=3

bilites, with the former having higher antioxidant properties than the latter.

The compounds found in the EOs of both *A. malaccensis* var. *nobilis* and *A. latilabris* were identified based on the GC-MS analysis of their total ion chromatogram together with further confidence obtained by converting retention time the compounds into kovats index to make compari-
sons in the GC-FID analysis as shown in Table 4. The analysis concluded that in EO of *A. malaccensis* var. *nobilis*, the major compound present was methyl cinnamate (63.00 %). Other major ones included 5.95 % of 1,8-cineole and 6.18 % of α-terpineole. In the EO of *A. latilabris*, phytol was the major compound (30.63 %) followed by carvone (6.88 %) and β-sesquiphellandrene (5.51 %).

**Discussion**

As expected of any ginger type essential oils the antimicrobial screening performed on *Alpinia* spp. EOs also worked well against the wide spectrum of microbes tested. Others who worked on these species found such similar findings. A research performed on EO of *A. latilabris* fruit proved that it worked against various microbes such as *S. aureus, B. subtilis, E. coli, P. aeruginosa* and etc. Similarly a research conducted on essential oil and methanol extracts of *A. malaccensis* leaf showed inhibition against *P. aeruginosa, S. aureus, C. albicans* and *A. niger*. The most significant findings related to EO of *A. malaccensis* var. *nobilis* is that, it is capable in inhibiting two different types of yeasts, namely *Candida tropicalis* and *Cryptococcus neoformans*. *C. tropicalis* is a type of opportunistic pathogen which is responsible for nosocomial candidaemia bloodstream infection among patients which have compromised immune system such as HIV patients. *C. neoformans* is a pathogen that causes cryptococcosis, a form of lung infection. Although more prevalent among patients with immune deficiency, the prolonged inhalation of infectious particle from the environment (particularly pigeon droppings) may also cause the pathogen to spread among person having normal immune system. The EO of *A. latilabris* was shown to have the strongest antimicrobial activity against *K. pneumoniae*. This Gram-negative bacteria had emerged as one of the strain that is highly resistant against a wide ranged spectrum of antibiotics which have been used clinically. Notably it is apparent that the ability of *A. malaccensis* var. *nobilis* as an antimicrobial agent goes beyond that of bacteria. However due to constraints in obtaining more yeasts types, the extent of its anti-yeasts capabilities was not able to be ascertained completely.

A study conducted on *Alpinia malaccensis* found in Bangladesh showed the IC₅₀ DPPH free radical scavenging activity to be 18.26 μg/mL. In a study on selected plants of *Elingera* spe-
Table 4. Chemical compounds of *A. malaccensis* var. *nobilis* and *A. latilabris* E Os

<table>
<thead>
<tr>
<th>Compound</th>
<th>KI T</th>
<th>KI C</th>
<th>Retention time</th>
<th>% Area</th>
<th>Identified by</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\alpha)-Terpineol</td>
<td>1195</td>
<td>1193</td>
<td>20.484</td>
<td>6.18</td>
<td>MS, KI</td>
</tr>
<tr>
<td>Methyl cinnamate</td>
<td>1379</td>
<td>1389</td>
<td>27.122</td>
<td>63.00</td>
<td>MS, KI</td>
</tr>
<tr>
<td>Aromadendrene</td>
<td>1475</td>
<td>1468</td>
<td>29.552</td>
<td>0.71</td>
<td>MS, KI</td>
</tr>
<tr>
<td>Caryophyllene oxide</td>
<td>1573</td>
<td>1562</td>
<td>32.888</td>
<td>0.36</td>
<td>MS, KI</td>
</tr>
<tr>
<td>Guaiol</td>
<td>1589</td>
<td>1586</td>
<td>33.810</td>
<td>1.17</td>
<td>MS, KI</td>
</tr>
<tr>
<td>Phytole</td>
<td>2128</td>
<td>2114</td>
<td>50.860</td>
<td>0.97</td>
<td>MS, KI</td>
</tr>
<tr>
<td>(\beta)-Bisabolene</td>
<td>1498</td>
<td>1510</td>
<td>30.993</td>
<td>3.39</td>
<td>MS, KI</td>
</tr>
<tr>
<td>(\beta)-Sesquiphellandrene</td>
<td>1519</td>
<td>1532</td>
<td>31.459</td>
<td>5.51</td>
<td>MS, KI</td>
</tr>
<tr>
<td>(\alpha)-Calacorene</td>
<td>1859</td>
<td>1848</td>
<td>43.714</td>
<td>4.52</td>
<td>MS, KI</td>
</tr>
<tr>
<td>Farnesyl acetate</td>
<td>1935</td>
<td>1921</td>
<td>45.880</td>
<td>3.79</td>
<td>MS, KI</td>
</tr>
<tr>
<td>Nerolidol</td>
<td>1539</td>
<td>2032</td>
<td>48.831</td>
<td>1.30</td>
<td>MS, KI</td>
</tr>
<tr>
<td>Phytole</td>
<td>2128</td>
<td>2116</td>
<td>50.917</td>
<td>30.63</td>
<td>MS, KI</td>
</tr>
</tbody>
</table>

Note: C refers to calculated KI, while T refers to theoretical KI taken in the range of ±10 of calculated KI. Identifications of all compounds achieved to the confidents of both GC-MS and GC-FID analysis.

The GC analysis of both essential oils showed that most of the compounds present in the EOs can be classified as terpenes and terpenoids. The results of this study showed that the main components detected in the EO of *A. malaccensis* var. *nobilis*, are methyl cinnamate (63.00 %), \(\alpha\)-ter-
pineol (6.18 %), and 1, 8-cineole (5.95 %). A study by Azah et al., reported a higher methyl cinnamate content of 88.00 % in the EO of the leaf of A. malaccensis var. nobilis. While the methyl cinnamate content of the EO of its rhizome was found to be 78.2 %.

Interestingly Bhuiyan et al., studied on A. malaccensis (uncertain on the variety studied as not mentioned) from Bangladesh but detected completely different compounds i.e. α-phellandrene (31.80 %), eucalyptol (13.76 %) and O-cymene (11.45 %). This difference in the compounds and its composition maybe most likely due to its geographical location and population variations in the samples studied. The major component of EO of A. latilabris in this study was phytol (30.63 %) followed by carvone and β-sesquiphellandrene (6.88 % and 5.51 % respectively). Phytol (3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol) is a type of isoprenoid component found in chlorophyl. In a previous research conducted by Pejin et al., phytol had been proven to have antimicrobial activity against sixteen different pathogenic microbes of infective endocarditis.

Interestingly in a recent study by Saensouk et al., there has been mention of Alpinia malaccensis in a survey identifying 38 species belonging to Zingibereae family in three regions of Thailand but not A. latilabris (or even any of its synonyms). Due to the morphological similarities of A. malaccensis var. nobilis and A. latilabris, relying merely on taxonomic keys may not be sufficient to differentiate and instead may lead easily to misidentification. Alternate or rather additional reliance on sensitive instruments or reliable as-
says had been warranted for surety of identification if very closely related species or variants are suspected to be involved. Some workers already clearly identified variations in substantial chemical composition among species of gingers. Nevertheless, limitations arise for such utilizations especially in attaining specialized equipment as well as readily available chemical database of all gingers in the wild.

Conclusion
Overall, it can be observed that EO of A. malaccensis var. nobilis exhibited better antimicrobial and antioxidant properties compared to A. latilabris. Most importantly A. malaccensis var. nobilis had significant anti-microbial activity against the two yeasts studied, with IC<sub>50</sub> against C. tropicalis and C. neoformans of 1.75±0.03 mg/mL and 1.97±0.32 mg/mL, respectively. Although here is a high level of methyl cinnamate (63.00 %) found in EO of A. malaccensis var. nobilis, however it is insufficient to substantiate with certainty of its role in exerting these biological activities unless isolated in the future to conclusively link the constituents to their respective biological activities.

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References


